

Biofilm formation (slime production) plays an important role in the pathogenesis of infections caused by different microorganisms . Samples collected from denture and orthodontic devices were diagnosed genetically after cultured and diagnostic microscopy using 16SrDNA sequencing from both devices. 16SrDNA sequence database provides high quality excellent identification at the species and subspecies levels. Screened these bacterial isolates for ability to form biofilm formation using CRA test, TCP test and icaAD gene test found gene test 100% was the best test for identified bacterial species to form biofilm for both devices . Moreover, in the present study four bacterial strains were recorded in European Nucleotide Archive (ENA), National Centre for Biotechnology (NCBI) and GeneBank as new strains in world and registered in the name of Iraq / Basrah.

Genetic Study of Biofilm Forming Bacteria

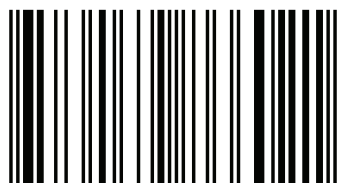


Kholoud Abdul Kareem Hussein

Study Biofilm of Bacteria Isolates, Identified by 16srRNA Gene Sequencing

Genetic Study of Biofilm Forming Bacteria, Isolated From Denture and Orthodontic Devices

Lecturer Kholoud Abdul Kareem Hussein has obtained her M.Sc. degree in Molecular Genetic of Medical Microbiology in 2013 from Basrah University , Science college, Biology department.



978-613-9-86602-1

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17 Meldrum Street, Beau Bassin 71504, Mauritius

Printed at: see last page

ISBN: 978-613-9-86602-1

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Acknowledgments

My heartfelt gratitude must be firstly offered to (Allah) for His merciful support and guidance to complete this study. All blessings and respects are for our beloved Prophet Muhammad whose teachings guide us.

I would like to thank and express my deep gratitude to my late father, martyr Assistant Professor Abdulkareem Hussein, lecturer in College of Agriculture, who taught me patience and guided me way, and for my mother whose love is boundless, my brother, my sister. Finally especial merciful love for my husband waleed and my lovely little children (Malak, Ahmed and Adam).

Kholoud

Summary

One hundred samples were collected from patients wearing dentures (n=50) using sterile cotton swabs and orthodontic devices (n=50) using sterile needle Under the supervision of a specialist doctor from the center of Al-shaheed Qais dental and private clinics in Basrah. All samples were collected randomly from both genders aged between 12-70 years.

The bacterial species were identified by molecular diagnosis using *16SrDNA* sequencing for only 94 bacterial samples from patient wearing denture (n=47) and orthodontic devices (n=47) revealing the following species in patient wearing denture : *Klebsiella pneumoniae* [8(40%)], *Proteus mirabilis* [7(35%)], *Proteus penneri* [5(25%)], *Enterobacter cloacae* [3(15%)], *morganella morganii* [2(10%)], *Hafnia alvei* [2(10%)], *Enterobacter aerogenes* [2(10%)], *Enterococcus faecalis* [1(5%)], *Enterobacter faecium* [1(5%)], *Bacillus cereus* [1(5%)], *Enterobacter mori* [1(5%)] and *Citrobacter freundii* [1(5%)], Some of these bacterial species were isolated at first time in the world from patient wearing denture as : *Proteus huseri* [1(5%)], *Klebsiella variicola* [1(5%)], *Lactococcus lactis* [1(5%)], *Streptococcus equinus* [1(5%)], *Acinetobacter baumannii* [1(5%)], *Chryseobacterium vietnamense* [1(5%)], *Klebsiella oxytoca* [1(5%)], and *Staphylococcus hominis* [1(5%)]. But the following species from patient wearing orthodontic devices : *Klebsiella pneumoniae* [8(40%)], *Staphylococcus aureus* [7 (35%)], *Bacillus cereus* [4(20%)], *Enterobacter cloacae* [3(15%)], *Enterococcus faecalis* [3(15%)], *Staphylococcus epidermidis* [2(10%)], *Proteus penneri* [2(10%)], *Enterobacter faecium* [2(10%)], *Enterobacter mori* [1(5%)], *Citrobacter freundii* [1(5%)], *Staphylococcus worneri* [1(5%)] and

Serratia marcescens [1(5%)]. Some of these bacterial species were isolated at first time in the world from patient wearing orthodontic devices as: *Staphylococcus pasteurii* [1(5%)], *Enterobacter ludwigii* [1(5%)], *Lactobacillus plantarum* [2(10%)], *Streptococcus anginosus* [2(10%)], *Pediococcus acidilactici* [1(5%)], *Bacillus subtilis* [2(10%)], *Escherichia fergusonii* [1(5%)], and *Proteus mirabilis* [1(5%)]. Furthermore, *Proteus penneri*, *Enterobacter mori* and *Citrobacter freundii* in both patient wearing denture and orthodontic devices were isolated at the first time.

The rooted neighbour joining (NJ) phylogenetic tree of isolates from present study (n=28) with 28 type strains from GeneBank showed that *Enterobacter cloacae* is the out group (root). Moreover, four bacterial strains were recorded in European Nucleotide Archive (ENA), National Centre for Biotechnology Information (NCBI) and Gene Bank as new strains in the world, these are: 71-*Chryseobacterium vietnamense* "IRQBAS3" (HG003648) and 74-*Morganella morganii* "IRQBAS4" (HG003649) isolated from patient wearing dentures, and 7-*Enterobacter ludwigii* "IRQBAS1" (HG003646), and 34-*Enterobacter cloacae* "IRQBAS2" (HG003647) isolated from patient wearing orthodontic devices . All these strains showed 99% sequence identity with their reference due to occur of point or frame shift mutation.

Biofilm formation of bacterial isolates from patient wearing dentures (n=47) and patient wearing orthodontic devices (n=47) were screened by Congo red agar (CRA), tissue culture plate (TCP) and *icaAD* gene method. In patient wearing dentures, CRA method appeared the frequency of negative isolates 37(78.7%) of 47 isolates was higher than positive isolates 8(17.02%) of 47 isolates with high significant ($p \leq 0.01$), and higher than intermediate 2(4.3%) of 47 isolates with high

significant ($p \leq 0.01$), while frequency of positive isolates was higher than intermediate isolates with high significant ($p \leq 0.01$).

The frequency in patient wearing orthodontic devices recovered very different results : 4(8.5%) of 47 isolates appeared positive result , 2(4.3%) of 47 isolates appeared intermediate result. In contrast, frequency of positive results was higher than intermediate with high significant ($p \leq 0.01$). 41(87.2%) of 47 isolates appeared negative results and frequency of this result was higher than positive and intermediate with high significant ($p \leq 0.01$). No significant differences were appeared between patient wearing dentures and orthodontic devices results for each test (positive, intermediate and negative) .

TCP method was recovered in patient wearing dentures, frequency of weak positive results [29(61.7%) of 47 isolate] appeared to be higher than high [8(17.02%) of 47 isolates] and moderate [7(14.9%) of 47 isolates] positive results with high significant difference ($p \leq 0.01$) , 3(6.4%) of 47 isolates showed negative results . However , the frequency of positive isolates 44(94%) of 47 isolates (high, moderate ,and weak) was higher ($p \leq 0.01$) than negative results.

The TCP method for patient wearing orthodontic devices , recovered that the weak positive [9(19.2%) of 47 isolates] result is higher than positive[2(4.3%) of 47 isolates] with high significant difference ($p \leq 0.01$). Furthermore, 36(76.6%) of 47 isolates showed negative results with high significant ($p \leq 0.01$) than positive (high, moderate and weak) results. However, frequency of positive result (high, moderate and weak) in patient wearing dentures was higher ($p \leq 0.01$) than in patient wearing orthodontic devices . Following, the frequency of negative result in patient wearing orthodontic devices is higher ($p \leq 0.01$) than inpatient wearing dentures .

icaAD gene method for isolates of patient wearing dentures showed that the present of *icaA* [37(78.7%) of 47 isolates] results was higher than the absent of *icaA* [10(21.3%) of 47 isolates] gene with high significant difference ($p \leq 0.01$). *icaD* gene was recovered 40(85.1%) of 47 isolates as positive results with high significant difference ($p \leq 0.01$) than negative results 7(14.9%) of 47 isolates.

In patient wearing orthodontic devices , the frequency of *icaA*[2(4.3%) of 47 isolates] gene negative results was higher than *icaA*[45 (95.8%) of 47 isolates] gene positive results with high significant difference ($p \leq 0.01$). While, the *icaD* gene was recovered 24(51.1%) of 47 isolates as positive results and 23(48.9%) of 47 isolates as negative results but without significant difference . Comparing ,in patient wearing dentures, TCP 44(93.6) and *icaA* and /or *icaD* gene 47(100%) are the best methods for detection of biofilm formation (no significant differences between these results), on the other hands, *icaA* and /or *icaD* gene were only the best method for detection biofilm formation in patient wearing orthodontic devices .

The frequency of bacteria was higher ($p \leq 0.01$) with patients aged >35 (70.1%), denture washing (in day) < 2 time (61.7%), without tonsillitis (100%), without gingivitis (100%), no cigarettes smoking (95.7%) and without dental caries (95.7%), but no significant difference with date of denture wearer (year). In orthodontic , the frequency of bacteria was higher ($p \leq 0.01$) in patients aged <18 (78.7%) , orthodontic washing > 2 (68.1%),tonsillitis (100%) , gingivitis (70.2%) , no cigarettes smocking (100%),without dental caries (100%), but no significant differences in patients with date of orthodontic insertion factor.

List of content

No.	content	Page
	Acknowledgement	I
	Summery	II
	List of contents	VI
	List of figures	XI
	List of tables	XVIII
	Abbreviations	XIX
No.	Chapter one : Introduction	1-5
1-1	Introduction	1
1-2	Aims of the study	5
No.	Chapter two : Literature Review	6-30
2-1	The role of oral cavity in disease	6
2-2	Common oral diseases	6
2-2-1	Dental caries	6
2-2-2	Periodontal diseases	9
2-3	Dentures	10
2-3-1	Type of denture	10
2-3-1-1	Removable partial dentures	10
	Removable complete dentures(full dentures)	
2-3-1-2		10
2-3-1-3	Implant over denture	11
2-3-2	Oral environment with denture wearer	12
2-3-3	Problems associated with denture plaque	13

2-3-3-1	Stomatitis and oral candidosis	13
2-3-3-2	Malodor	14
2-3-3-3	Reservoir of infection	14
2-4	Orthodontic	15
2-5	Dental plaque	17
2-6	Biofilm	17
2-7	Methods for detection of bacterial forming biofilms	20
2-8	Genes responsible for synthesis biofilm	21
2-9	Molecular genetic	23
2-9-1	Identification of bacterial species by <i>16SrDNA</i> sequencing	23
2-9-2	Phylogenetic tree	24
2-10	Bacterial species isolated in dentures and orthodontic	25
No.	Chapter three : Materials & Methods	31-49
3-1	Equipments and materials	31
3-2	Chemicals and dyes	32
3-3	Prepared media	34
3-4	Preparation of chemical solutions	34
3-4-1	Tris Borate – EDTA.(TBE) buffer preparation 5x	34
3-4-2	Tris EDTA (TE) buffer preparation	35

3-4-3	Ethidium bromide dye solution	35
3-4-4	Bromophenol blue dye solution	35
3-4-5	Phosphate buffer saline	36
3-4-6	Preparation of commercial culture media	36
3-5	Methods	36
3-5-1	Sample collection	36
3-5-2	Examination of specimen	38
3-5-2-1	laboratory diagnosis (Examination of specimens)	38
3-5-3	Molecular genetic identification	38
3-5-3-1	DNA extraction from bacteria	38
3-5-3-2	Examination of genomic DNA by agarose gel electrophoresis	40
3-5-3-3	Polymerase Chain Reaction (PCR) Technique	41
3-5-3-4	Universal <i>16SrDNA</i> primers	41
3-5-3-5	Reagents	42
3-5-3-6	Thermal cycling condition	43
3-5-3-7	Detection of PCR product by agarose gel electrophoresis	43
3-5-3-8	<i>16 SrDNA</i> gene sequences	44
3-5-3-9	Identification of bacteria	45
3-5-3-10	Phylogenetic tree	45
3-5-4	Detection the ability of adherence	46
3-5-4-1	Congo Red Agar (CRA) method	46

3-5-4-2	Preparation of Tissue Culture Plate (TCP) method	46
3-5-4-3	<i>icaAD</i> gene primers	47
3-5-4-3-1	Reagents	47
3-5-6-2	Thermal cycling condition	48
3-5-6-3	Detection of PCR product by agarose gel electrophoresis	48
3-5-7	Statistical analysis	49
No.	Chapter four: Results	50-107
4-1	Molecular Genetic Study	50
4-1-1	DNA extraction	50
4-1-2	Universal <i>16SrDNA</i> gene detection by PCR	50
4-1-3	Sequencing for universal <i>16S rDNA</i> gene	56
4-1-4	Phylogenetic tree of bacterial species	56
4-1-5	Detection of new bacterial strain from denture and orthodontic isolates	74
4-2	Detection of biofilm formation	86
4-2-1	Congo Red Agar (CRA) method	86
4-2-2	Tissue Culture Plate (TCP) method	88
4-2-3	Detection of <i>icaA</i> and <i>icaD</i> genes in denture and orthodontic	90
4-2-3-1	<i>icaAD</i> gene for adherence	90
4-3	The effectiveness of different bacterial species towards CRA , TCP, and <i>icaAD</i> genes Assays	92

4-4	Distribution of the bacterial species between denture and orthodontic devices	101
4-5	Detection of bacterial species isolates at first time from denture and orthodontic	103
4-6	The frequency of bacterial species according to some factors	103
No.	Chapter five : Discussion	108-126
5-1	primer for <i>16SrDNA</i> gene	108
5-2	Sequencing of <i>16SrDNA</i> gene and phylogenetic tree	108
5-3	The new recording of bacterial strain from denture and orthodontic	109
5-4	Detection of biofilm formation	111
5-5	The effectiveness of different bacterial species toward CRA, TCP and <i>icaAD</i> genes assays	113
5-6	Type of bacteria have the ability to produce slime	114
5-7	Distribution of the bacterial species between denture and orthodontic	116
5-8	Species isolated from denture and orthodontic	117
5-9	Species isolated from denture only	119
5-10	Species isolated from orthodontic only	122
5-11	Frequency of bacterial species from denture and orthodontic according to some factors	125

No.	Conclusion & recommendation	127-128
6-1	Conclusions	127
6-2	Recommendations	127
References		129-162
No.	Appendix	163-187
Appendix – 1	Alignment and concatenating of bacterial species obtained from the present study after sequencing data and reference strain by GeneBank using "CLUSTAL W "	163
Appendix – 2 A-1	<i>Enterobacter ludwigii</i> strain, IRQBAS1. GeneBink	180
Appendix – 2 B-1	<i>Enterobacter cloacae</i> strain IRQBAS2. GeneBink	182
Appendix – 2 C-1	<i>Chryseobacterium vietnamense</i> strain IRQBAS3. GeneBink	184
Appendix – 2 D-1	<i>Morganella morganii</i> strain IRQBAS4. GeneBink	186

List of figures

No.	Figure	Page
No.	Chapter two : Literature Review	6-30
2-1	Development Schematic representation of ecological dental caries	8

2-2	Removable partial dentures	10
2-3	Removable complete dentures	11
2-4	Implant over denture	11
2-5	Orthodontic	16
2-6	Steps of biofilm formation	20
2-7	Map of genomic organization of the <i>ica</i> <i>ADBC</i> gene cluster from <i>S. epidermidis</i> (O'Gara & Humphreys, 2001).	22
2-8	Biosynthesis of the exopolysaccharide poly-N-acetyl glucosamine (PIA).	23
No.	Chapter three : Materials & Methods	31-49
3 – 1	General layout of the study	37
No.	Chapter four : Results	50-107
4-1	Agarose (0.8%) gel electrophoresis for DNA bands (1-5) of random bacterial isolates from dentures and orthodontic under UV transilluminator	50
4-2	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator.Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-6: 1to5 of <i>16SrDNA</i> bands(1500bp) for bacterial isolates	51

4-3	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-6: 6 to 10 of <i>16SrDNA</i> bands (1500bp) for bacterial isolates	51
4-4	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-12: 11 to 21 of <i>16SrDNA</i> bands (1500bp) for bacterial isolates	52
4-5	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-13: 22 to 33 of <i>16SrDNA</i> bands (1500bp) for bacterial isolates	52
4-6	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-12: 34 to 44 of <i>16SrDNA</i> bands (1500bp) for bacterial isolates	53

4-7	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-12: 45 to 55 of <i>16SrDNA</i> bands (1500bp) for bacterial isolates	53
4-8	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-19: 56 to 73 of <i>16SrDNA</i> bands (1500bp) for bacterial isolates	54
4-9	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-12: (74 to 84 of <i>16SrDNA</i> bands (1500bp) for bacterial isolates	54
4-10	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-6: 85 to 89 of <i>16SrDNA</i> bands (1500bp) for bacterial isolates.	55
4-11	Agarose (1%) gel electrophoresis of universal <i>16SrDNA</i> PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-6: 90 to 94 of <i>16SrDNA</i> bands (1500bp) for bacterial isolates	55

4-12	Rooted neighbor joining phylogenetic tree constructed from concatenated sequences of (893bp)for bacterial species (derived from an alignment of <i>16SrDNA</i> gene sequences) then produced by (MAFFT)multiple alignment program for amino acid or nucleotide sequences and visulised using" forester" software. This NJ tree showing the distribution and phylogenetic relationships of 28 different species identified in this study and their refrence strains (T). The tree has been rooted with <i>Enterobacter mori</i> or bootstrap values = 1000. All horizontal branch length were drawn to scale	73
4-13	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 7-<i>Enterobacter ludwigii</i> identical (99%) to strain NRCG12 showed frame shift mutation (deletion nucleotide C) at the position 453bp changing all the following amino acid	76
4-14	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 34- <i>Enterobacter cloacae</i> identical (99%) to strain Nr.3 showed two point mutation type Transversion : G instead C at the position 462bp changing the amino acid Thr (ACC) to Ser (AGC) and C instead G at the position 471bp changing the amino acid Gly (GGT) to Ala (GCT).	77
4-15	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 71- <i>Chryseobacterium vietnamense</i> identical (99%) to strain GIMN1.005; showed point mutation type transversion A instead G at the position 207bp changing the amino acid Arg(CGC) to His (CAC).	78

4-16	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 71-<i>Chryseobacterium vietnamense</i> identical (99%) to strain GIMN1.005; showed point mutation type transversion T instead A at the position 102bp changing the amino acid Gln (CAG) to Lus(CTC),and point mutation type transition G instead A at the position 149bp changing amino acid Asn (AAT) TO Asp (GAT),and point mutation type transition C instead T at the position 160bp changing the stop codon (ATT) to Trp (ATC),and point mutation type transition G instead A at position 163bp without changing the type of amino acid	79
4-17	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 71-<i>Chryseobacterium vietnamense</i> identical (99%) to strain GIMN1.005; showed point mutation type transversion A instead C at the position 341bp without changing the type of amino acid	80
4-18	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 71-<i>Chryseobacterium vietnamense</i> identical (99%) to strain GIMN1.005; showed point mutation type transversion T instead G at the position 542bp changing the amino acid Gly (GGA) to stop codon (TGA)	81
4-19	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 71-<i>Chryseobacterium vietnamense</i> identical (99%) to strain GIMN1.005; showed point mutation type transversion C instead T at the position 631bp changing the amino acid Pro (TCC) to Ser(CCC).	82

4-20	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 74- <i>Morganella morganii</i> identical (99%) to strain MFS05 show frame shift mutation (deletion nucleotide G) at the position 554bp changing all following amino acids	83
4-21	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 47- <i>Morganella morganii</i> identical (99%) to strain MFS05 show frame shift mutation (deletion nucleotide C) at the position 58bp changing all the following amino acids	84
4-22	CLUSTALW for comparison of nucleotide sequences alignment for <i>16SrDNA</i> gene of 47- <i>Morganella morganii</i> identical (99%) to strain MFS05 show frame shift mutation (deletion nucleotide T) at the position 712bp changing all the following amino acids	85
4-23	Congo Red Agar (CRA) assay for bacterial isolates from dentures and orthodontics . Positive is black colonies with dry crystalline (slime producers) , intermediate is dark- pink colonies (weak slime producers), negative is pink-white as (not slime producers)	87
4-24	Tissue Culture Plate(TCP) assay of bacterial isolates from dentures and orthodontic . High: dark color, weak: poor color , moderate: medial color and negative : colorless.	89

4-25	Agarose (1%)gel electrophoresis showed PCR product of <i>icaA</i> gene for denture and orthodontic isolates. Lane 1: (100bp-1000bp) DNA ladder,Lane 2 to 6: <i>icaA</i> bands (188bp) of different isolates	91
4-26	Agarose (1%) gel electrophoresis showed PCR product of <i>icaD</i> gene to denture and orthodontic isolates. Lane 1: 1Kb (100bp-1000bp) DNA ladder (DNA marker).Lane 2 to 6: <i>icaD</i> bands(198bp) of different isolates	91
4-27	Percentage of bacterial species in denture and orthodontic	102

List of table

No.	Table	Page
No.	Chapter three : Materials & Methods	31-49
3-1	Equipments	31
3-2	Chemicals and dyes	33
3 -3	Prepared media	34
3-4	Universal <i>16SrDNA</i> primers used in PCR amplification	42
3 -5	Reagents and volum (25μl) used in PCR amplification or <i>16SrDNA</i> gene	42
3-6	Program used in PCR amplification for <i>16SrDNA</i> gene	43
3 - 7	<i>icaAD</i> primers sequence.	47
3 - 8	Reagents and volume (25μl) used in PCR amplification for <i>icaA</i> or <i>icaD</i> gene	48

3 - 9	Program use in PCR amplification for <i>icaA</i> or <i>icaD</i> gene	48
No.	Chapter four : Results	50-107
4 -1	Bacterial Species Identified By Sequencing Of Universal <i>16SrDNA</i> for Isolates from Dentures.	57
4-2	Comparison of Congo Red Agar (CRA) assay for bacterial isolates from denture and orthodontic	87
4 -3	Comparison of Tissue Culture Plate (TCP) assay for bacterial isolates from denture and orthodontic	89
4 - 4	Comparison of <i>icaAD</i> genes for bacterial species from denture and orthodontic.	92
4 - 5	Effectiveness of bacterial species from dentures toward slim formation assays.	94
4 - 6	Effectiveness of bacterial species from orthodontic toward slim formation assays	98
4 - 7	Frequency of bacterial species from denture according to some factors	104
4 -8	Frequency of bacterial species from orthodontic according to some factors	106

Abbreviations

Bp	Base pair
C	Celsius
CRA	Congo Red Agar
D.W.	Distal water
DNA	Deoxy ribonucleic acid

dNTPs	Deoxy ribonucleotide triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
ELISA	Enzyme Linked Immunosorbent Assay test
F	Forward
hr	Hour
Kb	Kilobase
min	Minute
ml	milliliter
NCBI	National Center for Biotechnology
Ng	Nanogram
No.	Number
OD	Optical density
PBS	Phosphate buffer Saline
PCR	Polymerase Chain Reaction
PEG	Poly Ethylene Glycol
pH	Power of hydrogen
pmol	Pico mole
R	Reverse
Sec	Second
TCP	Tissue Culture Plate
Tm	Melting temperature
μl	Microliter

μm	Micrometer
UV	Ultraviolet
V	Volt
μg	Microgram
<i>ica</i>	Intra cellular adhesion
<i>16S rDNA</i>	Sixteen subunit ribosomal deoxyribonucleic acid

Chapter one

Introduction

Oral cavity is one of the most complex microbial habitats in the human body that comprises of more than 600 diverse arrays of bacterial species (Kazor *et al.*, 2003). Oral microbiota beside its importance in oral health reflects the health and disease status of the host as it effects many other systemic diseases like bacterial endocarditis, pneumonia, preterm low birth weight and coronary heart disease gastrointestinal infection and chronic obstructive pulmonary (Thean *et al.*, 2007). Among many oral the dental caries and periodontal disease are the most common causes of tooth loss (Bowley, 2002 ; Petersen *et al.*, 2005). Tooth loss can result in diminished function, unbalanced diet, malnutrition,(Wöstman *et al.*, 2005), as well as loss of self-esteem(Jones *et al.*, 2003).Furthermore, dental caries and periodontal diseases have been historically considered the most important global oral health burdens, at present, the distribution and severity of oral diseases vary in different parts of the world and within the same country or region (Moimaz *et al.*, 2006).

Dental caries are commonly known as tooth decay However, it has been known for over 100 years that dental decay is caused by bacteria fermenting foods, producing acids and dissolving tooth mineral (Featherstone, 2008). At least two major groups of bacteria, namely the mutans streptococci and the lactobacilli species, are able to produce organic acids during metabolism of fermentable carbohydrates by these bacteria (Loesche, 1986; Marsh,1994) Furthermore, *Lactobacillus acidophilus* , and *Actinomyces viscosus* may be considered the main pathogenic species

involved in the initiation and development of dental caries (Shivakumar *et al.*, 2009). Periodontal disease is one of the most Common diseases of man and is responsible for most of the tooth loss in adults, bacterial species associated with periodontitis are *Porphyromonas gingivalis* , *Tannerella forsythia* and *Treponema denticola* , *Aggregatibacter actinomycetemcomitans*, associated with localized aggressive periodontitis, some Gram-positive species, such as *Peptostreptococcus micros* (Socransky *et al.*, 1998).

Oral health status declines with age and as a result the need for removable prostheses increases, denture usually made from poly methylmethacrylate (PMMA) materials called resin , these prostheses are generally attached to the remaining natural teeth by clasps that hold the denture in place (Jagger, 2002). Wearing removable dental prosthesis causes an alteration in the oral micro flora (Girard *et al.*, 1996). and dentures offer a reservoir for microorganisms associated with more systematic disease infections (Li *et al.*, 2000). There are three types of denture these are: Removable partial dentures, Removable complete dentures , Implant over denture (Pahlevan, 2005 ; Singla, 2007 ;Vecchiattini *et al.*, 2009). Oral cavity health is closely related with the dentures the patient wears, the latter cause retention of food particles, formation of plaque, as well as inflammation of oral cavity and periodontium, both, removable and fixed dentures, may to a great extent cause problems of bad breath, although the source of unpleasant breath is commonly related to location of bacteria colonies on the tongue, in pathological gingival pockets, on teeth and adjacent tissue (Jeng *et al.*, 1999) . The amount of cariogenic bacteria in persons with fixed prostheses is different from that of elderly persons with removable dentures (Tanaka

et al., 2003) There is an increase in the number of risk factors of oral diseases and missing teeth MT in the elderly, the type of prosthesis was decided by the number of MT in most cases, on the other hands , orthodontic treatment with fixed appliances resistance to undesirable tooth movement leads to increase biofilm accumulation and elevated levels of cariogenic and periodontal bacteria , the microbiological changes after bracket placement became a topic of interest during the late 1980, mainly because orthodontic brackets make good oral hygiene difficult, resulting in plaque accumulation and significantly increased risks for enamel demineralization or periodontal disease (Pellegrini *et al.*, 2009).Initially cariogenic species such as *Streptococcus mutans* and *Lactobacillus* species and the subsequent decalcification of enamel were the main fields of interest among investigators (Forsberg *et al.*, 1991; Rosenbloom and Tinanoff, 1991). Later on, the more complex system of periodontopathogenic microbes and the changes after bracket placement became the main topics of interest (Paolantonio *et al.*, 1996; Paolantonio *et al.*, 1999; Petti *et al.*, 1997). Naranjo *et al.*, (2006) observed a transition in subgingival dental plaque after the placement of brackets, the plaque index and gingivitis index increased significantly (Papaioannou *et al.* , 2007 ; . Pandis *et al.*, 2008).The presence of denture and orthodontic in mouth may lead to increase denture and dental plaque , dental plaque is a highly complex biofilm ,biofilm formation capacity is associated with antimicrobial resistance, and considered widely as a virulence factor (Forsberg *et al.*, 1991 ; Nikawa *et al.*, 1998 ; Marsh, 2005). Invasive isolates are more prone to produce biofilm than carriage isolates of healthy individuals (de Silva *et al.*,2002 ; Peetermans *et al.*, 2003).The principal component of biofilm is a polysaccharide intercellular adhesin {PIA} (Ziebuhr *et al.*, 1997; Lappin-Scott *et al.*, 2001). PIA is composed of a beta-1,6-N-acetylglucosamine polymer synthesized by an enzyme

codified by the *ica* operon found on the bacterial chromosome, that includes a regulating element of four genes "*A*, *B*, *C*, and *D* " (Kozitskaya *et al.*, 2004). It is known that the *icaA* gene codifies the N-acetylglucosamyl transferase enzyme responsible for synthesizing PIA, this enzyme is not very active *in vitro*, but co-expression of the *icaD* gene increases the activity, *IcaB* is the deacetylase responsible for the deacetylation of mature PIA and the transmembrane protein *IcaC* seems to be involved in externalization and elongation of the growing polysaccharide (Gerke *et al.*, 1998 ; Scott *et al.*, 2001).

Since the discovery of the polymerase chain reaction (PCR) and DNA sequencing, comparisons of the gene sequences of bacterial species have shown that the 16S ribosomal RNA (rRNA) gene has been widely used to study prokaryote diversity and allows for the identification and prediction of phylogenetic relationships (Weisburg *et al.*, 1999).

1-2- Aims of the study

Because of increased the problems of bacteria associated with presence of denture and orthodontic in mouth and causes other systematic diseases, the present study was designed to achieve the following aims:

- Genetic identification all different bacterial species from denture and orthodontic without focusing on a limited type listed in previous studies .
- Comparison between the bacterial species and their frequencies in dentures and orthodontic sources.
- Looking for new strains in these two sources , as a result of the limited precise identification methods in previous studies especially in Iraq.
- detection the ability of bacteria to produce biofilm and thus ability. on adhesion . Furthermore, which the best type for biofilm forming test.
- comparison among the best test to detect biofilm formation.
- Detect which the best test for biofilm formation to each identified species.

Chapter two

Literature Review

2-1- The role of oral cavity in disease:-

The oral cavity as an integral part of the digestive system has various specific functions , the impact of common oral diseases extends beyond the oral cavity (Thorstensson and Johansson, 2009). Oral infection has been found to be associated with death risk in studies among middle-aged individuals (Soikkonen *et al.*, 2000; Jansson *et al.*, 2002). The relative importance of oral health as a predictor of survival has also been analyzed and the common oral diseases have shown significant influence on survival (Semba *et al.*, 2006; Morita *et al.*, 2006).

2-2- Common oral diseases:-

Dental caries and periodontal disease are the most common bacterial diseases of man which result from an interaction between a susceptible host, commensal microbiota and the environment, Although some specific microorganisms have been implicated in the pathogenesis of these conditions, it is now recognized that they are not classical infectious diseases but rather a complex of diseases resulting from a breakdown in the homeostasis between the human host and microbiota (Stamatova, 2010).

2-2-1- Dental caries :-

Dental caries can be defined as localised destruction of the tissues of the tooth by acids generated from bacterial fermentation of dietary carbohydrates

(Marsh,1994). Caries is a result of the complex interaction between carbohydrates in food and cariogenic microorganisms in oral biofilms, influenced by the quality and quantity of saliva and clinically manifested by demineralization and destruction of dental hard tissues(Stamatova , 2010). The development in molecular analyses have shown that all the bacteria that have been associated with caries belong to the normal microbiota of the oral cavity and dental caries is regarded as an endogenous infection (Takahashi and Nyvad , 2008).

The ecological plaque hypothesis caries is a result of a shift in the balance of resident microbiota driven by changes in local environmental conditions, it is generally believed that all three parameters (microorganisms, host and environment) must “act” simultaneously for carious lesions to develop and progress to become visually detectable (Aas *et al.*, 2008). Wide group of microorganisms are identified from carious lesions of which *Streptococcus mutans* , *Lactobacillus acidophilus* and *Actinomyces viscosus* may be considered the main pathogenic species involved in the initiation and development of dental caries (Shivakumar *et al.*, 2009). *Streptococcus mutans*, initially isolated in 1924,has been primarily implicated in this disease and extensively studied throughout several decades (Loesche, 1986) .According to (Belli and Marquis, 1991; Li and Burne, 2001; Kuramitsu, 2003; Scheie and Petersen,2004) Some significant virulent traits of *S.mutans* that contribute to caries initiation and progression are :-

A- Initiation of biofilm formation by adherence and accumulation on the tooth surface that is promoted by its synthesis of insoluble, extracellular polysaccharides.

B- Production of numerous bacteriocins that kill other species, favouring its competition in dental biofilms.

C- High efficiency in catabolizing carbohydrates and producing acids.

D- The ability to tolerate low pH . fig.(2-1).

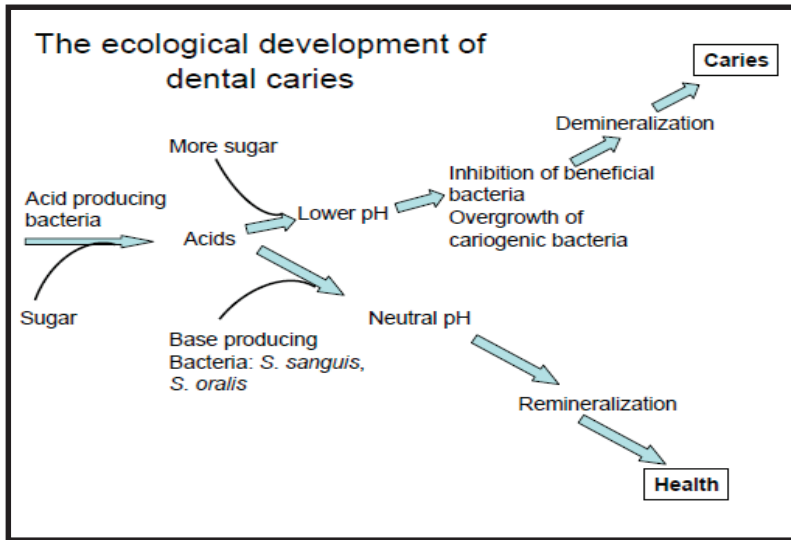


Figure (2-1): Development Schematic representation of ecological dental caries (March ,2004).

Molecular biology techniques have shown that more than 50% of the oral species are uncultivable by conventional methods, researches recognized that caries results not solely because of the presence of *S.mutans* or any single organism in dental plaque, but it is rather the interaction of multiple acid-producing organisms such as low-pH non-mutans streptococci, *Veilonella*, *Lactobacillus*, *Propionibacterium*,

Bifidobacterium that may be involved in the initiation of the disease (Aas *et al.*, 2008; He *et al.*, 2009; Mantzourani *et al.*, 2009).

2-2-2-Periodontal diseases :-

Gingivitis and periodontitis are the most common diseases with a microbial etiology affecting the periodontium, the gingivitis is an inflammation of the marginal periodontal tissues associated with an accumulation of dental plaque, the inflammation is reversible and there is no destruction of the periodontal attachment of the teeth, although inflammation may be found at sites with a prior attachment loss, in contrast to gingivitis, periodontitis is characterized by a progressive destruction of the supporting structures of the teeth (Haukioja *et al.*, 2008). Bacteria may also directly cause tissue damage due to virulence factors, such as toxins and enzymes (Smalley, 1994). Furthermore, the capacity of micro-organisms to induce the production and / or activation of matrix metalloproteinase in host tissues is important in the pathogenesis of periodontitis (Okamoto *et al.*, 1997). The inflammatory response including an increased flow of gingival crevice fluid (GCF), a rise in pH favours the Gram-negative and proteolytic species which leading to an ecological shift as suggested by the ecological plaque hypothesis (Marsh, 2005).

Most bacterial species associated with periodontitis are Gram - negative, obligate anaerobes : *Porphyromonas gingivalis* , *Tannerella forsythia* and *Treponema denticola* form the red complex of bacteria associated with increased pocket depth and bleeding on probing (Socransky *et al.*, 1998). *Aggregatibacter actinomycetemcomitans*, associated with localised aggressive periodontitis is a

facultative anaerobe , some Gram-positive species, such as *Parvimonas micra* (*Peptostreptococcus micros*) (Socransky *et al.*, 1998), and viruses, such as *Epstein-Barr* virus, are also associated with this disease (Slots, 2007).

2-3-Dentures:-

Dentures, artificial teeth, are prosthetic devices constructed to replace missing teeth , which are supported by surrounding soft and hard tissues of the oral cavity, usually made from polymethylmethacrylate (PMMA) materials called resin, these materials are the most widely used non-metallic denture base materials (Price, 1994 ; Jagger, 2002).

2-3-1-Type of denture :-

2-3-1-1-Removable partial dentures :

Removable partial dentures are removable appliances used for the person missing some of the teeth but still having a number of natural teeth (Pahlevan, 2005). As figure (2-2)



Figure (2-2): Removable partial dentures. (Pahlevan , 2005)

2-3-1-2-Removable complete dentures(full dentures):-

Removable complete dentures or known as a full dentures are removable appliances used for a person missing all teeth (Singla, 2007).As figure (2-3)



Figure (2-3): Removable complete dentures . (Singla , 2007).

2-3-1-3- Implant over denture:-Implant over denture is a denture of precision dental attachment of cylindrically shape of pore titanium that can be placed in tooth roots by surgically method (Vecchiadini *et al.*, 2009).As figure (2-4).



Figure (2-4): Implant over denture .(Vecchiadini *et al.*, 2009).

2-3-2-Oral environment with denture wearer:-

The adherence of microbial species to denture and other dental restorative materials ,furthermore , subsequent formation of biofilms on these surfaces are contributory factors to plaque-related oral and systemic disease, the mouth of the denture wearer presents additional hard, non-shedding areas and new environments (tissue-fitting surfaces) to support the growth of microorganisms and the development of plaque, denture may also act as a reservoir of infection for respiratory and systemic opportunistic pathogens (Sumi *et al.*, 2003), and presents a niche for antibiotic-resistant bacteria(Smith *et al.*, 2003). Literature review Sumi *et al.*,(2003) suggests respiratory pathogens preferentially colonies teeth or dentures, rather than soft tissue. The oral flora comprises a diverse group of microorganisms including bacteria, fungi, mycoplasmas, protozoa, viruses and bacteria predominate with an estimate of over 600 different species present in the oral cavity(Kazor *et al.*, 2003). However, only half of these species can be cultured in the laboratory(Wade *et al.*,1997).

Denture plaque is a dense, complex heterogeneous layer of microorganisms and their metabolites(Nikawa *et al.*, 1998). Denture plaque develops from adherence, aggregation and growth of microbes from saliva, oral mucosa and possibly fingers in the absence of denture hygiene (Theilade *et al.*, 1988),plaque accumulates preferentially at stagnant sites offering protection from flow and mechanical removal forces in the mouth (March, 2004).There is general agreement that denture plaque composition is broadly similar to that of dental plaque (Theilade *et al.*, 1983),with Gram positive cocci and short rods predominating (Koopmans *et al.*, 1988) whereas Gram-negative rods are relatively few in number (Budtz-Jorgensen *et*

al., 1988). Plaque microflora varies between individuals and sites in the mouth and on the denture, where differences between the flange, denture tooth, tooth gum interface and the fitting surface have been identified (Budtz-Jorgensen *et al.*, 1983).

The predominant cultivable microflora of denture plaque includes *Streptococcus spp.* (*S. sanguinis* [formerly *S. sanguis*], *S. oralis*, *S. anginosus*, *S. salivarius*), *Staphylococcus spp.* (*S. aureus*, *S. epidermidis*), Gram-positive rods (*Actinomyces spp.* [*A. israelii*, *A. naeslundii*, *A. odontolyticus*], lactobacilli, *Propionibacterium spp.*), *Veillonella spp.*, Gram-negative rods and yeasts (Budtz-Jorgensen *et al.*, 1983). In addition, a higher nutrient concentration, low salivary flow rates and roughened topography support and protect plaque (Verran, 2005). Denture plaque in comparison to dental plaque was reported to have a large proportion of obligate anaerobic *Actinomyces spp.* (*A. israelii*), low proportions of Gram negative rods and the regular presence of the skin bacterium *Staphylococcus aureus* (Theilade *et al.*, 1983).

2-3-3- Problems associated with denture plaque:-

2-3-3-1- Stomatitis and oral candidosis:-

Most of the literature on denture plaque focuses on *Candida Spp.* and its association with denture stomatitis, *Candida* is isolated more frequently from denture plaque than from dental plaque (Davenport, 1970).

2-3-3-2 Malodor:-

Oral malodor is a common and often distressing condition which is poorly explored in denture wearers, due to the artificial nature of the denture, many edentulous patients express concern that they may produce a distinct malodor (Fiske *et al.*,1995), dirty dentures contribute to malodor (Neill, 1968), which is generally acknowledged in the dentate to be caused in part by volatile sulphur compounds (VSCs), including hydrogen sulphide, methyl mercaptan and dimethyl sulphide (Tonzetich, 1977). These VSCs cause a fetid or putrid odour producing by Gram negative bacteria, particularly anaerobic species such as *Porphyromonas spp.*, *Prevotella spp.* and *Fusobacterium spp.* (Rolla *et al.*,1999), by proteolytic degradation of sulphur-containing peptides and amino acids present in saliva , shed

epithelium, food debris, gingival crevicular fluid (GCF), plaque and blood, these bacteria also have an association with periodontal disease (Tonzetich ,1977), other Gram negative bacteria found in denture plaque, such as *Klebsiella spp.*, may be potential pathogens in respiratory or systemic diseases arising from the oral reservoir of microorganisms (Verran,2005).

2-3-3-3.Reservoir of infection :-

There has been an increase in the number of studies investigating the link between oral and systemic diseases in the dentate (Sumi *et al.*, 2007) and edentate (Senpuku *et al.*, 2003) .Oral bacteria have been implicated in bacterial endocarditis (Berbari *et al.*, 1997) , aspiration pneumonia(Scannapieco, 2006), gastrointestinal infection (Sumi *et al.*, 2003), and chronic obstructive pulmonary disease (Thean *et*

al., 2007), among others, dentures offer a reservoir for microorganisms associated with these infections, dentures may spend time in a non-hygienic environment when out of the mouth and may also harbor microorganisms not normally associated with the oral flora, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, certain Enterobacteriaceae including *E. coli*, *Klebsiella* spp. (Sumi *et al.*, 2002), *Pseudomonas* spp., and staphylococci including, but rarely, MRSA (Smith *et al.*, 2003), Such organisms may be considered respiratory pathogens and have been reported to colonise the denture plaque in 46% of the dependent elderly (Sumi *et al.*, 2002),

2-4-Orthodontic:-

Orthodontic anchorage can be defined as resistance to undesirable tooth movement, traditional metal wired braces are stainless steel and sometimes in combination with titanium are the most widely used (Freitas *et al.*, 2012),figure(2-5). The placement of orthodontic appliances is known to create a favourable environment for the accumulation of microbiota and food residues which in time, may cause caries or exacerbate any preexisting periodontal disease (Ristic *et al.*, 2007). The microbiological changes after bracket placement became a topic of interest during the late 1980's, initially cariogenic species such as *Streptococcus mutans* , *Lactobacillus* species and the subsequent decalcification of enamel were the main fields of interest among investigators (Forsberg *et al.*, 1991). Later on, the more complex system of periodontopathogenic microbes and the changes after bracket placement became the main topics of interest (Petti *et al.*, 1997).

Naranjo *et al.*, (2006) observed a transition in subgingival dental plaque after the placement of brackets, the plaque index and gingivitis index increased significantly. *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Tannerella forsythia* and *Fusobacterium* species were significantly elevated in the experimental group after bracket placement compared with the control group without orthodontic therapy, including superinfecting microorganisms such as *Enterobacter cloacae*, *Klebsiella oxytoca*, *Klebsiella pneumonia* and *Serratia marcescens* were also found (Naranjo *et al.*, 2006). Lee *et al.*, (2005) succeeded in detecting significant differences in the subgingival dental plaque retrieved from gingivitis lesions in patients with and without orthodontic fixed appliances, *Tannerella forsythia*, *Treponema denticola*, and *Prevotella nigrescens* were significantly more common in the samples obtained from the orthodontic patients than in the samples obtained from the non orthodontic control patients (Lee *et al.*, 2005) . The presence of orthodontic bands and brackets, therefore, cannot affect on the microbiologic condition of the whole mouth (Naranjo *et al.*, 2006).

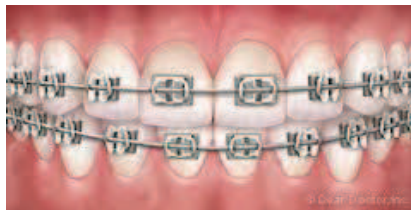


Figure (2-5):Orthodontic.(Freitas *et al.*, 2012).

2-5-Dental plaque:-

Dental plaque is a highly complex biofilm that provides nutrients and protection for periodontopathogenic bacteria (Marsh, 2005). It is the primary cause of gingivitis and the possible transition to periodontitis (L  e, 1965). The Gram positive and mostly aerobic microorganisms that initially colonize intra-oral hard surfaces are replaced by predominantly Gram negative and anaerobic microorganisms (Socransky and Haffajee, 2005). *Tannerella forsythia*, *Porphyromonas gingivalis*, *Actinobacillus Actinomycetemcomitans* and *Prevotella intermedia* are found more frequently in patients with gingivitis and periodontitis than in healthy subjects (Darveau *et al.*, 1997). Thus both the quantity as well as the quality of plaque, are important factors in the onset of periodontal disease and are influenced by many factors including surface characteristics (Quirynen *et al.*, 1990). Especially surface roughness and surface free energy were found to be positively correlated with the plaque growth rate (Quirynen & Bollen, 1995), additionally, the presence of gingival inflammation will further increase plaque growth (Ramberg *et al.*, 1995).

2-6-Biofilm:-

A biofilm is a community of cells attached to either a biotic or abiotic surface enclosed in a complex exopolymeric substance (EPS) (Mah & O'Toole, 2001). The oral cavity is constantly contaminated by a complex diversity of microbial species that have a strong tendency to colonize surfaces, however, the major components involved in biofilm formation are bacterial cells, a solid surface, and a fluid medium biofilm formation occurs on all hard surfaces, e.g. the tooth surface,

restorative materials and implant components. In the formation of a biofilm to a non-shedding surface the following stages have been described according to (Scheie,1994; . Bos *et al.*,1999) :-

- **Stage 1: Conditioning layer formation:-**

The first stage in the development of biofilm is the adsorption of organic and inorganic molecules to the solid surface, this conditioning layer in the oral cavity, called pellicle, consists of numerous components including glycoproteins, proline-rich proteins, phosphoproteins, histidine-rich proteins, enzymes, and other molecules that can function as receptors for bacteria.

- **Stage 2: Transport of bacteria to the substrate surface:-**

The initial transport of microbes to the substrate may occur through brownian motion, through liquid flow, or through active bacterial movement (chemotactic activity) which may be influenced by many factors include pH, temperature, flow rate of the fluid, surface energy of the substrate, bacterial growth stage, surface hydrophobicity.

- **Stage 3: Bacterial adhesion :-**

The next step in biofilm formation is the adhesion of microbial cells to the conditioning layer by the following phase:

- ❖ **Phase 1:** Initial non-specific microbial-substrate adhesion, the bacterial surface structures form bridges between the bacteria and the conditioning layer (Grenier and Mayrand, 1986). Initially, these bridges may not be strong, however with time the bacteria-substrate bonds gain in strength.

❖ **Phase 2:** Specific microbial-substrate adhesion, in this phase polysaccharide adhesion or ligand on the bacterial cell surface bind to receptors on the substrates (Miron *et al.*, 2001).

- **Stage 4: Bacterial colonization and biofilm maturation:-**

In this stage, the monolayer of microbes attracts secondary colonizers forming microcolony (Costerton *et al.*, 1999), the firmly attached microorganisms start growing, newly formed cells remain attached and biofilms can develop, the physicochemical surface properties of a pellicle are largely dependent on the physical and chemical nature of the underlying hard surface (Sipahi *et al.*, 2001). Thus, the characteristics of the underlying hard surface will influence on the initial bacterial adhesion.(figure 2-6) .

It is known from in vitro studies that monolayer of oral bacteria release enzymes that mediate their detachment (Lee *et al.*, 1996). It is likely that localized detachment of microorganisms starts after initial adhesion and increases with time as it is related to the number of microorganisms present in the biofilms, the fact that microorganisms detach regularly has implications for their spreading and colonization to other sites (Auschill *et al.*, 2001)

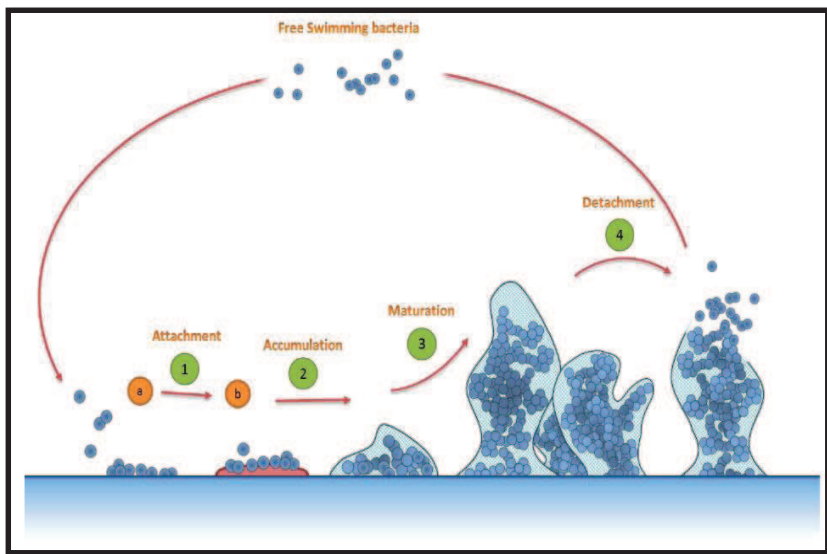


Figure (2-6) : Steps of biofilm formation. Biofilm formation is divided into four steps: 1) initial adhesion of bacterial cells to surfaces, a) attachment to biomaterial surfaces, b) attachment to host matrix proteins coated the surfaces, 2) aggregation into multicellular structures, 3) formation of mature stable biofilms, and 4) dispersal of bacterial cells from the biofilms (Otto *et al.*, 2009) .

2-7-Methods for the detection of bacterial forming biofilms:-

There are two methods for the detection of bacterial forming biofilms:

1. The Phenotypic method:-

Qualitative methods, such as the tube adherence (TM) test described by Christensen *et al.*, (1982) , Congo red agar (CRA) method described by Freeman *et*

al., (1989), and quantitative methods, such as the tissue culture plate (TCP) assay described by Christensen *et al.*, (1985) are used in routine laboratories

2. The Genotypic method:-

PCR amplification methods have been shown to improve the detection of biofilms, biofilm non producers are negative for *icaA* and *icaD* and lack the entire *icaADBC* operon, but this requires specialized equipments and techniques (Arciola *et al.*, 2001; O' Gara *et al.*, 2001).

2-8-Genes responsible for synthesis biofilm:-

After the initial adhesion, cell-cell interaction prevents the dissemination of the initial colonizing bacteria and facilitates the formation of mature biofilms, *S. epidermidis* releases various surface macromolecules and proteins that facilitate the intercellular aggregation, polysaccharide intercellular adhesin (PIA) is involved in the cell-to-cell adhesion during *S. epidermidis* biofilm formation (Mack *et al.*, 1994). PIA is a linear homoglycan composed of β -1,6-linked N-acetylglucosamine residues and carries up to 15% deacetylated amino groups, the positive and negative charges can be simultaneously introduced to the polysaccharide by substitution with ester-linked succinate and phosphate residues, then the ionic interaction caused by these positive and negative charges within the polysaccharide could explain its function in linking different cells within the biofilm (Mack *et al.*, 1996), PIA biosynthesis is directed by enzymes encoded by the *icaADBC* gene operon, the intercellular adhesion (*ica*) locus is composed of four open reading

frames (ORFs) *icaA*, *icaD*, *icaB* and *icaC* in an operon (Heilmann *et al.*, 1996 ; Gerke *et al.*, 1998), Figure (2-7)

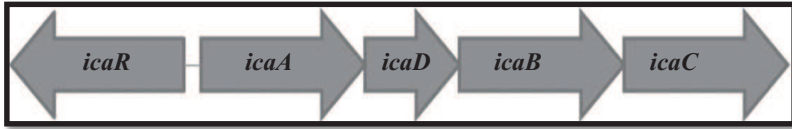


Figure (2-7): Map of genomic organization of the *icaRADBC* gene cluster from *S. epidermidis* (O’Gara & Humphreys, 2001).

IcaA and *IcaD* are transmembrane proteins involved in the synthesis of N-acetylglucosamine (GlcNAc) polymers, while elongation and export of the resultant polymers is believed to be controlled by the *IcaC* membrane protein, after export, the *IcaB* deacetylase enzyme is responsible for de-acetylation of some of the GlcNAc residues needed to provide the polymer with the cationic character that is essential for surface attachment (Rohde *et al.*, 2007), In addition, it has been demonstrated that *IcaR* represses *ica* expression by binding to the *icaA* promoter region (Jefferson *et al.*, 2004), as Figure (2-8).

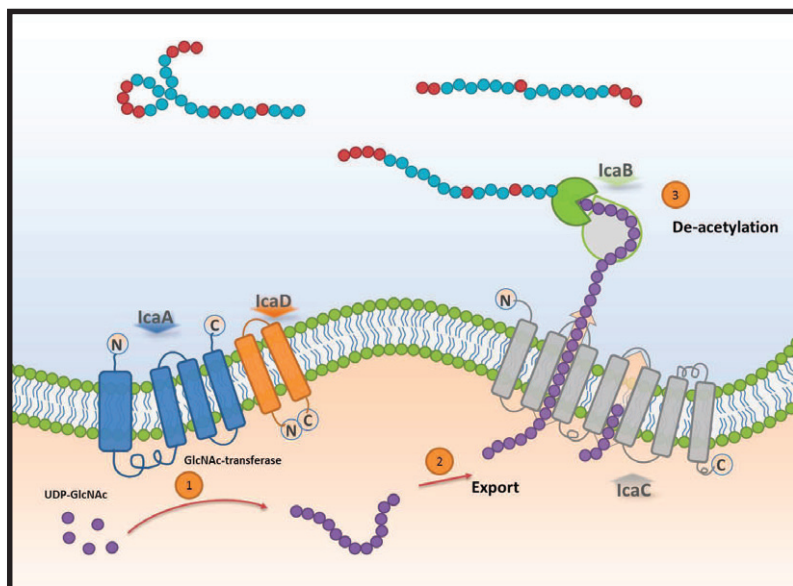


Figure.(2-8): Biosynthesis of the exopolysaccharide poly-N-acetylglucosamine (PIA). *IcaA* and *IcaD* are trans-membrane proteins involved in the synthesis of N-acetylglucosamine (GlcNAc) polymers (1). The *IcaC* membrane protein controls the elongation and export of the polymer (2). The *IcaB* protein is responsible for de-acetylation of GlcNAc residues (3), (Otto., 2009) .

2-9-Molecular genetic:-

2-9-1-Identification of bacterial species by *16SrDNA* sequencing:-

Within the biological world, ribosome share many similarities, indicating the conservative nature of its structure in prokaryotic ribosomes contain three types of RNA :(5S, 16S, and 23S) (Harmsen and Karch, 2004). Both 5S and *16SrDNA* have

been used to determine relatedness, since the 16S molecule is large (with about 1500 bases), it contains more information and the smaller 5S molecule with only 120 bases, where as little work has been done on the 23S molecule because it's so longer (about 3000 nucleotides) and it's therefore more difficult to study, thus, scientists interested in the classification and evolution of bacteria has concentrated on the 5S and 16S (Mendoza *et al.*, 1998). The more highly conserved region permit one to compare distantly related organisms and the more variable domains are used to examine the more closely related organisms (Harmsen and Karch, 2004). In previous studies, polymerase chain reaction (PCR) analysis was used to detect pathogens and many primers have been developed to detect species-species genes (Skow *et al.*, 2005). The different primers for different species is impractical for routine analysis of cultures that may contain one or more of many possible pathogens, but this can be avoided by using a single pair of universal primers designed to amplify conserved stretches of *16SrDNA* from any bacterium (Khamis *et al.*, 2005) .

2-9-2-Phylogenetic tree:-

The discovery of the polymerase chain reaction (PCR) and DNA sequencing, comparisons of the gene sequences of bacterial species have shown that the 16S ribosomal RNA (rRNA) gene is highly conserved within a species and among species of the same genus, hence can be used as the new gold standard for the speciation of bacteria, using this new standard, phylogenetic trees, based on base differences between species, are constructed and bacteria are classified and reclassified into new genera (Olsen and Woese, 1993; Schmidt and Relman 1994). Phylogeny is the study of the evolutionary history of organisms (Delsuc *et al.*,

2005). Cladistic relationships indicate the degree of relatedness between microorganisms as shown by pathways of ancestry (Cain & Harrison, 1960). Consequently, classifications which are based on perceived evolutionary relationships between organisms reflect the extent of change over time, therefore , Phylogenetic relationships between organisms are represented by evolutionary trees and are inferred from various types of phonetic relationships based on assumptions of how evolution occurs ,although, evolutionary systems are sometimes seen merely as simple branching over time but this is an oversimplification as *in vivo* hybridisation and lateral gene transfer lead to conceptual as well as to computational difficulties (Maynard , 1990). Bacterial systematics is increasingly being based on phylogenetic information, notably that derived from macromolecules such as DNA, RNA and proteins (Olsen *et al.*, 1994). Classification, the one of basic disciplines of bacterial systematic , is becoming increasingly dependent on the use of molecular sequence data, notably on information generated from 16S rRNA analyses (Woese *et al.*, 1991).

2-10-Bacterial species isolated in dentures and orthodontic:-

Bacterial species isolated from denture and orthodontic are *Staphylococcus aureus* *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus warneri* normally found on the skin of humans and animals (Cimiotti *et al.*, 2007), in nasal cavities (Rasmussen *et al.*,2000) and in the mouth (Ohara-Nemoto *et al.*, 2008).

Staphylococcus pasteurii is a Gram positive organism which is emerging as an agent of nosocomial infections and a blood derivatives contaminant, though its role in causing human disease mostly remains controversial (Savini *et al.*, 2009).

Staphylococcus hominis is one of the major staphylococcal species inhabiting the skin of humans and on most of the people that have been examined, it produces large populations in the axillae and inguinal and perineal areas(Kloos, 1986).

Staphylococcus warnerii found as part of the skin flora on humans , animals and cause infection in patients whose immune system is compromised (Barigye *et al.*, 2007) and in the mouth (Ohara-Nemoto *et al.*, 2008).

Morganella morganii is a gram-negative rod commonly found in the environment and in the intestinal tracts of humans, mammals, and reptiles as normal flora.(Samonis *et al.*, 2001).

Klebsiella pneumoniae is a Gram-negative found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human lungs if aspirated (Tu *et al.*, 2009).

Klebsiella oxytoca is a Gram-negative found opportunistic in nature this specie tends to colonize along the mucosa membranes of the colon and nasopharynx , and skin; however, they can be found colonizing on all parts of the body (Ménard *et al.*, 2010).

Klebsiella variicola as a new species isolated in Mexico from plants (rice, maize, sugar cane and banana) and hospitals (Rosenblueth *et al.*, 2004) .

Serratia marcescens is involved in nosocomial infections, particularly catheter-associated bacteremia, urinary tract infections and wound infections, and is responsible for 1.4% of nosocomial bacteremia cases in the United States ,it is commonly found in the respiratory and urinary tracts of hospitalized adults and in the gastrointestinal system of children (Hejazi and Falkine 1997) .

Proteus hauseri exist in manure, soil, polluted water, and in intestines of human and wide variety of animals (Garrity *et al.*, 2005).

Proteus mirabilis is one of the most common gram-negative pathogens encountered in clinical specimens and can cause a variety of community- or hospital-acquired illnesses, including urinary tract, wound, and blood stream infections "BSI" (O'Hara *et al.*,2000).

Proteus penneri usually infects urinary tract, blood, abdominal wound, groin, neck and ankle and has been isolated mostly from urine (50%), wound and soft tissue exudates (25%), and blood cultures (15%) (O'Hara *et al.*, 2000 ; Cantón *et al.*, 2006).

Enterococcus faecalis is a gram-positive bacterium that can cause a variety of nosocomial infections of which urinary tract infections are the most common , these

infections can be exceptionally difficult to treat because of drug resistance of many *Enterococcus faecalis* isolates (Kau *et al.*, 2005).

Enterococcus faecium causes nosocomial bacteremia, surgical wound infection, endocarditis, and urinary tract infections (Paulsen *et al.*, 2003).

Escherichia fergusonii is belong to Enterobacteriaceae, infect open wounds in humans and may also cause bacteraemia or urinary tract infections (Mahapatra *et al.*, 2005).

Bacillus cereus, which is a well-known cause of food poisoning and a dreaded cause of posttraumatic endophthalmitis ,and can also cause opportunistic infections, mainly in the immunocompromised host (Drobniewski,1993).

Bacillus subtilis is non-pathogenic , they can contaminate food and caused food poisoning (Perez, 2000).

Enterobacter aerogenes is found in soil, water, dairy products and inhabits a natural flora in the gastrointestinal tract of animals as well as humans and causes disease in humans through inadvertent bacteria transfer in hospital settings ((Burchard *et al.*,1986 ; Janda *et al.*, 2006).

Enterobacter cloacae has emerged as an important nosocomial pathogen which could cause a wide spectrum of infections including respiratory system disease,

urinary tract infections, involving mostly patients with impaired host deficiency, and bacteremia (Wisplinghoff *et al.*, 2004; Galani *et al.*, 2005) .

Enterobacter mori is plant-pathogenic enterobacterium responsible for the bacterial wilt of *Morus alba* L. (Li *et al.*, 2010) .

Enterobacter ludwigii is gram-negative, fermentative, motile rods which were isolated from clinical specimens (Hoffmann *et al.*, 2005) .

Hafnia alvei can be isolated from various anatomical sites in humans and from various environmental sources and not normally pathogenic, but may cause disease in immunocompromised patients (Janda *et al.* , 2006).

Citrobacter freundii is an opportunistic pathogen cause of a variety of nosocomial infections of the respiratory tract, urinary tract, blood and several other normally sterile sites in patients (Whalen *et al.*, 2007).

Lactobacillus plantarum is found in dairy, meat and much vegetable fermentations, it is also found in the human gastrointestinal tract (De Vries *et al.*, 2006).

Lactococcus lactis is nonpathogenic bacteria used widely for industrial production of fermented dairy products such as milk, cheese and yogurt, however, is nonpathogenic bacteria (Tanous *et al.*, 2007).

Streptococcus anginosus is part of the human bacterial flora, but can cause diseases including brain and liver abscesses under certain circumstances, the habitat of *S. anginosus* is a wide variety of sites inside the human body such as mouth, throat, feces, and vagina (Ruoff *et al.*, 1988).

Streptococcus equinus makes up the majority of the bacterial flora in horse feces (Boone *et al.*, 1991) and is seldom found in humans (Noble, 1978).

Acinetobacter baumannii is an opportunistic pathogen which has caused nosocomial infections as its ability of to live on a variety of hospital surfaces (Franco *et al.*, 2004), commonly found in fermented vegetables, fermented dairy products and meat (Barros *et al.*., 2001).

Chryseobacterium vietnamense was isolated only from a forest soil sample in Vietnam (Li and Zhu, 2011).

Chapter three

Materials and Methods

3-1- Equipments and materials:-

The equipments used in this study are described in Table (3-1).

Table (3-1) : Equipments

No.	Equipments	Company	Country
1	Autoclave	Tuttnauer Brinkama	USA
2	Centrifuge	Human	Germany
3	Cooling incubator	Binder	Germany
4	Deep freezer (-18°C)	Nuair	Japan
5	Digital balance	Mettlertoledo	Swistezerland
6	Distillatory	GFL	Germany
7	Electrophoresis system (mini horizontal unit)	Fisher scientific	USA
8	ELISA microplate reader	Human	Germany
9	Eppendorf tubes	Eppendorf	Germany
10	Finn tips , 100µl , 500µl , 1000µl	Fisher	USA
11	Light microscope	Human	Germany
12	Micropipettes , 0.5 - 10µl 10 - 100µl , 100 - 1000µl	Fisher scientific	USA
13	Microplate titter (96 well – flatbottom)	Himedia	India

No.	Equipments	Company	Country
14	Mini vortex	Fisher scientific	USA
15	Minifuge	Fisher scientific	USA
16	Oven	Memmert	Germany
17	PCR sprint thermal cycler	Thermo	USA
18	PCR tubes	Fisher	USA
19	pH meter	Thermo	USA
20	Platinum wire loop	Himedia	India
21	Stirrer/hotplate	Coming	USA
22	Ultra centrifuge	Thermo	USA
23	U.V transilluminator	Velber Lourmat	EEC France
24	Water bath	Memmert	Germany
25	Eppendorf concentrator 5301	Hamburg	Germany

3-2- Chemicals and dyes:-

The Chemical and dyes used in this study are described in Table (3-2)

Table (3-2): Chemicals and dyes

No.	Chemicals	Company	Country
1	Agarose	Promega	USA
2	Absolute ethanol	Lab M	UK
3	Boric acid	Fisher	USA
4	DNALadder 100 bases	BIONEER	Korea
5	DNA ladder 1kb	Promega	USA
6	EDTA (ethylene diamine tetra acetate)	BDH	England
7	Ethanol (96%)	AL-Tharthar	Iraq
8	Formalin	BDH	England
9	Lysozym	Promega	USA
10	Master mix	Promega	USA
11	Phosphate buffers saline	Oxoid	England
12	Primers	BIONEER	Korea
13	Sucrose	Merck	Germany
14	Tris – HCl	Fisher	USA
15	Tris base	Fisher	USA
16	Triton X–100	Fisher	USA
17	Bromophenol blue	Fisher	USA
18	Congo red	DAB.6	Germany
19	Ethidium bromide	Fisher	USA

No.	Chemicals	Company	Country
20	Gram's stain	Fara	Iran
21	Genomic DNA mini kit (blood /cultured cell)	Geneaid	Taiwan

3-3-Prepared media:-

The media using in this study were prepared according to their companies are described in Table (3-3)

Table (3 -3) : Prepared media

No.	Media	Company	Country
1	Blood agar base	Himedia	India
2	Brain heart infusion broth	Oxoid	England
3	Nutrient agar	LAB	UK
4	Tryptic soy broth	Alpha	USA
5	Congo red agar	prepared media	

3-4- Preparation of chemical solutions:-

3-4-1- Tris Borate – EDTA.(TBE) buffer preparation 5x:-

1. EDTA (1.86 gm) was dissolved in 20ml of distilled water pH :8.
2. Tris base (45 gm) .
3. Baric acid (27.5 gm) .

The mixture was dissolved in distilled water and completes the volume to 1 liter. The pH was adjusted to 8, autoclaved at 121°C for 15min, and stored at 4°C (Sambrook and Rusell, 2001) .

3-4-2-Tris EDTA (TE) buffer preparation :-

TE buffer it consists of :-

A. 1.214 gm of Tris – HCL in 10ml distilled water, (1M) as astock .

B. 1.861 gm of EDTA in 10ml distilled water (0.5M) as astock and dissolved by heating .

TE buffer was prepared by mixing 1ml of solution (A) and 0.2 ml of solution (B) the volume was completed to 100 ml with distilled water and the pH was adjusted to 8, autoclaved at 121°C for 15 min and stored at 4°C to be use later . (Sambrook and Rusell, 2001) .

3-4-3-Ethidium bromide dye solution:-

Dissolving 0.05 gm of Ethidium bromide in 10 ml distilled water (stirred until dissolved) , and stored in a dark reagent bottle or folded by aluminum foil sheet – at 4°C (Sambrook and Rusel, 2001) .

3-4-4-Bromophenol blue dye solution:-

It is prepared of the following :

A. Bromophenol blue 0.25% (w/v) .

B. Sucrose in H₂O 40% (w/v) .

Store at 4°C .

3-4-5- Phosphate buffer saline:-

Dissolving one tablet of PBS in 100 ml distilled water and pH was adjusted to 7.2 .

3-4-6- Preparation of commercial culture media:-

Blood agar base , NA , BHIB , tryptic soy broth were prepared according to manufacturer and sterilized by autoclaving at 121°C for 15 min .

3-5- Methods:-

3-5-1-Sample collection:-

One hundred sample were collected from patients dentures (n=50) using sterile cotton swabs and from orthodontic (n=50) using sterile needle from Al-shaheed Qais center in Basrah after confirm from patient's denture and orthodontic washing. All samples were from both genders aged between 12-70 years (Figure 3- 1). Collected samples were placed in sterile tubes containing (5ml) brain heart infusion broth (BHIB) and transferred to the laboratory (Talan *et al* . , 1989) , to incubate at 37°C for 12 – 24 hr then streaked onto blood agar using a loop and incubated at 37°C for 24 hr. All colonies that appeared were gram stained and detected by light microscope ,then subcultured onto nutrient agar plate (for biological studies) and nutrient agar slants (as stock) then incubated at 37°C for 24 – 48 hr .

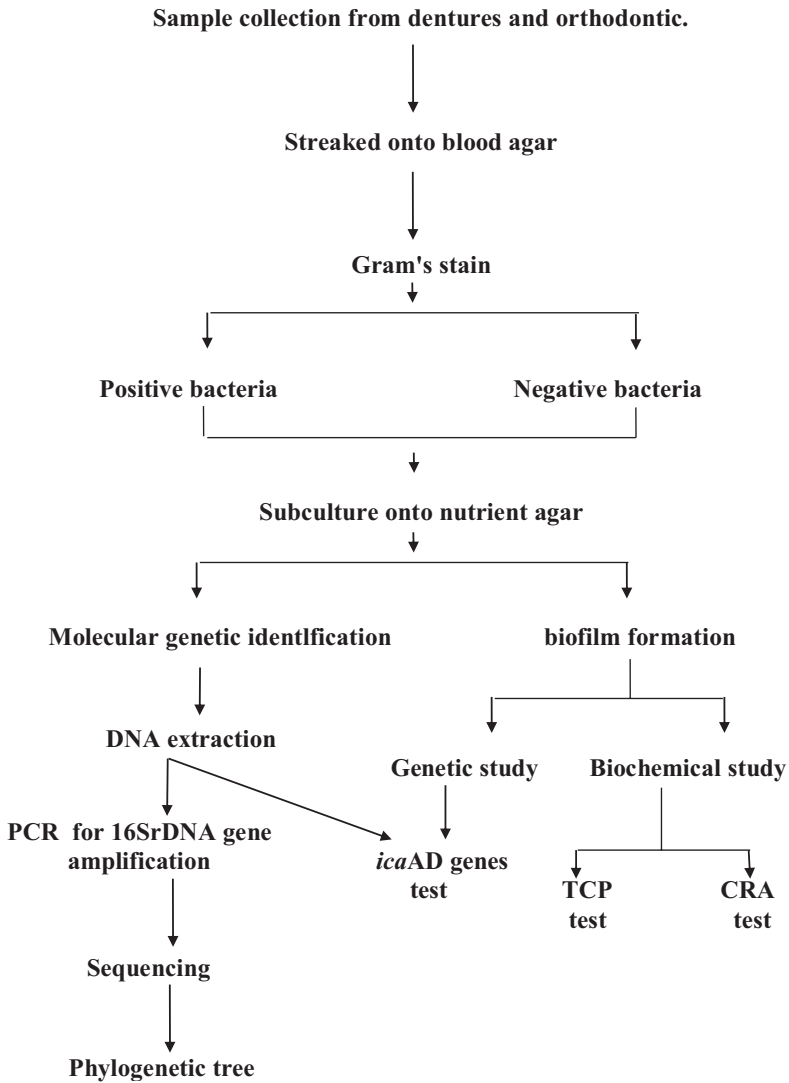


Figure: (3 - 1) : General layout of the study.

3-5-2-Examination of specimen:-

3-5-2-1-laboratory diagnosis:-

All Gram negative and positive cocci and Gram negative and positive rod bacteria were identified according to molecular study.

3-5-3- Molecular genetic identification:-

3-5-3-1-DNA extraction from bacteria :-

DNA was extracted by genomic DNA mini kit (blood /cultured cell) Geneaid ,according to the manufactured instruction as follow:

1. Cultur bacterial cells from BHIB tube containing activated bacteria was transferred to a 1.5 ml microcentrifuge tube .
2. 200 µl of lysozyme buffer (20 mg / ml lysozyme , 20mM Tris-Hcl , 20mM EDTA , 1% Triton x-100 , pH 8.0 , prepare fresh lysozyme buffer immediately prior to use) was added to the tube above and resuspend the cell pellet by shaking with pipet
3. Incubated at room temperature for 10 min . During incubation the tube was inverted every 2-3 min .
4. 200 µl of GB buffer was added to the sample and mixed by shaking vigorously for 5 sec .
5. Incubated at 70°C for 10 min or until the sample lysate is clear . During incubation , the tube was inverted every 3 min , At this time , the required elution Buffer (200 µl per sample) was incubated at 70°C (for step 19 DNA elution) .

6. After 70°C incubation ,200 µl of absolute ethanol was added to the sample lysate and immediately mixed by shaking vigorously . when precipitate appears , break it up by pipetting .
7. The mixture (including any precipitate) was transferred to the GD column .
8. GD column was placed in a 2 ml collection tube .
9. Centrifuged at 12,000 xg for 5 minutes .
10. The 2 ml collection tube containing the flow through was discarded and the GD column was placed in a new 2 ml collection tube .
11. 400 µl of W1 buffer was Added to the GD column .
12. Centrifuged at 12,000 xg for 3 min .
13. The flow through was discarded and the GD column was placed again in the 2 ml collection tube .
14. 600 µl of wash buffer (absolute ethanol added) was added to the GD column .
15. centrifuged at 12,000 xg for 3 min .
16. The flow through was discarded and the GD column was placed again in the 2 ml collection tube .
17. Centrifuged for 5 min at 12,000 xg to dry the column matrix .
18. The dried GD column was transferred to a clean 1.5 ml microcentrifuge tube .
19. 100 µl of preheated elution buffer was added to the center of the column matrix .

20. The column was left stand for 3.5 min or until the elution buffer was absorbed by the matrix .

21. centrifuged at 12,000 xg for 7 min . to elute the purified DNA .(DNA was stored at - 20° C) .

3-5-3-2-Examination of genomic DNA by agarose gel electrophoresis:-

DNA was visualized by agarose gel electrophoresis (Sambrook and russel , 2001)

Agarose gel was prepared as follow:

1. 0.2 gm agarose was dissolved with 25 ml of TBE buffer (1 X) in a beaker , the mixture was melting by hot plate agarose ,then 0.2 µl of ethidium promide was added , and mixture was left to cool for 50 - 60°C.

2. Melted agarose gel was poured into the casting tray of the electrophoresis apparatus and the comb was placed at one end of the tray to form wells for loading .

3. After the agarose solidified at room temperature , the cast was pushed gently upwards out of the tray , and the comb was lifted up out of the gel . the gel was gently replaced on the electrophoresis tray.

4. The electrophoresis apparatus was filled with TBE buffer until the entire gel surface was covered with the buffer .

5. 9 µl of DNA sample was mixed with 3 µl of bromophenol blue and the mixture was transferred carefully in the wells of agarose gel .
6. The gel was subjected to equal electric current by connecting to a power supplier .
7. The cathode was connected to the well sides of the tray while the anode on the other side and the gel was run at 60V until the bromophenol blue tracking dye migrated to the end of the gel .
8. DNA bands were detected and examined under UV. Transilluminator.

3-5-3-3-Polymerase Chain Reaction (PCR) technique :-

PCR method for amplification the universal *16SrDNA* gene were accomplished according to Miyoshi *et al.*(2005).

3-5-3-4-Universal *16SrDNA* gene primers:-

The bacteria in the clinical specimens were identified by using PCR in order to amplify universal bacterial *16SrDNA* gene which is listed in table (3 -4) .

Table (3-4): Universal *16SrDNA* primers used in PCR amplification

Gene	Primer type	Primer sequence (5'-3')	Size of product bp	TM	TA
Universal bacterial <i>16SrDNA</i>	B 27 F	5'-AGAGTTTG ATCCTGGC-3'	1500bp	48°C	51.8°C
	U 1492R	5'-GGTTACCT TGTTACGACTT-3'		42°C	51.8°C

* TM=Melting temperature

* TA=Annealing temperature

3-5-3-5- Reagents:-

The reagents and their volumes were used for PCR amplification are described in table (3-5) .

Table (3 -5): Reagents and volume (25µl) used in PCR amplification or *16SrDNA* gene

No	Reagent	Volume
1	DNA template	5 µl
2	Forward primer	1 µl (10 pmol)
3	Reverse primer	1 µl (10 pmol)

No	Reagent	Volume
4	Master mix	12.5 µl
5	Nuclease free water	5.5 µl
Volumes		25 µl

3-5-3-6 -Thermal cycling condition :-

The program is described in table (3-6) .

Table (3-6): Program used in PCR amplification for *16SrDNA* gene

Steps	Temperature	Time	No . Of cycles
Initial denaturation	92°C	2min	1
Denaturation	94°C	30 sec	30
Annealing	51.8°C	45 sec	
Extension	72°C	1.5 min	
Final extension	72°C	5 min	1

3-5-3-7-Detection of PCR product by agarose gel electrophoresis :-

The solution, procedure and viewing were identical to those for the detection of DNA bands. However, there are few exceptions:

1- 0.5 gm agarose was dissolved in 25 ml from TBF buffer (5 X)

2-Without using promophenol blue .

3-10µl of PCR product was transferred into the wells of agarose gel, and in the first well was for 10µl DNA ladder (1kb).

3-5-3-8- 16 SrDNA gene sequences:-

A-Purification of DNA product:-

Purification of PCR products was carried out to remove any unreacted primers and/or leftover dNTPs (Embley, 1991).

Procedure:-

1-20 µl of PCR product (*16SrDNA*) was transferred from the PCR tube to eppendorf tube 1.5 ml.

2-60 µl of 20% PEG was added to each tube and mixed by Vortex.

3-Tubes were incubated at room temperature for 3-4hr or overnight at 4°C.

4-The precipitated PCR product was pelleted by centrifugation at 12000 rpm for 20 min.

5-The supernatant was carefully removed by a micropipette leaving some solution in the eppendorf tube, as the DNA is gelatinous at this stage and could easily be removed accidentally.

6-0.5 ml of 70% chilled ethanol was added, and centrifuged at 12000 rpm for 10 min.

7-The supernatant was removed by a micropipette and ethanol precipitation was repeated by addition of 0.5 ml of 70% chilled ethanol and centrifuged at 12000 rpm for further 10 min.

8-The supernatant was removed from each tube by a micropipette and

precipitated DNA was dried in a vacuum drier for 30 min.

9-Pellet was resuspended in 15 µl (depending on the brightness of the band on the gel) of free nuclease water, then left overnight at 4°C.

B-Agarose gel electrophoresis:-

The electrophoresis was similar to its solutions and procedure to that of *16SrDNA* produced (previously) from PCR, the electrophoresis was important to ensure that PCR product is pure after purification

C-Sending to BIONEER:-

The *16SrDNA* products sequencing and its preparation were done according to BIONEER Co. DNA concentration [45 ng/ul] and sample volume [15 ul] and for primer (for each) concentration (5pmol/ul (5uM) and volume (5ul) then sending to BIONEER for sequencing .

3-5-3-9-Identification of bacteria:-

All bacterial species were identified (using the *16SrDNA* sequencing products) in "BLAST " provided by the National Center for Biotechnology Information Service (NCBI) <http://www.ncbi.nlm.nih.gov> after treatment and recorection (Kerbaudy *et al.*, 2011).

3-5-3-10- Phylogenetic tree:-

The sequences data obtained from the present study and from type strains by Gen Bank (Sung *et al.*, 2006) were aligned and concatenated at least in 893 bp and

compared to assign the differences using "CLUSTALW" <http://www.ebi.ac.uk/clustalw/> (Kerbaui *et al.*, 2011) (appendix-1), then a phylogenetic tree by Neighbour Joining method was constructed using MAFFT(Multiple Alignment using Fast Fourier Transform) viewed by (Kazutaka *et al.*, 2005) and "forester"(Zmasek and Eddy, 2001).

3-5-4-Detection the ability of adherence:-

3-5-4-1-Congo Red Agar (CRA) method:-

This agar was prepared by blood agar base supplemented with 0.8 gm congo red and 50 gm sucrose dissolved in 1 liter distilled water and adjusted pH to 8, autoclaved at 121°C for 15 min, a positive result is black colonies with a dry crystalline (slime producers), intermediate results are dark-pink colonies (weak slime producers), and negative results are pink-white (not slime producers) (Freeman *et al.*, 1989).

3-5-4-2-Preparation of Tissue Culture Plate (TCP) method :-

A log-phase culture (18-24hr) of the isolates were inoculated into tryptic soy broth was placed in the wells of micro ELISA auto reader sterilized plate and incubated overnight at 37°C. The content of each well was gently removed and discarded. The wells were washed four times with phosphate buffer saline to remove free floating "planktonic" bacteria. Biofilms formed by adherent "sessile" bacteria in plate were fixed with 25% formalin and stained with crystal violet (0.1% w/v). Excess stain was rinsed off through washing with deionized water. Plates were kept for drying

.Optical density (OD) of stained adherent bacteria were determined with micro ELISA auto reader at wavelength of 490nm (OD) These OD values were considered as an index of bacteria adhering to surface and forming biofilms (Christensen *et al.*,1985).

3-5-4-3- *icaAD* gene primers:-

The PCR method for amplification of *icaA* and *icaD* to detect the biofilm (as slim formation) were done according to Arciola *et al.* (2001) as in table (3 - 7).

Table (3 - 7): *icaAD* primers sequence.

Gene		Primer sequence (5'-3')	Size of product bp	TM	TA
<i>IcaA</i> gene	F	5'-TCTCTTGCAGGAGCAAT CAA-3'	188bp	58°C	55.5°C
	R	5'-TCAGGCACTAACATCC AGCA-3'		60°C	
<i>IcaD</i> gene	F	5'-ATGGTCAAGCCCAGAC AGAG-3'	198bp	62°C	55.5°C
	R	5'CGTGTTTCAACATTTAA TGCAA-3'		60°C	

3-5-4-3-1- Reagents:-

The reagents and their volumes were used for PCR amplification are described in table (3- 8).

Table (3 - 8): Reagents and volume (25µl) used in PCR amplification for *icaA* or *icaD* gene

No	Reagent	Volume
1	DNA template	5 µl
2	Forward primer	1 µl (10 pmol)
3	Reverse primer	1 µl (10 pmol)
4	Master mix	12.5 µl
5	Nuclease free water	5.5 µl
Volumes		25 µl

3-5-6-2 -Thermal cycling condition :-

The program was described in table (3 - 9) .

Table (3 - 9): Program use in PCR amplification for *icaA* or *icaD* gene

Steps	Temperature	Time	No . Of cycles
Initial denaturation	94°C	5min	1
Denaturation	94°C	30 sec	50
Annealing	55.5°C	30 sec	
Extension	72°C	30 sec	
Final extension	72°C	1 min	1

3-5-6-3-Detection of PCR product by agarose gel electroforiasis :-

The solution, procedure and viewing were identical to those for the

detection of universal *16srDNA* bands . However there are few exceptions using 100 bp DNA ladder .

3-5-7-Statistical analysis:-

Statistical analysis was performed by Chi-square (X^2) test. P-value less than 0.05 was considered as statistically significant and P-value less than 0.01 considered as highly significant (Al-Mohammed *et al.*, 1980).

Chapter four

Results

4-1- Molecular Genetic Study:-

4-1-1-DNA extraction:-

The DNA from 94 isolates (47 from dentures and 47 from orthodontic) was extracted and examined by agarose gel electrophoresis (Figure 4-1).

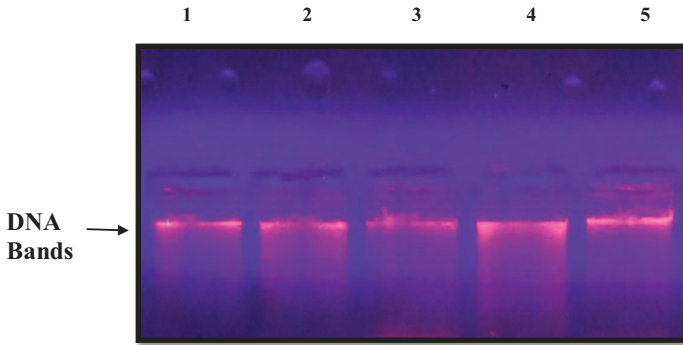


Figure (4 - 1) : Agarose (0.8%) gel electrophoresis for DNA bands (1-5) of random bacterial isolates from dentures and orthodontic under UV transilluminator .

4-1-2- Universal *16SrDNA* gene detection by PCR:-

The extracted DNA for all isolates was subjected to PCR for amplifying universal *16SrDNA* gene .PCR products for the universal *16SrDNA* primers gave bands on agarose gel at the position 1500bp when compared with standard molecular DNA ladder (Figure 4 - 2 to 4

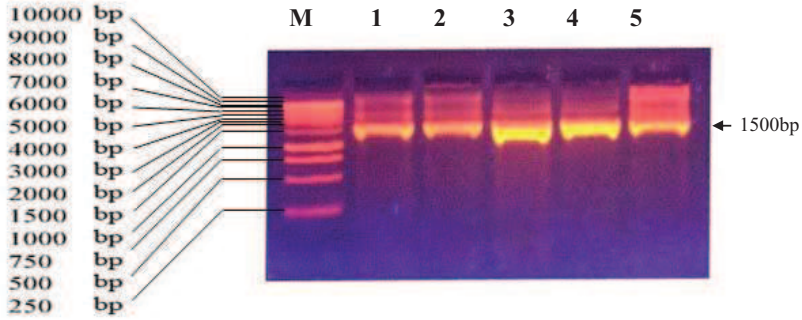


Figure (4 - 2) : Agarose (1%) gel electrophoresis of universal *16SrDNA* PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-6: 1to5 of *16SrDNA* bands(1500bp) for bacterial isolates .

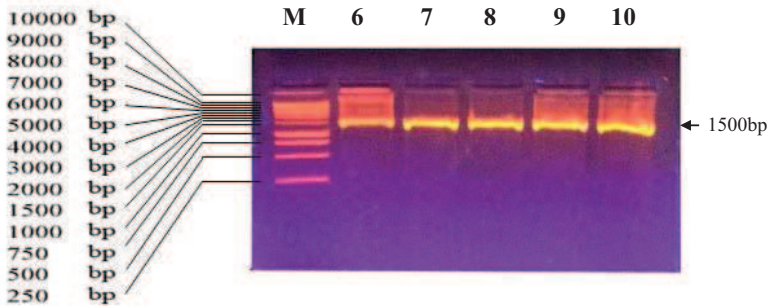


Figure (4 - 3) : Agarose (1%) gel electrophoresis of universal *16SrDNA* PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-6: 6 to 10 of *16SrDNA* bands (1500bp) for bacterial isolates .

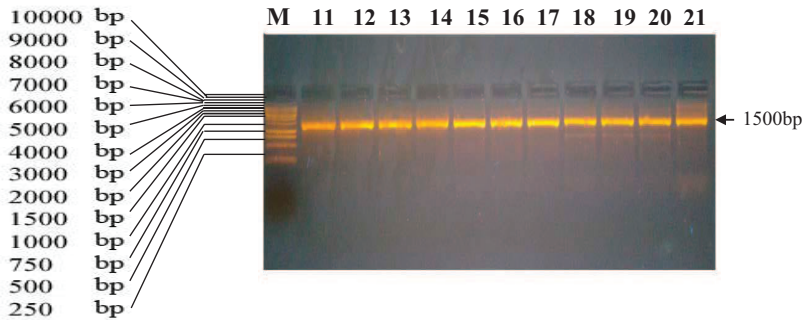


Figure (4 - 4) : Agarose (1%) gel electrophoresis of universal *I6SrDNA* PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-12: 11 to 21 of *I6SrDNA* bands (1500bp) for bacterial isolates .

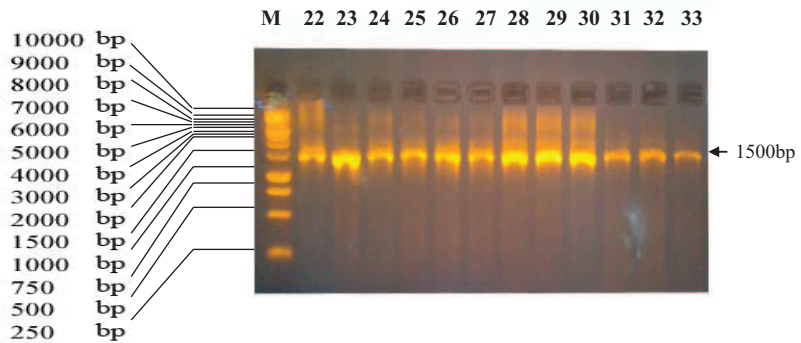


Figure (4 - 5) : Agarose (1%) gel electrophoresis of universal *I6SrDNA* PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-13: 22 to 33 of *I6SrDNA* bands (1500bp) for bacterial isolates .

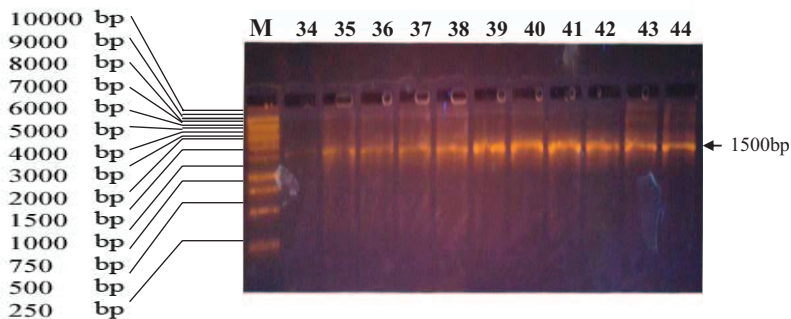


Figure (4 - 6) : Agarose (1%) gel electrophoresis of universal *16SrDNA* PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-12: 34 to 44 of *16SrDNA* bands (1500bp) for bacterial isolates .

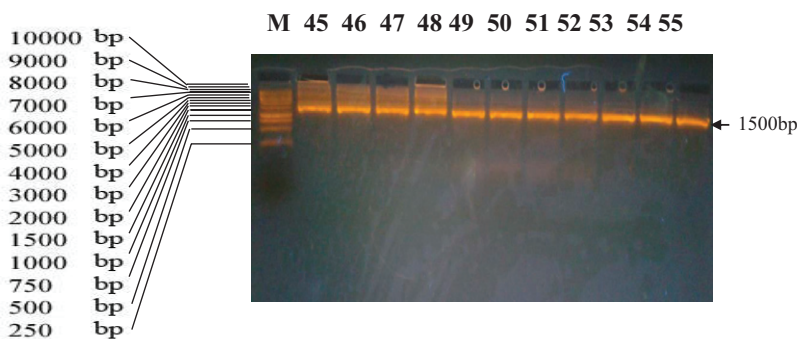


Figure (4 - 7) : Agarose (1%) gel electrophoresis of universal *16SrDNA* PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-12: 45 to 55 of *16SrDNA* bands (1500bp) for bacterial isolates .

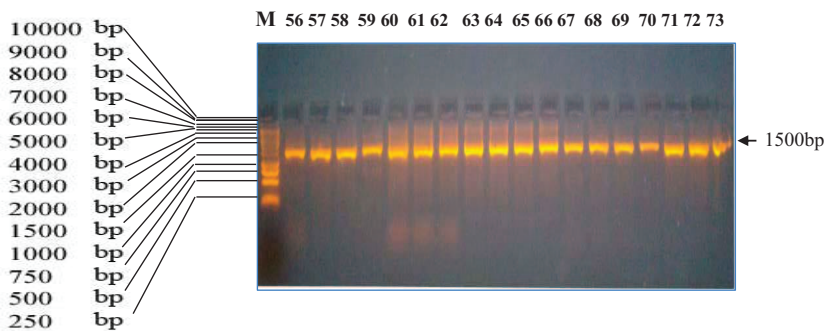


Figure (4 - 8) : Agarose (1%) gel electrophoresis of universal *16SrDNA* PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-19: 56 to 73 of *16SrDNA* bands (1500bp) for bacterial isolates .

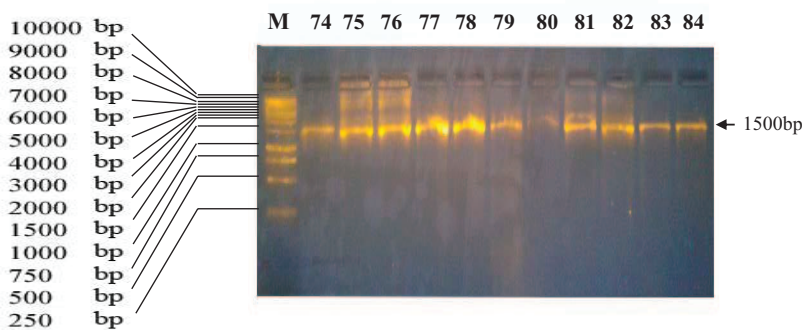


Figure (4 - 9) : Agarose (1%) gel electrophoresis of universal *16SrDNA* PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-12: (74 to 84 of *16SrDNA* bands (1500bp) for bacterial isolates

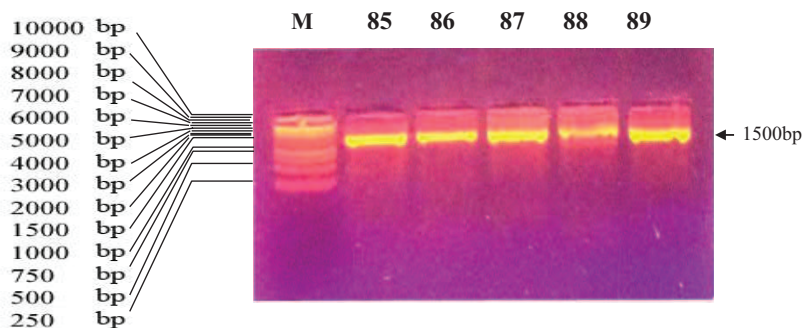


Figure (4 - 10) : Agarose (1%) gel electrophoresis of universal *16SrDNA* PCR products for bacterial isolates from denture and orthodontic under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. Lane 2-6: 85 to 89 of *16SrDNA* bands (1500bp) for bacterial isolates.

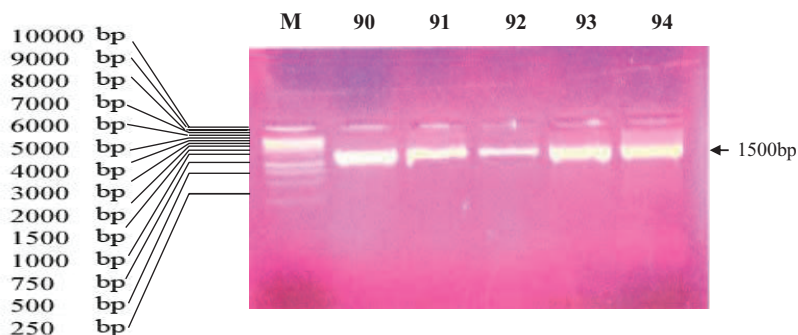


Figure (4 - 11) : Agarose (1%) gel electrophoresis of universal *16SrDNA* PCR products for bacterial isolates from denture and under UV transilluminator. Lane 1: (M) : 1Kb (250 bp – 10000bp) DNA ladder. orthodontic Lane 2-6: 90 to 94 of *16SrDNA* bands (1500bp) for bacterial isolates

4-1-3- Sequencing for universal 16S rDNA gene:-

Table (4-1) showed out of 94 alignments 16SrDNA gene sequences for all isolates, 31 species of bacterial isolates were identified by 16SrDNA gene sequencing (comparing with identical reference strain) from denture and orthodontic. These are: *Klebsiella pneumoniae* (n=16), *Proteus mirabilis* (n=8), *Proteus penneri* (n=7), *Staphylococcus aureus* (n=7), *Enterobacter cloacae* (n=6), *Bacillus cereus* (n=5), *Enterococcus faecalis* (n=4), *Enterobacter faecium* (n=3), *Morganella morganii* (n=2), *Hafnia alvei* (n=2), *Enterobacter aerogenes* (n=2), *Enterobacter mori* (n=2), *Citrobacter freundii* (n=2), *Staphylococcus epidermidis* (n=2), *Bacillus subtilis* (n=2), *Lactobacillus plantarum* (n=2), *Streptococcus anginosus* (n=2), *Proteus houserii* (n=1), *Acinetobacter baumannii* (n=1), *Klebsiella oxytoca* (n=1), *Lactococcus lactis* (n=1), *Chryseobacterium vietnamense* (n=1), *Streptococcus equinus* (n=1), *Klebsiella variicola* (n=1), *Escherichia fergusonii* (n=1), *Pediococcus acidilactici* (n=1), *Staphylococcus pasteurii* (n=1), *Staphylococcus worneri* (n=1), *Serratia marcescens* (n=1), *Enterobacter ludwigii* (n=1), *Staphylococcus hominis* (n=1). Other isolates (n=6) were failed sequencing but recognized only by morphological and gram's stain. These are: *Streptococcus* spp. (n=2), *Bacillus* spp. (n=1), *Staphylococcus* spp. (n=3).

4-1-4- Phylogenetic tree of bacterial species:-

The resultant tree rooted with *Enterobacter cloacae* as the out group, is presented in figure (4-13). This tree shows the distribution and phylogenetic relationships among the studied species and type strains.

Table (4 -1) : Bacterial Species Identified By Sequencing Of Universal *16SrDNA* For Isolates From Dentures.

No.	No. of isolate	Bacterial species	Nucleotide sequence	source	Identical with Strain	Length (bp)
1	93	<i>Klebsiella pneumoniae</i>	GGTATAGCCTACTCTCTTTTGCAACCACCTCCCATGGTGTGACGGCGGGTGTGTACAAAGGCCG GGGACGTATTACCTAGCATTTGTATCTACGATTATAGCATCTGATCTGATCTGATGAGTCCGA GTTCAGACTCCAACTCGAGCTAGCAGCATACTTATGAGGTCCGGCTGTCTCTCGAGGATCGCTCT CTCTCTATATGCTATTGATAGACCTGTGACCTGTGAGATCTGAGGAGGACATGATGATGATGAT CATLCCAGCTTCTCCAGTTTATCTACGAGCACTCTCTTGATGTTCCGGCTAACGCTGGGACA CAAGAGTAAAGGTTGTGGCTGCTTCCGGGACTTAAACCAATCTCTACACAGAGCTGACGAA AGGCTACGAGCACTGTCTCAAGTTCCGGAAGGACGAATCCATCTCGGAAGTTCTGTGGAT TGACATGAGCAGGTGAGGTTCTGCGCTTCCATGATTAACCAATCTGACCACTGCTCCAGCTCTGTG GGCCCGCCGATCAATTTGATGTTTAACTTGTGGGCGCTACTCCAGGCGGTGATTAATACGC GTATGCTGGGAGGACGAGCTCAAGGAGCAAACTCCATATGGATGAGTGTTCAGAGCTGGGAG TACAGGGGTATCTAATCTGTTGTGCCACGCTTTCGCACTGAGGCGTGAAGTTCTGTCTCCAGG GGGGCTCTGGCACGGGTATCTCGAGATCTTACGATTAATGACCTGCTGCTGGAATTT ACCCCTCTCTACAGACTAGCTCGCGATTTCCGATGCAATGCTCCAGGTTGAGCCGGGGAT TGATATCGGCTGACAGAGCGCTGGGTGCTTTAGGCGAATTAATGATTAAGCTGCTGCTCA CTCTGATTATCCCGGCTGTGCGACGAGGTAGCGCGTGTCTCTCTGGGTAAGCTCMA TGATAGAGTTATTAACTTACCGCTCTCTCTCTCGGATGCTTTACAACTCGAGGGCT CTTCAACAGCGGGCTAGCTGATCAGGCTTGGCGCATGTGCAATGATGCTCCAGCTGGCTCT CTGTAGAGATCTGAGCGCTTCTAGTGTAGTGGTGGTATCTCTCAAGAGAGTAG CTCTGCTGCTTAGGTGAGCGCTTACCACTTACAGCAATATCGATCTGGGACATCTGATGGT GTGAGGCGCAAGGTTCTCTTTGCTTTCAGCTTTATGGGTATTGACAGGCTCTGCTCTG AGTTATCCCTCTCATATAGGAGATCTCCAGCAATATLCAACCGTCTGGCGCTGTGTACAGGAG AGGAGGCTCTGTCTGCTAGCTGGGTGGTATGTT	D*	SY-1T 100%	1419bp
2	48	<i>Klebsiella pneumoniae</i>	CAAGATGAAGTGGTAAGCGCTCCCGAAGGTTAAGTACTACTCTTTTGCAACCACTCC ATGGTGTGACGGGGGTGTGTACAAAGCCGGGAGCATATTACCGTAGCATCTGATGATGAG ATTAGAGCATTCGATCTAGGATGAGGTTGAGATCCCATCTCGATCTGGATACGATACATCTT ATGAGGTCCCGCTGTGCTTCGGGAGGCTGTCTCTTTGTATATGCCATTGACAGCATGTGTAGC CTGTGCTTAGGGGCTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT CTCTTTGATTTGCTGCGGACGACCTGGGCTCAAGGATAGAGGTTGTGCTCTGTGGGAT TAACTCAATCTTCAACACGAGCTGACAGAGCATGATGAGGACTGTCTCACTGCTGCTGCTG TGGAATTAACCACTATGCTCCAGCTGTGCTGGGCGCCCTCAATTTGATGATTTAACTGTT GGGATCTCTCAAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG ACCTTCAATGACATGCTTTAGGGGTGACACTACAGGATCTAATCTTGTGCTCCAGC CTTGCATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG TTAGCGATTACCGCTGATCTGCGGATCTTACCGCTCTACAGACTAGCTGCTGCTGCTGCTG CTTGCATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG TTTAGCGCGATTAATCGATTAACTGCTGACCTCTGATTAACGCGGTGCTGGACGGAGT ACCGGCTGCTTCTTGTGAGTAACTAGCGAGGATTAATTAAGTTAGCTGCTGCTGCTGCTG CGCTTAAGAGCTTTACAACTGAGGCGCTTCTCAACACGCGGAGTGGCTGATGAGGCTG CTG TTGTGG CTGAGTATGCGGATTTAGCTACGCTTCCAGTAGTATATCCCTCTCAAGGAGGTTCCGACAG ATTATACCGCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG GTGTAGCTGGCTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG	D	DM-8T 100%	1419bp
3	57	<i>Klebsiella pneumoniae</i>	CAAGATGAAGTGGTAAGCGCTCCCGAAGGTTAAGTACTACTCTTTTGCAACCACTCCGA ATGGTGTGACGGGGGTGTGTACAAAGCCGGGAGCATATTACCGTAGCATCTGATGATGAG TACTAGGATTCGATCTAGGATGAGGTTGAGATCCCATCTCGATCTGGATACGATACATCTT TAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG TTGTGATGAGGCGGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATG CTTTGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG TCTTGAATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG ACCCAACTTTACACAGAGAGCTGACGAGCACTGACAGACTGTCTCAAGTTTCCGAAGG GACATGATCTGAGGAGTTTGTGATGCTGAGAGATTAAGTTTGTGATGCTGCTGCTGCTGCTG AATTAACAGCATGCTCCAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG GGCTACTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG CACTAAATGACATGCTTTAGGCGGTGACACTACAGGATCTAATCTGCTGCTGCTGCTGCTG TAGGCTATTACGCTGATCTGCGGATCTTACCGCTCTACAGACTAGCTGCTGCTGCTGCTG GTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG ACCGGCTGCTTCTTGTGAGTAACTAGCGAGGATTAATTAAGTTAGCTGCTGCTGCTGCTG GAAAGGCTTTACAACTGAGGCGCTTCTCAACACGCGGAGTGGCTGATGAGGCTG GATTTGCAATTTCCAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG GTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG CTAATTCATCTGGGAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATG TTATCGGATTTAGCTACGCTTCCAGTAGTATATCCCTCTCAAGGAGGTTTCCGACAGATAC TACCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG ACCGCGGATTTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG	D	sks1T 100%	1411bp
4	61	<i>Klebsiella pneumoniae</i>	CCCATGTATCAGTAGGGGGGCGCTCCGAAGGTTAAGTACTACTCTTTTGCAACCACTCCGA TGGTGTGACGGGGGTGTGTACAAAGCCGGGAGCATATTACCGTAGCATCTGATGATGAG ATTAGGATCCCATTTAGGATTCGATTTGCAATCTTACCTCACTCGATGAGAGATTTATGA AGATTGCTTGGATCTCCGAGGCACTTCTTGGGAAGTGGCTTGGAGCTGGGGAAGCC AGGCTCAAGGAGATGAGGATTTAGCTGCTTCCCTCTCTCTCGGTAGGCTGGGAGTCT CTTTGAATCTCCAGCGAATGATGAGGCACTAAGGAAGGGTGGCGCTGTGGGGGAGG CTTTGAATCTTGTAGCAAGAGGATGACAGCACTGAGGAGGCTGCTTTTGAATCTT CGAAGGGGAAGCTTATCTGATCTGTCAAGGATGTTCAAGACTAGGATGAGTTCTTCCG GTGCTGCTGATTTAAACCACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG ACCTG AGGCTCAAGGATCTTAACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG AGGCTCAAGGATCTTAACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG GCTTCAAGATCTTAACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG CTTCAAGATCTTAACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG CTTCAAGATCTTAACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG GATTTAAAGCTTGGACGCTACGATTACCGGGGCTGGGAGCGATGATGATGCTGCTGCTGCTG TCTTGGT	D	KSST 100%	1030bp
5	62	<i>Klebsiella pneumoniae</i>	GGCATATCAAGTGGTAAGCGCTCCCGAAGGTTAAGTACTACTCTTTTGCAACCACTC CATGATTTGACGGGGGTGTGTACAAAGCCGGGAGCATATTACCGTAGCATCTGATGATGAG GATTATAGCATTCGATCTCATGAGTCTGAGTCTGAGACTCCATCGGAGCTAGCATATATT TATGAGGATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG CTGCTGCTGATGAGGCGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATG TCTCTGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG TTAACTCAATCTTCAACACGAGCTGACAGAGGCTGACGAGCTGTCTCAAGTTTCCGAAG GGGACATGATCTGAGGAGTCTGTGATGATGATGATGATGATGATGATGATGATGATGATGATG TGGAATTAACCACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG GGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG ACCTTCAATGACATGCTTTAGGGGTGACACTACAGGATCTAATCTTGTGCTCCAGC CTTGCATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG TTAGCGATTACCGCTGATCTGCGGATCTTACCGCTCTACAGACTAGCTGCTGCTGCTGCTG CTTGCATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG TTTAGCGCGATTAATCGATTAACTGCTGACCTCTGATTAACGCGGTGCTGGACGGAGT ACCGGCTGCTTCTTGTGAGTAACTAGCGAGGATTAATTAAGTTAGCTGCTGCTGCTGCTG CGCTTAAGAGCTTTACAACTGAGGCGCTTCTCAACACGCGGAGTGGCTGATGAGGCTG CTG TTGTGG CTGAGTATGCGGATTTAGCTACGCTTCCAGTAGTATATCCCTCTCAAGGAGGTTTCCGACAG ATTATACCGCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG GTGTAGCTGGCTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG	D	KST 100%	1435bp

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[illegible]

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[illegible]

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[illegible]

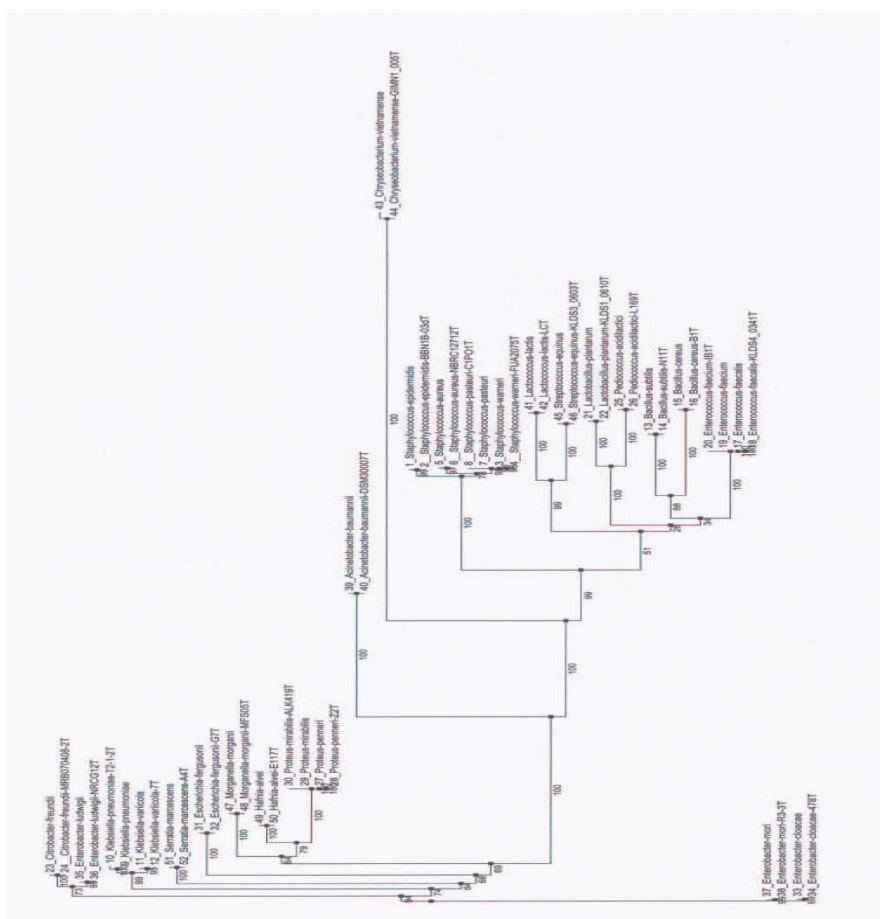
[illegible]

[illegible]

[illegible]

[illegible]

[illegible]



T: type strain, the sequence of each is available from <http://blast.ncbi.nlm.nih.gov>.

Figure (4 -12):Rooted neighbor joining phylogenetic tree constructed from concatenated sequences of (893bp)for bacterial species (derived from an alignment of *16SrDNA* gene sequences) then produced by (MAFFT)multiple alignment program for amino acid or nucleotide sequences and visualized using" forester" software. This NJ tree showing the distribution and phylogenetic relationships of 28 different species identified in this study and their reference strains (T). The tree has been rooted with *Enterobacter cloacae* or

4-1-5-Detection of new bacterial strain from denture and orthodontic isolates:-

In the present study four bacterial strains were identified and recorded to be new in the world . Therefore, they were tested in European Nucleotide Archive (ENA),National Centre for Biotechnology Information (NCBI) and Gene Bank (appendix-2) . These are 7-*Enterobacter ludwigii* "IRQBAS1" (HG003646), 34-*Enterobacter cloacae* "IRQBAS2" (HG003647) isolated from orthodontic , 71-*Chryseobacterium vietnamense* "IRQBAS3" (HG003648) and 74-*Morganella morganii* "IRQBAS4" (HG003649) isolated from dentures, showed 99% sequence identity with reference strains NRCG12 , Nr.3, GIMM1.005 and MFS05 respectively . 7- *Enterobacter ludwigii* was different from strain NRCG12 as a result to a frame shift mutation (deletion of base C) from the sequence at position 453 bp changing all the following amino acids , (Figure 4-13). While,34-*Enterobacter cloacae* was different from strain Nr.3 in two positions of nucleotide sequences; the first one was by point mutation (transversion) of base G instead of C at position 462 bp changing the amino acid Thr (ACC) to Ser (AGC) , and the second was point mutation (transversion) of base C instead of G at position 471bp changing the amino acid Gly (GCT) to Ala (GGT), (Figure 4 -14) .

On the other hand , 71- *Chryseobacterium vietnamense* was different from strain GIMN1.005 in eight positions of nucleotide sequences, the first one was point mutation (transition) of base A instead of G at position 208bp changing the amino acid Arg (CGC) to His (CAC) as Figure (4 - 15), the second was point mutation (transversion) of base T instead of A at position 102bp changing the amino acid

Gln (CAG) to Leu (CTG), the third was point mutation (transition) of base G instead of A at position 149bp changing amino acid Asn (TAG) to Asp (TGG) , the fourth was point mutation (transition) of base C instead of T at position 160bp changing stop codon (TTT) to amino acid Trp (TCT) ,the fifth and sixth were mutation changing of base G instead of A at position 163bp and A instead C at position 341bp respectively, but without changing the type of amino acids Tyr (GGT) or (GAT) and Arg (CGA) or (AGA) respectively (Figure 4-16 and 4-17), the seventh mutation was point mutation (transversion) of base T instead of G changing amino acid Gly (GGA) to stop codon (TGA) figure (4-18), the last mutation was point mutation (transition) of base C instead of T at position 361bp changing amino acid Pro (TCC) to Ser (CCC), as Figure (4 -19). While 74-*Morganella morganii* was different from strain MFS05 in three positions of nucleotide sequences ,all of them were frame shift mutation (deletion of base G , C and T) from the sequence position 534bp , 58 bp and 712bp respectively, changing all the following amino acids (Figure 4 – 20 , 4 – 21 and 4-22) respectively .

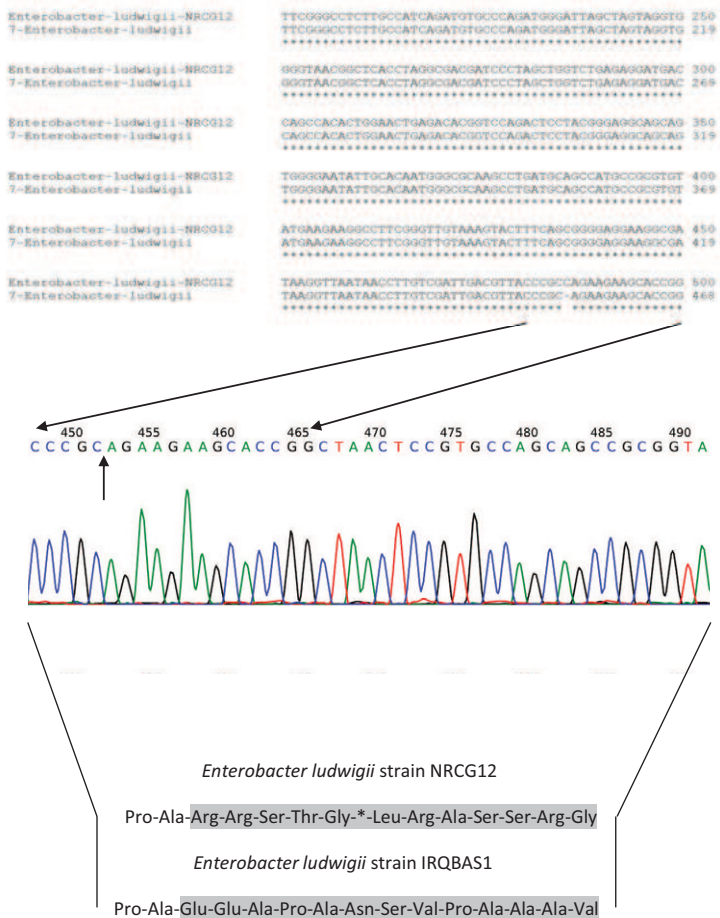


Figure (4 - 13): CLUSTALW for comparison of nucleotide sequences alignment for *16SrDNA* gene of 7-*Enterobacter ludwigii* identical (99%) to strain NRCG12 showed frame shift mutation (deletion nucleotide C) at the position 453bp changing all the following amino acid

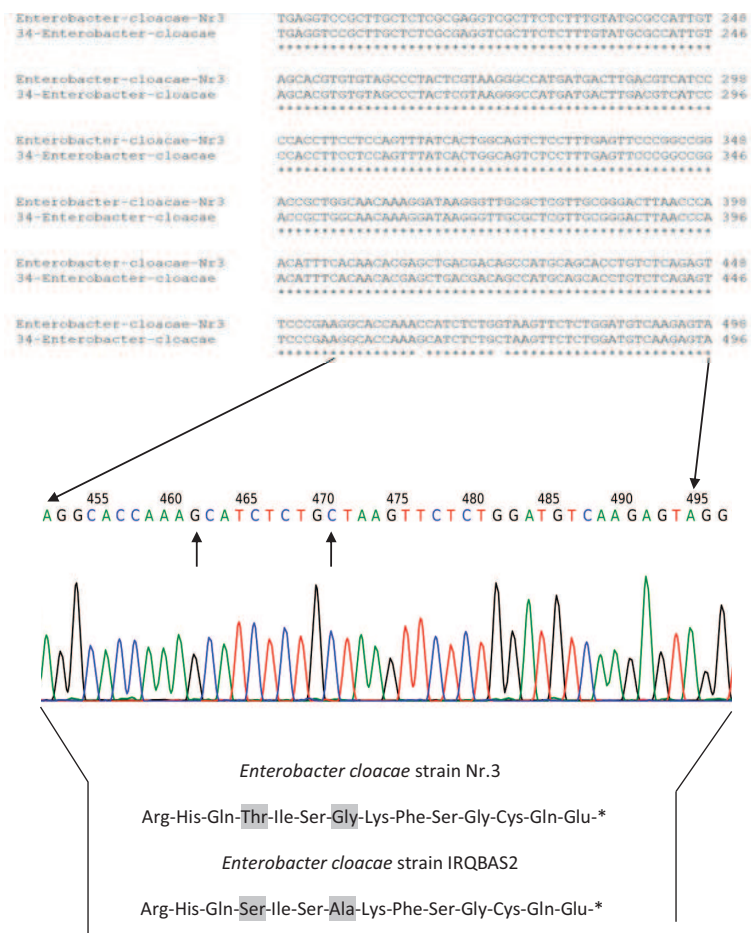


Figure (4 -14) : CLUSTALW for comparison of nucleotide sequences alignment for 16SrDNA gene of 34- *Enterobacter cloacae* identical (99%) to strain Nr.3 showed two point mutation type Transversion : G instead of C at the position 462bp changing the amino acid Thr (ACC) to Ser (AGC) and C instead of G at the position 471bp changing the amino acid Gly (GGT) to Ala (GCT).

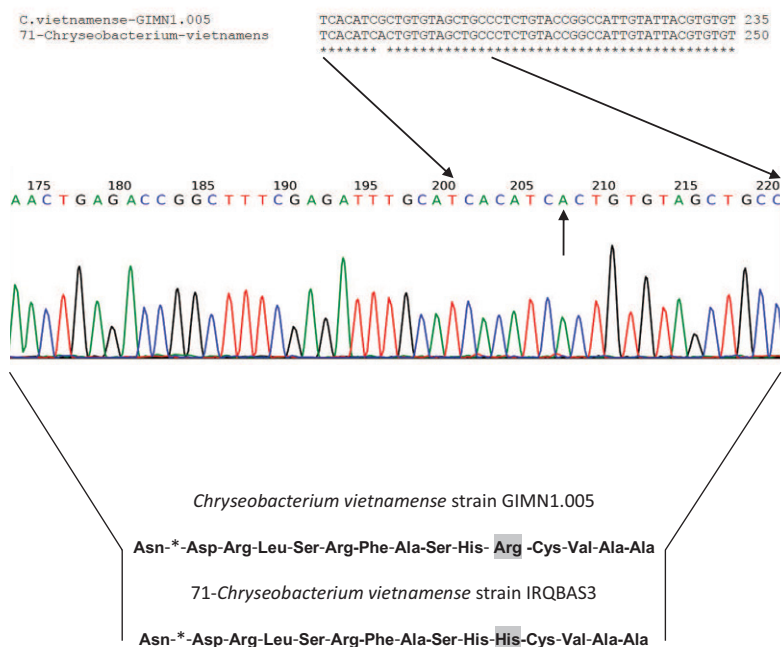


Figure (4 - 15): CLUSTALW for comparison of nucleotide sequences alignment for *16SrDNA* gene of 71- *Chryseobacterium vietnamense* identical (99%) to strain GIMN1.005; showed point mutation type transversion A instead of G at the position 207bp changing the amino acid Arg(CGC) to His (CAC).



Figure (4 – 16) : CLUSTALW for comparison of nucleotide sequences alignment for 16S rDNA gene of 71- *Chryseobacterium vietnamense* identical (99%) to strain GIMN1.005; showed point mutation type transversion T instead of A at the position 102bp changing the amino acid Gln (CAG) to Leu (CTC), and point mutation type transition G instead of A at the position 149bp changing amino acid Asn (AAT) to Asp (GAT), and point mutation type transition C instead of T at the position 160bp changing the stop codon (ATT) to Trp (ATC), and point mutation type transition G instead of A at position 163bp without changing the type of amino acid .

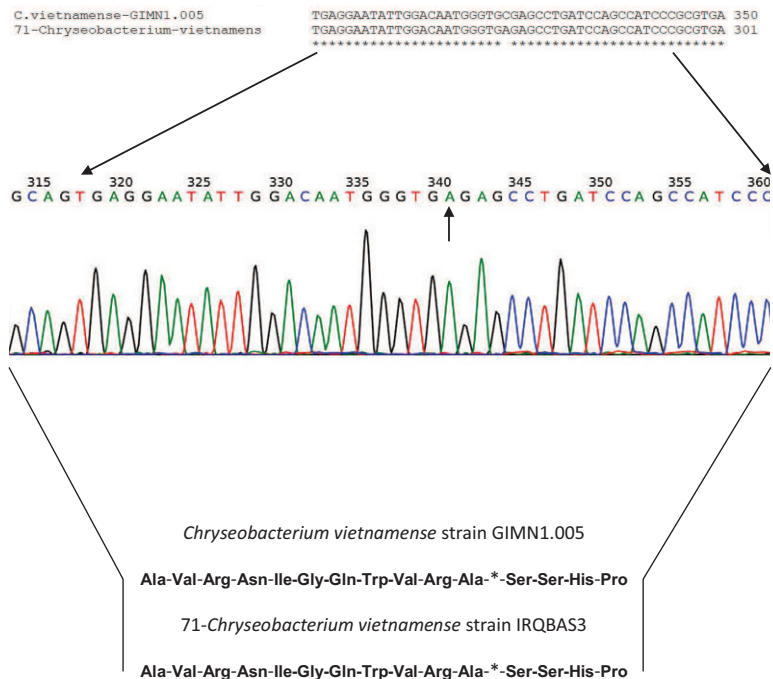


Figure (4 – 17): CLUSTALW for comparison of nucleotide sequences alignment for 16S rDNA gene of 71- *Chryseobacterium vietnamense* identical (99%) to strain GIMN1.005; showed point mutation type transversion A instead of C at the position 341bp without changing the type of amino acid .

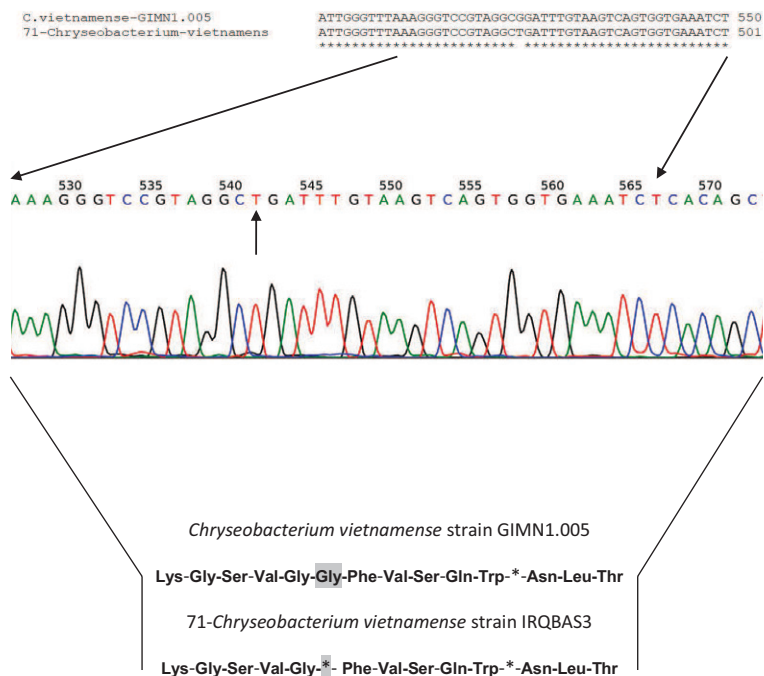


Figure (4 – 18): CLUSTALW for comparison of nucleotide sequences alignment for 16S rDNA gene of 71- *Chryseobacterium vietnamense* identical (99%) to strain GIMN1.005; showed point mutation type transversion T instead of G at the position 542bp changing the amino acid Gly (GGA) to stop codon (TGA)

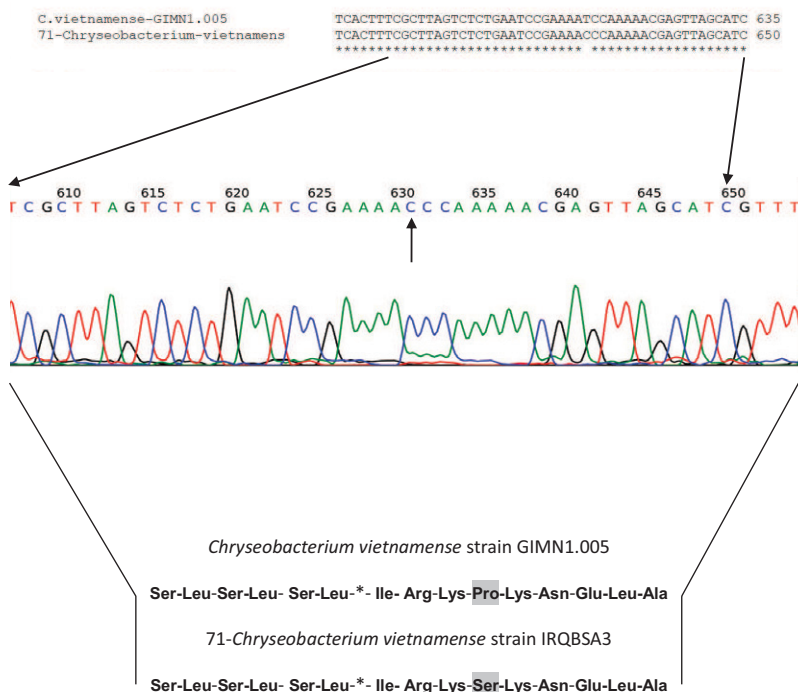


Figure (4 -19): CLUSTALW for comparison of nucleotide sequences alignment for *16SrDNA* gene of 71- *Chryseobacterium vietnamense* identical (99%) to strain GIMN1.005; showed point mutation type transversion C instead of T at the position 631bp changing the amino acid Pro (TCC) to Ser(CCC).

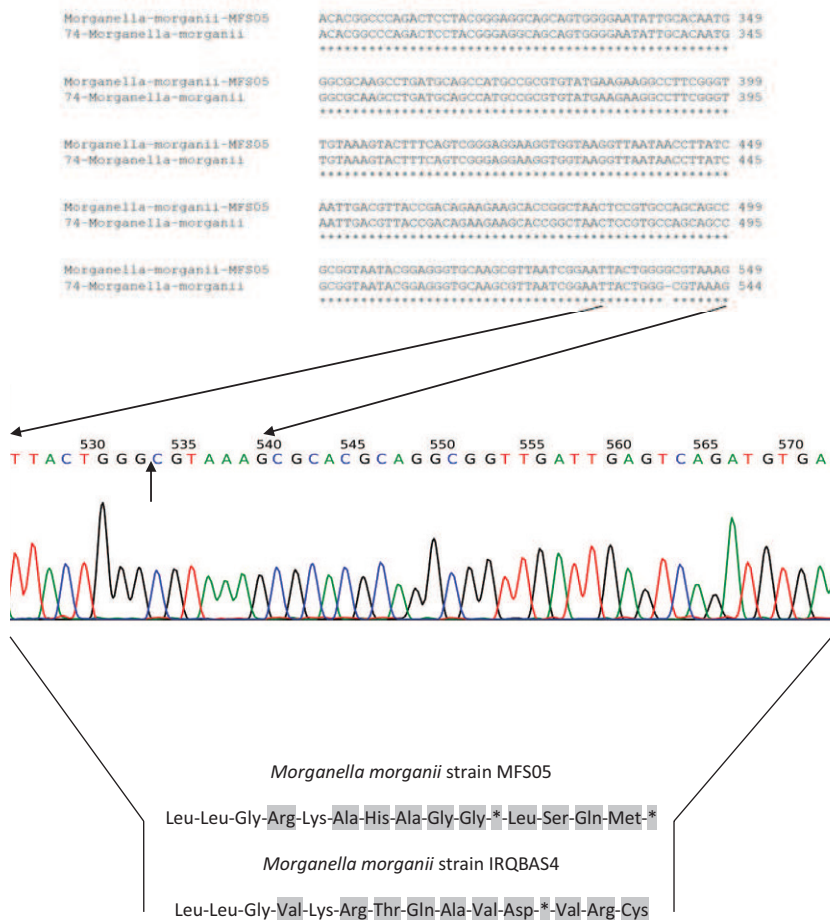


Figure (4 -20). CLUSTALW for comparison of nucleotide sequences alignment for 16SrDNA gene of 74- *Morganella morganii* identical (99%) to strain MFS05 show frame shift mutation (deletion nucleotide G) at the position 554bp changing all following amino acids

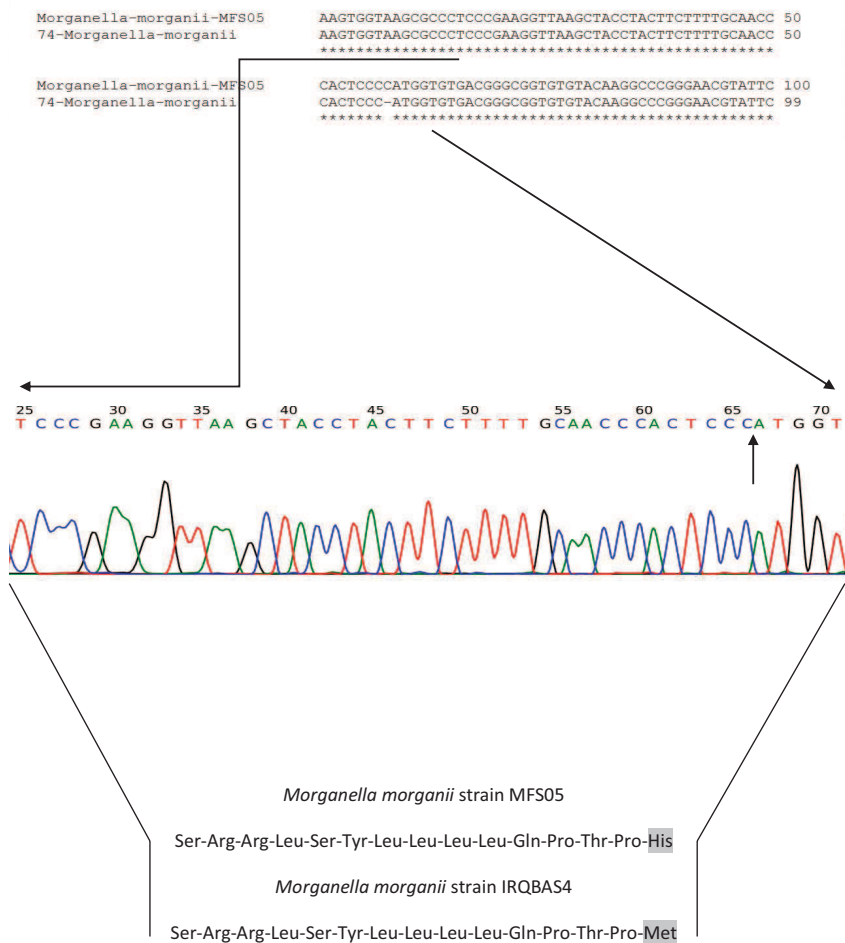


Figure (4 -21): CLUSTALW for comparison of nucleotide sequences alignment for 16SrDNA gene of 47-Morganella morganii identical (99%) to strain MFS05 show frame shift mutation (deletion nucleotide C) at the position 58bp changing all the following amino acids

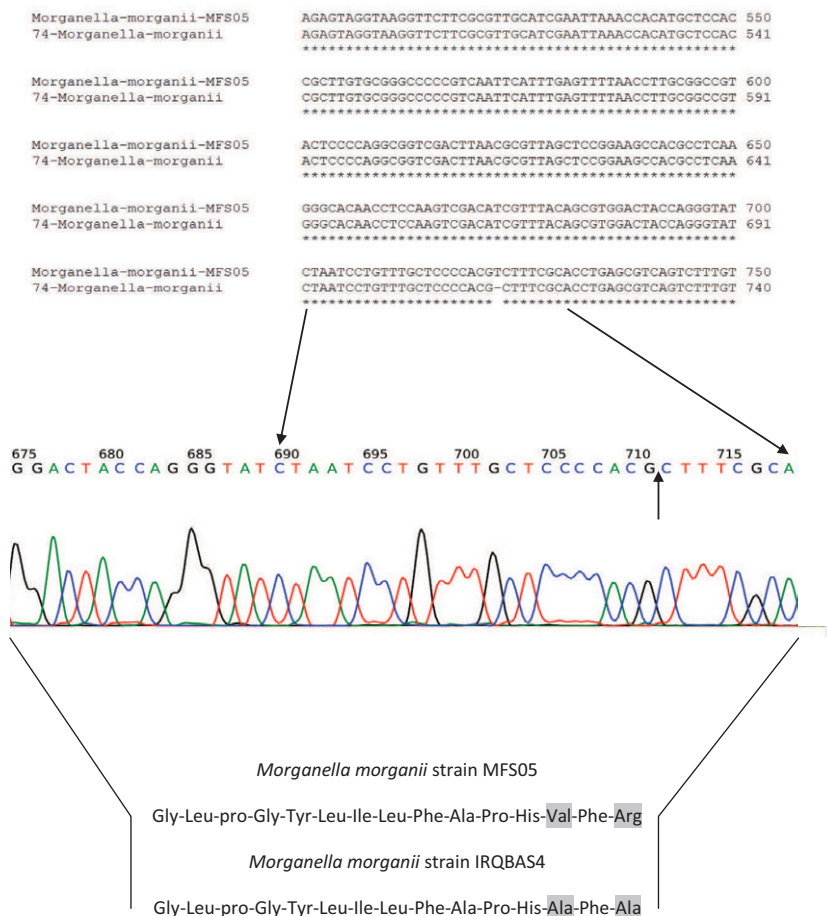


Figure (4 -22) : CLUSTALW for comparison of nucleotide sequences alignment for 16SrDNA gene of 47- *Morganella morganii* identical (99%) to strain MFS05 show frame shift mutation (deletion nucleotide T) at the position 712bp changing all the following amino acids.

4-2- Detection of biofilm formation:-

4-2-1- Congo Red Agar (CRA) method:-

CRA method showed very different results. In dentures 8(17.02%) of 47 isolates showed black colonies with a dry crystalline as positive results (slime producers) as Figure (4-23). 2(4.3%) of 47 isolates showed dark-pink colonies as intermediate results (weak slime producers), and 37(78.7%) of 47 isolates showed pink-white as negative results (not slime producers). Frequency of negative isolates was higher than positive isolates with high significant ($p \leq 0.01$), and higher than intermediate with high significant ($p \leq 0.01$), while frequency of positive isolates was higher than intermediate isolates with high significant ($p \leq 0.01$). As summarized in Table (4-2). In orthodontic recovered very different results, 4(8.5%) of 47 isolates appeared positive result, 2(4.3%) of 47 isolates appeared intermediate result. In contrast, frequency of positive results was higher than intermediate with high significant ($p \leq 0.01$). 41(87.2%) of 47 isolates appeared negative results and frequency of this result was higher than positive and intermediate with high significant ($p \leq 0.01$). No significant differences appeared between denture and orthodontic results for each test (positive, intermediate, negative).

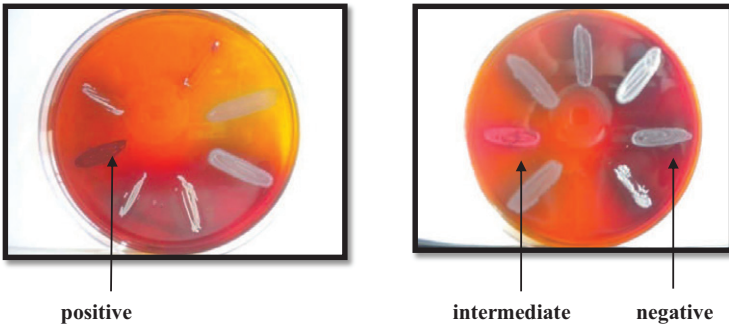


Figure (4-23): Congo Red Agar (CRA) assay for bacterial isolates from dentures and orthodontics . Positive is black colonies with dry crystalline (slime producers) , intermediate is dark- pink colonies (weak slime producers), negative is pink-white as (not slime producers)

Table (4-2) Comparison of Congo Red Agar (CRA) assay for bacterial isolates from denture and orthodontic

source of bacterial isolates	Positive n(%)	Intermediate n(%)	Negative n(%)
Denture	8(17.02%)	2(4.3%)	37(78.7%)
Orthodontic	4(8.5%)	2(4.3%)	41(87.2%)

($p \leq 0.01$)

4-2-2-Tissue Culture Plate (TCP) method:-

TCP method Figure (4 -24) was recovered in denture, 8(17.02%) of 47 isolates showed high positive results for slime producing ,7(14.9%) of 47 isolates showed moderate positive results and 29(61.7%) of 47 isolate showed weak positive results (Table 4-3). Frequency of weak positive results appeared to be higher than high and moderate positive results with high significant difference ($p \leq 0.01$) , 3(6.4%) of 47 isolates showed negative results , However , the frequency of positive isolates 44(94%) of 47 isolates (high, moderate ,and weak) was higher ($p \leq 0.01$) than negative results . In orthodontic TCP method recovered 2(4.3%) of 47 isolates showed high positive results ,and 9(19.2%) of 47 isolates showed weak positive results, with high significant difference ($p \leq 0.01$). 36(76.6%) of 47 isolates showed negative results with high significant ($p \leq 0.01$) than positive (high, moderate and weak) results . Frequency of positive result (high, moderate and weak) in denture was higher ($p \leq 0.01$) than in orthodontic . Following, the frequency of negative result in orthodontic is higher ($p \leq 0.01$) than in denture .

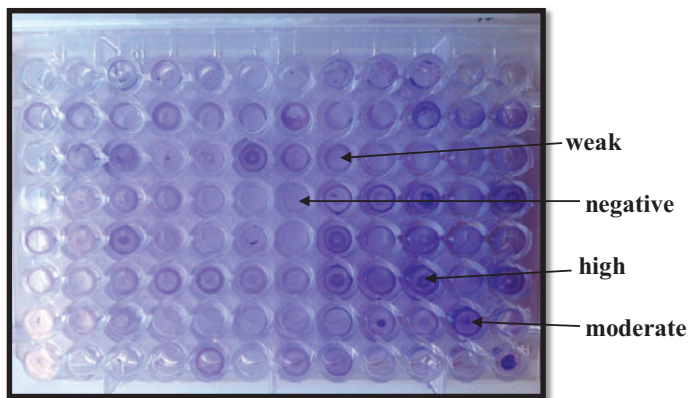


Figure (4 - 24): Tissue Culture Plate(TCP) assay of bacterial isolates from dentures and orthodontic . High: dark color, weak: poor color , moderate: medial color and negative : colorless

Table (4 -3): Comparison of Tissue Culture Plate (TCP) assay for bacterial isolates from denture and orthodontic

Sources of bacterial isolates	High OD ^a ≥0.131 n(%)	Moderate OD= 0.0655 0.0983 n(%)	Weak OD ≤0.0655 n(%)	Negative OD= 0 n(%)
Dentures	*8(17%)	*7(14.9%)	*29(61.7)	3(6.4%)
Orthodontic	2(4.3%)	Zero	9(19.2%)	*36(76.6%)

*($p \leq 0.01$)

a:Optical Density(OD

4-2-3-Detection of *icaA* and *icaD* genes in denture and orthodontic:-

PCR products for *icaA* and *icaD* genes were gave bands on agarose gel electrophoresis at 188bp and 198bp (respectively) position when compared with standard molecular DNA ladder (100 – 1000 base pair) as in figure (4 -26 and 4 -27) respectively.

4-2-3-1- *icaAD* gene for adherence :-

icaAD gene assay in denture showed that *icaA* gene showed 37(78.7%) of 47 isolates as positive results and 10(21.3%) of 47 isolates as negative results ,(Table 4-4) .Frequency of *icaA* gene positive was higher than *icaA* gene negative with high significant difference ($p \leq 0.01$). *icaD* gene was showed 40(85.1%) of 47 isolates as positive results and 7(14.9%) of 47 isolates as negative with high significant ($p \leq 0.01$) .

In orthodontic ,the *icaA* gene was showed 45 (95.8%) of 47 isolates was positive results , and only 2(4.3%) of 47 isolates was negative with high significant difference ($p \leq 0.01$). While, the *icaD* gene was showed 24(51.1%) of 47 isolates as positive results and 23(48.9%) of 47 isolates as negative results but without significant difference .

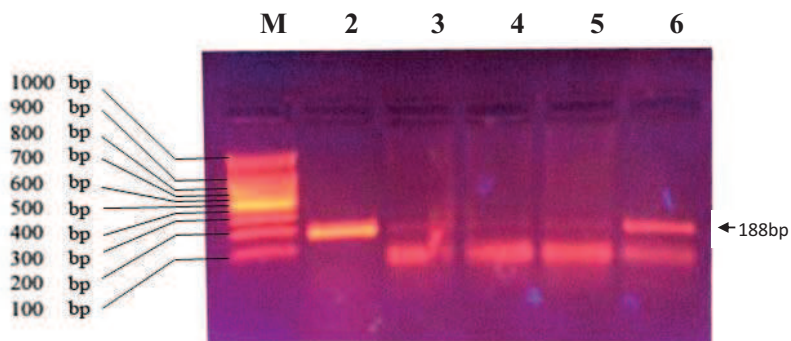


Figure (4- 25): Agarose (1%)gel electrophoresis showed PCR product of *icaA* gene for denture and orthodontic isolates. Lane 1: (100bp-1000bp) DNA ladder, Lane 2 to 6: *icaA* bands (188bp) of different isolates.

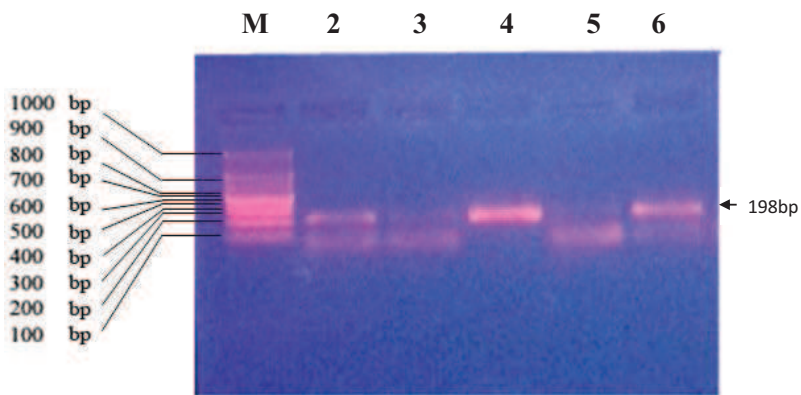


Figure (4 -26): Agarose (1%) gel electrophoresis showed PCR product of *icaD* gene to denture and orthodontic isolates. Lane 1: 1Kb (100bp-1000bp) DNA ladder (DNA marker).Lane 2 to 6: *icaD* bands(198bp) of different isolates

Table (4 - 4) : Comparison of *icaAD* genes for bacterial species from denture and orthodontic.

source of bacterial isolates	<i>Ica A</i>		<i>Ica D</i>	
	Positive n(%)	Negative n(%)	Positive n(%)	Negative n(%)
Denture	37 (78.7%)	10 (21.3%)	40* (85.1%)	7 (14.9%)
Orthodontic	45* (95.8%)	2 (4.3%)	24 (51.1%)	23* (48.9%)

*($p \leq 0.01$)

discrimination between these method (CRA,TCP, *icaAD* genes) for detection biofilm formation in denture isolates showed that the frequency of *icaAD* genes and TCP results were higher than positive CRA with high significant difference ($p \leq 0.01$) and no significant difference between the results of TCP and *icaAD* genes .In orthodontic isolates the frequency of *icaAD* genes was higher than both CRA and TCP results with high significant difference ($p \leq 0.01$), but no significant difference was found between TCP and CRA results .

4-3-The effectiveness of different bacterial species towards CRA , TCP, and *icaAD* genes Assays:-

Table (4 - 5) and (4-6) showed the effectiveness of each bacterial species isolated from denture and orthodontic respectively , toward congo red agar , tissue

culture plate and *icaAD* genes assays with which the best assay for each species to detect the ability of adherence .

In denture (Table 4-5) , TCP and *icaAD* assay were the best assays for *Klebsiella Pneumoniae* (100%,100%), *Proteus mirabilis* (100%,100%), *Proteus penneri* (100%,100%) , *Bacillus cereus* (100%,100%), *Enterobacter cloacae* (100%,100%), *Enterobacter aerogenes* (100%,100%) ,*Acinetobacter baumannii* (100%,100%), *Proteus houserii* (100%,100%) , *Enterobacter mori* (100%,100%), *Klebsiella variicola* (100%,100%) and *Staphylococcus hominis* (100%,100%) respectively comparing to CRA with high significant difference ($p \leq 0.01$), while *icaAD* genes were the best assay for *Morganella morganii* (100%) ,*Hafnia alvei* (100%), *Enterococcus faecium* (100%) than TCP and CRA assays with high significant difference ($p \leq 0.01$), no significant difference ($P \leq 0.01$) between CRA TCP ,and *icaAD* genes for *Klebsiella oxytoca* (100%,100%,100%), *Lactococcus lactis* (100%,100%,100%) *Enterococcus faecalis* (100%,100%,100%), *Chryseobacterium vietnamense* (100%,100%,100%), *Streptococcus equines* (100%,100%,100%), *Citrobacter freundii* (100%,100%,100%), *Bacillus spp.* (100%,100%,100%), *Streptococcus spp.* (100%,100%,100%) respectively , all different bacterial species from denture showed *icaAD* 46(97.9%) gene and TCP 44(93.6%) are the best assays for detection biofilm formation with higher significant ($p \leq 0.01$) than CRA but no significant difference between *icaAD* genes and TCP assays.

Table (4 - 5):Effectiveness of bacterial species from dentures toward slim formation assays.

No.	Isolate No.	Bacterial species In denture	(CRA)**	(TCP)***	IcaA and/or icaD genes
1	93	<i>Klebsiella pneumoniae</i>	—	+	+
2	48	<i>Klebsiella pneumoniae</i>	—	+	+
3	57	<i>Klebsiella pneumoniae</i>	—	+	+
4	61	<i>Klebsiella pneumoniae</i>	—	+	+
5	62	<i>Klebsiella pneumoniae</i>	—	+	+
6	78	<i>Klebsiella pneumoniae</i>	—	+	+
7	80	<i>Klebsiella pneumoniae</i>	—	+	+
8	89	<i>Klebsiella pneumoniae</i>	—	+	+
n(%)		8(17%)	0	8(100%)	8(100%)
9	50	<i>Proteus mirabilis</i>	—	+	+
10	51	<i>Proteus mirabilis</i>	—	+	+
11	52	<i>Proteus mirabilis</i>	—	+	+
12	54	<i>Proteus mirabilis</i>	—	+	+
13	58	<i>Proteus mirabilis</i>	—	+	+
14	83	<i>Proteus mirabilis</i>	—	+	+
15	84	<i>Proteus mirabilis</i>	—	+	+
n(%)		7(14.9%)	0	7(100%)	7(100%)
16	53	<i>Proteus penneri</i>	—	+	+
17	85	<i>Proteus penneri</i>	—	+	+
18	86	<i>Proteus penneri</i>	—	+	+
19	87	<i>Proteus penneri</i>	—	+	+
20	88	<i>Proteus penneri</i>	—	+	+
n(%)		5(10.6%)	0	5(100%)	5(100%)
21	56	<i>Bacillus cereus</i>	—	+	+
22	66	<i>Bacillus cereus</i>	—	+	+
23	67	<i>Bacillus cereus</i>	—	+	+
24	70	<i>Bacillus cereus</i>	+	+	+
n(%)		4(8.5%)	1(25%)	4(100%)	4(100%)
25	92	<i>Enterobacter cloacae</i>	—	+	+
26	55	<i>Enterobacter cloacae</i>	—	+	+

No.	Isolate No.	Bacterial species In denture	(CRA)**	(TCP)***	IcaA and/or icaD genes
27	73	<i>Enterobacter cloacae</i>	—	+	+
n(%)		3(6.4%)	0	3(100%)	3 (100%)
28	91	<i>Morganella morganii</i>	+	—	+
29	74	<i>Morganella morganii</i>	—	+	+
n(%)		2(4.3%)	1(50%)	1(50%)	2(100%)
30	94	<i>Hafnia alvei</i>	—	—	+
31	77	<i>Hafnia alvei</i>	—	+	+
n(%)		2(4.3%)	0	50%	2(100%)
32	65	<i>Enterobacter aerogenes</i>	—	+	+
33	81	<i>Enterobacter aerogenes</i>	—	+	+
n(%)		2(4.3%)	0	2(100%)	2(100%)
34	59	<i>Acinetobacter baumannii</i>	—	+	+
n(%)		1(2.1%)	0	1(100%)	1(100%)
35	60	<i>Klebsiella oxytoca</i>	+	+	+
n(%)		1(2.1%)	1(100%)	1(100%)	1(100%)
36	49	<i>Proteus houserii</i>	—	+	+
n(%)		1(2.1%)	0	1(100%)	1(100%)
37	68	<i>Lactococcus lactis</i>	+	+	+
n(%)		1(2.1%)	1(100%)	1(100%)	1(100%)
38	69	<i>Enterococcus faecalis</i>	+	+	+
n(%)		1(2.1%)	1(100%)	1(100%)	1(100%)

No.	Isolate No.	Bacterial species In denture	(CRA)**	(TCP)***	IcaA and/or icaD genes
39	71	<i>Chryseobacterium vietnamense</i>	+	+	+
n(%)		1(2.1%)	1(100%)	1(100%)	1(100%)
40	72	<i>Streptococcus equines</i>	+	+	+
n(%)		1(2.1%)	1(100%)	1(100%)	1(100%)
41	75	<i>Enterobacter mori</i>	—	+	+
n(%)		1(2.1%)	0	1(100%)	1(100%)
42	76	<i>Klebsiella variicola</i>	—	+	+
n(%)		1(2.1%)	0	1(100%)	1(100%)
43	74	<i>Staphylococcus hominis</i>	—	+	+
n(%)		1(2.1%)	0	1(100%)	1(100%)
44	82	<i>Citrobacter freundii</i>	+	+	+
n(%)		1(2.1%)	1(100%)	1(100%)	1(100%)
45	90	<i>Enterococcus faecium</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)
46	64	<i>Bacillus spp</i>	+	+	+
n(%)		1(2.1%)	1(100%)	1(100%)	1(100%)
47	63	<i>Streptococcus spp</i>	+	+	+
n(%)		1(2.1%)	1(100%)	1(100%)	1(100%)
total n(%)		47(50%)	10(21.3%)	44(93.6)*	47(100%)*

*(p<0.01)

**CRA:Congo Red Agar assay (positive and intermediate

***TCP:Tissue Culture Plate assay (high , moderate and weak)

In orthodontic (Table 4-6), *icaAD* genes were the best assay for *Klebsiella pneumoniae* (100%) , *Staphylococcus aureus* (100%) , *Enterobacter cloacae* (100%), *Enterococcus faecalis* (100%) , *Staphylococcus epidermidis* (100%) , *Bacillus subtilis* (100%) , *Lactobacillus plantarum* (100%) , *Proteus penneri* (100%) , *Enterococcus faecium* (100%) , *Streptococcus anginosus* (100%) , *Escherichia fergusonii* (100%) , *Proteus mirabilis* (100%) , *Pediococcus acidilactici* (100%) , *Citrobacter freundii* (100%) , *Enterobacter mori* (100%) , *Staphylococcus pasteurii* (100%) , *Staphylococcus wernerii* (100%) , *Enterobacter ludwigii* (100%) , *Staphylococcus Spp.* (100%) , *Streptococcus Spp.* (100%) , than TCP and CRA assays with high significant difference ($p \leq 0.01$), while *icaAD* genes and TCP were the best assay for *Serratia marcescens* (100%,100%) and *Cereus* (100%,100%,) respectively than CRA with high significant ($p \leq 0.01$). All different bacterial species in orthodontic showed that *icaAD* genes were the best assay for detection biofilm formation against to TCP and CRA assays with high significant ($p \leq 0.01$) .

Table (4 - 6):Effectiveness of bacterial species from orthodontic toward slim formation assays

No.	Isolate No.	Bacterial species In orthodontic	(CRA)**	(TCP)***	IcaA and/or icaD genes
1	2	<i>Klebsiella pneumoniae</i>	—	+	+
2	12	<i>Klebsiella pneumoniae</i>	—	—	+
3	19	<i>Klebsiella pneumoniae</i>	—	—	+
4	20	<i>Klebsiella pneumoniae</i>	—	+	+
5	28	<i>Klebsiella pneumoniae</i>	—	—	+
6	36	<i>Klebsiella pneumoniae</i>	—	—	+
7	39	<i>Klebsiella pneumoniae</i>	—	+	+
8	42	<i>Klebsiella pneumoniae</i>	+	—	+
n(%)		8(17%)	1(12.5%)	3(37.5%)	8(100%)
9	9	<i>Staphylococcus aureus</i>	—	—	+
10	14	<i>Staphylococcus aureus</i>	—	—	+
11	15	<i>Staphylococcus aureus</i>	—	—	+
12	22	<i>Staphylococcus aureus</i>	+	—	+
13	29	<i>Staphylococcus aureus</i>	—	—	+
14	38	<i>Staphylococcus aureus</i>	—	+	+
15	45	<i>Staphylococcus aureus</i>	+	—	+
n(%)		7(14.9%)	2(28.6%)	1(14.3%)	7(100%)
16	5	<i>Enterobacter cloacae</i>	—	+	+
17	6	<i>Enterobacter cloacae</i>	—	+	+
18	34	<i>Enterobacter cloacae</i>	—	—	+
n(%)		3(6.3%)	0	2(66.7%)	3(100%)
19	17	<i>Enterococcus faecalis</i>	—	—	+
20	40	<i>Enterococcus faecalis</i>	—	—	+
21	41	<i>Enterococcus faecalis</i>	—	—	+
n(%)		3(6.3%)	0	0	3(100%)
22	1	<i>Staphylococcus epidermidis</i>	—	—	+
23	16	<i>Staphylococcus epidermidis</i>	—	—	+
n(%)		2(4.2%)	0	0	2(100%)
24	13	<i>Bacillus subtilis</i>	—	—	+
25	44	<i>Bacillus subtilis</i>	+	—	+
n(%)		2(4.2%)	1(50%)	0	2(100%)

No.	Isolate No.	Bacterial species In orthodontic	(CRA)**	(TCP)***	IcaA and/or icaD genes
26	24	<i>Lactobacillus plantarum</i>	+	—	+
27	26	<i>Lactobacillus plantarum</i>	—	—	+
n(%)		2(4.2%)	1(50%)	0	2(100%)
28	32	<i>Proteus penneri</i>	—	—	+
29	47	<i>Proteus penneri</i>	+	—	+
n(%)		2(4.2%)	1(50%)	0	2(100%)
30	35	<i>Enterococcus faecium</i>	—	—	+
31	43	<i>Enterococcus faecium</i>	—	—	+
n(%)		2(4.2%)	0	0	2(100%)
32	18	<i>Sterptococcus anginosus</i>	—	+	+
33	37	<i>Sterptococcus anginosus</i>	—	—	+
n(%)		2(4.2%)	0	1(50%)	2(100%)
34	33	<i>Escherichia fergusonii</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)
35	46	<i>Proteus mirabilis</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)
36	30	<i>Pediococcus acidilactici</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)
37	27	<i>Citrobacter freundii</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)
38	25	<i>Enterobacter mori</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)

No.	Isolate No.	Bacterial species In orthodontic	(CRA)**	(TCP)***	IcaA and/or icaD genes
39	23	<i>Bacillus cereus</i>	—	+	+
n(%)		1(2.1%)	0	1(100%)	1(100%)
40	31	<i>Staphylococcus pasteurii</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)
41	11	<i>Staphylococcus worneri</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)
42	8	<i>Serratia marcescens</i>	—	+	+
n(%)		1(2.1%)	0	1(100%)	1(100%)
43	7	<i>Enterobacter ludwigii</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)
44	3	<i>Staphylococcus Spp.</i>	—	—	+
45	4	<i>Staphylococcus Spp.</i>	—	+	+
46	10	<i>Staphylococcus Spp.</i>	—	+	+
n(%)		3(6.3%)	0	2(66.7%)	3(100%)
47	21	<i>Streptococcus Spp.</i>	—	—	+
n(%)		1(2.1%)	0	0	1(100%)
total n(%)		47(50%)	6(12.8%)	11(23.4%)	47(100%)*

*(p≤0.01)

**CRA: Congo Red Agar assay (positive and intermediate)

***TCP: Tissue Culture Plate assay (high , moderate and weak)

4-4-Distribution of the bacterial species between denture and orthodontic devices :-

Nine species were found in both denture and orthodontic sources : *Klebsiella pneumoniae* [8(40%) and 8(40%)] , *Proteus mirabilis* [7(35%) and 1(5%)] , *Proteus penneri* [5(25%) and 2(10%)] , *Enterobacter cloacae* [3(15%) and 3(15%)], *Enterococcus faecalis* [1(5%) and 3(15%)] , *Enterobacter faecium* [1(5%) and 2(10%)], *Bacillus cereus*[1(5%) and 4(20%)], *Enterobacter mori* [1(5%) and (5%)], *Citrobacter freundii* [1(5%) and 1(5%)] respectively. While there were 11 species in denture only : *morganella morganii* [2 (10%)], *Hafnia alvei* [2(10%)], *Enterobacter aerogenes* [2(10%)], *Proteus houserii* [1(5%)], *Acinetobacter baumannii* [1(5%)], *Klebsiella oxytoca*[1(5%)], *Lactococcus lactis* [1(5%)], *Chryseobacterium vietnamense* [1(5%)], *Streptococcus equines* [1(5%)], *Klebsiella variicola* [1(5%)], *Staphylococcus hominis* [1(5%)] . In contrast, there were 11 species in orthodontic only : *Staphylococcus aureus* [7 (35%)], *Staphylococcus epidermidis* [2(10%)], *Bacillus subtilis* [2(10%)], *Lactobacillus plantarum* [2(10%)], *Streptococcus anginosus* [2(10%)], *Escherichia fergusonii* [1(5%)], *Pediococcus acidilactici* [1(5%)], *Staphylococcus pasteurii* [1(5%)], *Staphylococcus warneri* [1(5%)], *Serratia marcescens* [1(5%)], *Enterobacter ludwigii* [1(5%)].As summarized in Figure (4-27).

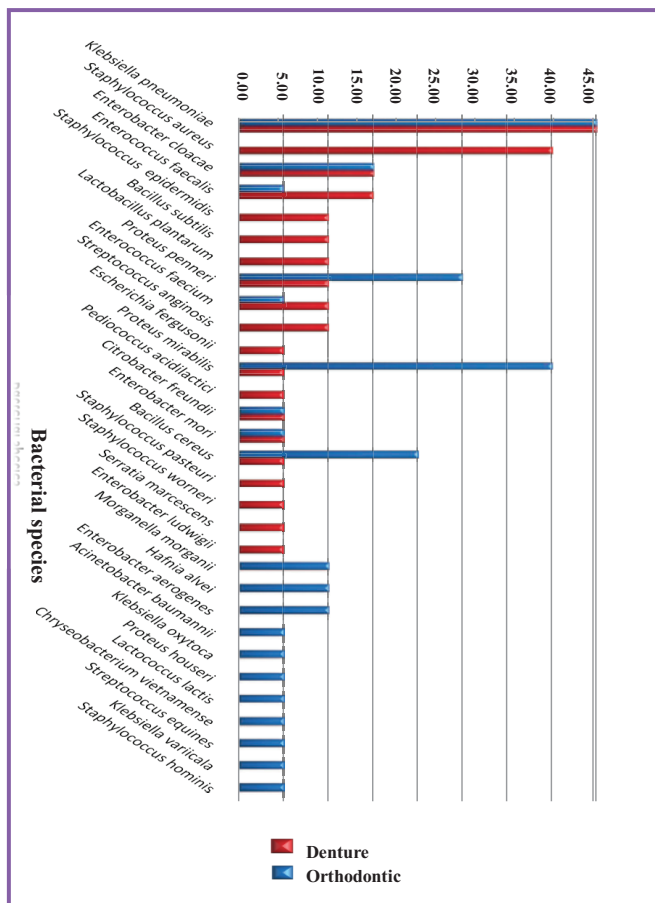


Figure (4-27) percentage of bacterial species in denture and orthodontic

4-5- detection of bacterial species isolates at first time from denture and orthodontic:-

Some bacterial species were detected and recorded from denture and orthodontic at the first time in world these are: *Proteus penneri*, *Enterobacter mori* and *Citrobacter freundii* found in both denture and orthodontic .While *Proteus houser*, *Klebsiella variicola*, *Lactococcus lactis*, *Streptococcus equinus* ,*Acinetobacter baumannii* , *Chryseobacterium vietnamense*, *Klebsiella oxytoca* and *Staphylococcus hominis* found in denture only. But *Staphylococcus pasteurii* , *Enterobacter ludwigii*, *Lactobacillus plantarum*, *Streptococcus anginosus*, *Pediococcus acidilactici* , *Bacillus subtilis*, *Escherichia fergusonii* and *Proteus mirabilis* found in orthodontic only.

4-6-The frequency of bacterial species according to some factors:-

In denture (Table 4-7), the frequency of bacteria was higher ($p \leq 0.01$) with patients aged > 35 (70.1%) denture washing (in day) < 2 time (61.7%), without tonsillitis (100%), without gingivitis (100%), no cigarettes smoking (95.7%) and without dental caries (95.7%), no significant difference with date of denture wearer (year). In orthodontic , (Table 4-8), the frequency of bacteria was higher ($p \leq 0.01$) with patients aged < 18 (78.7%) , orthodontic washing > 2 (68.1%), tonsillitis (100%) , gingivitis (70.2%) , no cigarettes smoking (100%), without dental caries (100%), but there was no significant differences in patients with date of orthodontic insertion factor .

Table (4 -7): frequency of bacterial species from denture according to some factors

Bacterial Isolates from denture n(%)	Age n(%)		Date of denture wearer (year) n(%)		Times of Denture washing (in day) n(%)		Dental caries n(%)		Smoking cigarettes n(%)		Gingivitis n(%)		Tonsillitis n(%)	
	35 < 35		3 < 3		2 < 2		Yes	NO	Yes	NO	Yes	NO	Yes	NO
<i>Klebsiella pneumonia</i> 8(17%)	3 37.5%	5 62.5%	7 87.5%	1 12.5%	6 75%	2 25%	0	8 100%	0	8 100%	0	8 100%	0	8 100%
<i>Proteus mirabilis</i> 7(14.9%)	2 28.6%	5 71.2%	4 57.1%	3 42.9%	6 85.7%	1 14.3%	0	7 100%	0	7 100%	0	7 100%	0	7 100%
<i>Proteus penneri</i> 5(10.6%)	1 20%	4 80%	4 80%	1 20%	3 60%	2 40%	0	5 100%	0	5 100%	0	5 100%	0	5 100%
<i>Bacillus cereus</i> 4(8.5%)	1 25%	3 75%	2 50%	2 50%	0	4 100%	0	4 100%	0	4 100%	0	4 100%	0	4 100%
<i>Enterobacter cloacae</i> 3(6.4%)	1 33.3%	2 66.7%	0	3 100%	1 33.3%	2 66.7%	0	3 100%	0	3 100%	0	3 100%	0	3 100%
<i>Morganella morganii</i> 2(4.3%)	0	2 100%	2 100%	0	1 50%	1 50%	0	2 100%	0	2 100%	0	2 100%	0	2 100%
<i>Hafnia alvei</i> 2(4.3%)	0	2 100%	0	2 100%	2 100%	0	0	2 100%	0	2 100%	0	2 100%	0	2 100%
<i>Enterobacter aerogenes</i> 2(4.3%)	0	2 100%	2 100%	0	1 50%	1 50%	0	2 100%	0	2 100%	0	2 100%	0	2 100%
<i>Acinetobacter baumannii</i> 1(2.1%)	0	1 100%	0	1 100%	1 100%	0	1 100%	0	0	1 100%	0	1 100%	0	1 100%
<i>Klebsiella oxytoca</i> 1(2.1%)	0	1 100%	0	1 100%	1 100%	0	0	1 100%	1 100%	0	0	1 100%	0	1 100%
<i>Proteus houleri</i> 1(2.1%)	1 100%	0	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%
<i>Lactococcus lactis</i> 1(2.1%)	0	1 100%	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%
<i>Enterococcus faecalis</i> 1(2.1%)	1 100%	0	1 100%	0	0	1 100%	1 100%		0	1 100%	0	1 100%	0	1 100%
<i>Staphylococcus hominis</i> 1(2.1%)	0	1 100%	0	1 100%	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%
<i>Chryseobacterium vietnamense</i> 1(2.1%)	0	1 100%	0	1 100%	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%
<i>Streptococcus equinus</i> 1(2.1%)	1 100%	0	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%
<i>Enterobacter mori</i> 1(2.1%)	1 100%	0	1 100%	0	0	1 100%	0	1 100%	1 100%	0	0	1 100%	0	1 100%

Bacterial Isolates from denture n(%)	Age n(%)		Date of denture wearer (year) n(%)		Times of Denture washing (In day) n(%)		Dental caries n(%)		Smoking cigarettes n(%)		Gingivitis n(%)		Tonsillitis n(%)	
	35 < 35		3 < 3		2 < 2		Yes	NO	Yes	NO	Yes	NO	Yes	NO
<i>Klebsiella varicola</i> 1(2.1%)	1	0	1	0	1	0	0	1	0	1	0	1	0	1
	100%		100%		100%			100%		100%		100%		100%
<i>Staphylococcus hominis</i> 1(2.1%)	0	1	0	1	1	0	0	1	0	1	0	1	0	1
		100%		100%	100%			100%		100%		100%		100%
<i>Citrobacter freundii</i> 1(2.1%)	0	1	0	1	1	0	0	1	0	1	0	1	0	1
		100%		100%	100%			100%		100%		100%		100%
<i>Bacillus spp.</i> 1(2.1%)	0	1	0	1	1	0	0	1	0	1	0	1	0	1
		100%		100%	100%			100%		100%		100%		100%
<i>Streptococcus spp.</i>	1	0	0	1	1	0	0	1	0	1	0	1	0	1
	100%			100%	100%			100%		100%		100%		100%
Total	14	33*	27	20	29**	18	2	45*	2	45*	0	47*	0	47*
	29.9%	70.1%	57.4%	42.6%	61.7%	38.3%	4.3%	95.7%	4.3%	95.7%		100%		100%

*p≤0.01

**p≤0.05

Table (4 -8): frequency of bacterial species from orthodontic according to some factors

Bacterial Isolates from denture n(%)	Age n(%)		Date of orthodontic wearer (year) n(%)		Times of orthodontic washing (in day) n(%)		Dental caries n(%)		Smoking cigarettes n(%)		Gingivitis n(%)		Tonsillitis n(%)	
	18 < 18		1.5 < 1.5		2 < 2		Yes	NO	Yes	NO	Yes	NO	Yes	NO
<i>Klebsiella pneumonia</i> 8(17%)	5 62.5%	3 37.5%	4 50%	4 50%	3 37.5%	5 62.5%	0	100%	0	8 100%	6 75%	2 25%	0	8 100%
<i>Staphylococcus aureus</i> 7(14.9%)	6 85.7%	1 14.3%	4 57.1%	3 42.9%	2 28.5%	5 71.4%	0	7 100%	0	7 100%	4 57.1%	3 42.9%	0	7 100%
<i>Enterobacter cloacae</i> 3(6.3%)	2 66.7%	1 33.3%	3 100%	0	33.3%	2 66.6%	0	3 100%	0	3 100%	2 66.7%	1 33.3%	0	3 100%
<i>Enterococcus faecalis</i> 3(6.3%)	2 66.7%	1 33.3%	0	3 100%	0	3 100%	0	3 100%	0	3 100%	3 100%	0	0	3 100%
<i>Staphylococcus epidermidis</i> 2(4.2%)	2 100%	0	0	2 100%	1 50%	1 50%	0	2 100%	0	2 100%	2 100%	0	0	2 100%
<i>Bacillus subtilis</i> 2(4.2%)	2 100%	0	0	2 100%	2 100%	0	0	2 100%	0	2 100%	2 100%	0	0	2 100%
<i>Lactobacillus plantarum</i> 2(4.2%)	2 100%	0	2 100%	0	0	2 100%	0	2 100%	0	2 100%	1 50%	1 50%	0	2 100%
<i>Proteus penneri</i> 2(4.2%)	2 100%	0	2 100%	0	1 50%	1 50%	0	2 100%	0	2 100%	0	2 100%	0	2 100%
<i>Enterococcus faecium</i> 2(4.2%)	2 100%	0	1 50%	1 50%	1 50%	1 50%	0	2 100%	0	2 100%	2 100%	0	0	2 100%
<i>Streptococcus anginosus</i> 2(4.2%)	2 100%	0	1 50%	1 50%	0	2 100%	0	2 100%	0	2 100%	1 50%	1 50%	0	2 100%
<i>Enterococcus faecium</i> 1(2.1%)	1 100%	0	1 100%	0	0	1 100%	0	1 100%	0	1 100%	1 100%	0	0	1 100%
<i>Proteus mirabilis</i> 1(2.1%)	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%	1 100%	0	0	1 100%
<i>Pediococcus acidilactici</i> 1(2.1%)	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%	1 100%	0	0	1 100%
<i>Citrobacter freundii</i> 1(2.1%)	1 100%	0	1 100%	0	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%
<i>Enterobacter mori</i> 1(2.1%)	1 100%	0	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%
<i>Bacillus cereus</i> 1(2.1%)	1 100%	0	0	1 100%	1 100%	0	0	1 100%	0	1 100%	1 100%	0	0	1 100%
<i>Staphylococcus pasteurii</i> 1(2.1%)	0	1 100%	0	1 100%	1 100%	0	0	1 100%	0	1 100%	1 100%	0	0	1 100%
<i>Staphylococcus warneri</i> 1(2.1%)	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%

Bacterial Isolates from denture n(%)	Age n(%)		Date of orthodontic wearer (year) n(%)		Times of orthodontic washing (in day) n(%)		Dental caries n(%)		Smoking cigarettes n(%)		Gingivitis n(%)		Tonsillitis n(%)	
	18 < 18		1.5 < 1.5		2 < 2		Yes	NO	Yes	NO	Yes	NO	Yes	NO
<i>Serratia marcescens</i> 1(2.1%)	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%	1 100%	0	0	1 100%
<i>Enterobacter ludwigii</i> 1(2.1%)	1 100%	0	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%
<i>Staphylococcus Spp.</i> 3(6.4%)	2 66.7%	1 33.3%	2 66.7%	1 33.3%	1 33.3%	2 66.7%	0	3 100%	0	3 100%	3 100%	0	0	3 100%
<i>Streptococcus Spp.</i> 1(2.1%)	1 100%	0	0	1 100%	0	1 100%	0	1 100%	0	1 100%	1 100%	0	0	1 100%
Total	37* 78.7%	10 21.3%	23 48.9%	24 51.1%	15 31.9%	32* 68.1	0	47 100%	0	47 100%	33* 70.2%	14 29.8	0	47 100%

*p≤0.01

Chapter Five

Discussion

5-1-primer for *16SrDNA* gene:-

In the present study, DNA of all bacterial isolates from denture (n=47) and orthodontic (n=47) were extracted and electrophoreses (Figure 4-1), then subjected to PCR for amplifying their *16S rDNA* gene with universal primers (F27 and R1492). Since, the primer amplify the *16SrDNA* (16S ribosomal RNA) gene for all bacteria species preventing to lose any possible or new species (Mellmann *et al.*, 2006), as Figures (4-2 to 4-11). The use of *16SrDNA* gene to study bacterial phylogeny and taxonomy has been the most common genetic marker because : Its presence in almost all bacteria, often existing as a multigene family, or operon , the function of the *16SrDNA* gene over time has not changed, suggesting that random sequence changes are more accurate measure of time (evolution) and the *16SrDNA* gene (1,500 bp) is large enough for informatics purposes (Patel, 2001 ;Janda and Abbott, 2007).

5-2-Sequencing of *16SrDNA* gene and phylogenetic tree:-

The amplified *16SrDNA* gene was purified , it was important to obtain a clear single band and because the unpurified gene will cause problems during sequencing (Barker, 2006). Sequencing of the *16SrDNA* gene has served as an important tool for identifying and determining phylogenetic relationships between bacterial species from dentures and orthodontic (figure 4-12) .Since features of this molecular target (*16SrDNA* gene sequencing) that make it useful for phylogenetic tool , also make it useful for bacterial detection and identification in the clinical laboratory, sequence analysis of the *16SrDNA* gene is a powerful mechanism for identifying new

pathogens in patients with suspected bacterial disease and more recently this technology is being applied in the clinical laboratories for routine identification of bacterial isolates, on the other hand, several studies have shown that sequence identification is useful for slow-growing, unusual, and fastidious bacteria as well as for bacteria that are poorly differentiated by conventional methods (Patel, 2001).

Furthermore, Conventional biochemical tests and commercial identification system as well as phenotypic variants are not included in the level of subspecies and often miss identified (Seifert *et al.*, 2003). In contrast, the high quality of *16SrDNA* sequence database provides excellent identification at the species and subspecies levels; moreover, it can lead to the recognition of novel pathogens and non cultured bacteria (Clarridge, 2004; Mellmann *et al.*, 2006). Nucleotide sequences of the *16SrDNA* gene of the 28 different species identified in this study with their reference strains from Gene were concatenated in at least length 898bp depending on the shorter sequence exhibited when aligned using CLASTALW "[http://www .ebi. ac.uk/clustalw/](http://www.ebi.ac.uk/clustalw/)" (Kerbaux *et al.*, 2011), then compared phylogenetically based on phylogenetic tree analysis. the phylogenetic tree in the present study rooted with *Enterobacter cloacae* as the out group, conserved with its genetic characteristics (Barker, 2006).

5-3- The new recording of bacterial strain from denture and orthodontic:-

The present study recorded four new bacterial strains in orthodontic [7- *Enterobacter ludwigii* (HG003646)"IRQBAS1" , 34- *Enterobacter cloacae*

(HG003647)"IRQBAS2"] and in denture [71-*Chryseobacterium vietnamense* (HG003648) "IRQBAS3" , 74-*Morganella morganii* (HG003649)"IRQBAS4"] as in Figures (4-13 to 4-22) .These strains were identical with each their reference strains at 99% (1% difference with reference strain for each) because the occurrence of mutation changing nucleotide sequence of genome of an organism, mutations are caused by either unrepaired damage to DNA or to RNA genomes (typically caused by radiation or chemical mutagens),errors in the process of replication or insertion or deletion of segments of DNA by mobile genetic elements (Bertram , 2000 ; Burrus and Waldor , 2004 ; Aminetzach *et al* ., 2005) , these mutations are : point mutation often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another , these changes are classified as 1- transitions or 2-transversions , most common is the transition that exchanges a purine to a purine (A ↔ G) or a pyrimidine to a pyrimidine, (C ↔ T) ,less common is a transversion, which exchanges a purine to a pyrimidine or a pyrimidine to a purine (C/T ↔ A/G) and 3-frame shift mutation which is caused by insertion or deletion of a number of nucleotides, the insertion or deletion can disrupt the reading frame, or the grouping of the codons, resulting in a completely different translation from the original (Dunnen , 2000; Ernst, 1959). Since, according to some guidelines, a range of about a 0.5% to 1% difference (99.5 to 99% similarity) is often used for classification (Song *et al.*, 2003). Bosshard *et al.* (2003) used ≥ 99% similarity to define species and ≥ 95 to < 99% to define a genus whereas, Hall *et al.* (2003) adopted a distance score of 0.00 to less than 1% as the criterion for species identity. While, Tang *et al.* (1998, 2000), suggested a 0.5% difference as the limit for species designation. Furthermore, a strain with a small genotypic difference (less than 0.5%) has been considered as subspecies (Chen *et al.*, 2002) . When there is a clear phenotypic

uniqueness, genogroups with less than 1% differences in sequence have in fact been named as a new species (Kattar *et al.*, 2001; Roth *et al.*, 2003; Tortoli, 2003). However, a comparison of sequences for several subspecies shows differences from 1 to 14 bp (Clarridge , 2004). bacterial isolates at the first time in the world may be due to a change in the Iraqi environment or may be due to the quality of feed person.

5-4- Detection of biofilm formation:-

Biofilm formation (slime production) plays an important role in the pathogenesis of infections caused by different microorganisms (Hall-Stoodley *et al.*, 2004 ; Aparna *et al.*, 2008). The major virulence factor is that they can form biofilm on polymeric surfaces and adherence to catheters and other artificial materials during the early phase of biofilm development (Chaieb *et al.* , 2005) . Further more, investigations to understand the pathogenesis of these infections have focused upon the process of adherence of these microorganisms on biomedical devices, using various methods of quantify number of microorganisms adhering to surfaces (Donlan , 2001; Donlan *et al.*, 2001) .

Tested 94 clinical isolates n=47 from denture and n=47 from orthodontic by three *in vitro* screening procedures for their ability to form biofilm ,these are Congo Red Agar (CRA), Tissue Cultur Plate (TCP), *icaAD* genes methods in denture and orthodontic . Congo Red Agar was simple and reliable to determine whether an isolate has the potential for biofilm production or not (Jain and Agarwal, 2009). Tissue Cultur Plate has been determined according to well-established protocols

and can be modified for various biofilm formation assays, this test is fast, efficient, reliable, and reproducible method and it gives a quantitative result (Djordjevic *et al.*, 2002; Tumbarello *et al.*, 2007). Other investigators also reported PCR to be an important tool for the identification of *ica* genes since the technique is simple, rapid and reliable and only requires minimal amounts of DNA (Cafiso *et al.*, 2004 ; Arciola *et al.*, 2001). PCR was used in this study as a reference for the phenotypic method based on several studies (Cafiso *et al.*, 2004 ; Arciola *et al.*, 2001; Gad *et al.*, 2009) that demonstrated the efficiency of this technique in detecting the genes of the *ica* operon.

By Congo red agar method we obtained very different results as Table (4-2) and Figure (4-23) most of bacterial isolates displayed in denture and orthodontic positive [8(17.02%) and 4(8.5%)] intermediate [2(4.3%) and 2(4.3%)] and negative isolates [37(78.7%) and 41(87.2%)] ,based on the observations in the present results don't recommend the CRA method as a suitable method for detection of biofilm formation, these results are in agreement with Knobloch *et al.*, (2002). By tissue culture plate most of bacterial isolates as Table (4-3) and Figure (4-24) displayed in denture and orthodontic positive [8(17%) and 2(43%)] , moderate [7(14.9%) and zero], weak [29(61.7%) and 9(19.2%)] and negative [3(6.4%) and 36(76.6%)]. In denture results correlate well with those reported by Gökçe *et al.*, (2007) but in orthodontic results are in disagreement with Gökçe *et al.*, (2007) but they are in agreement with Oliveira and Cunha, (2010) demonstrated the tube adherence test can be indicated for the routine detection of biofilm production in CNS, because the strongly adherent is very weak when comparison TCP results assay with *icaA* and *icaD* results assay . The relationship between strongly adherent for bacterial isolates and the concomitant presence of *icaA*, and *icaD* detected by PCR , because of the slime production

increase depended on co-expression of *icaA* with *icaD* gene (Cafiso *et al.*, 2004) ,in denture no significant difference between *icaA* and *icaD* gene positive but in orthodontic high significant difference between *icaA* and *icaD* gene positive as Table (4-4),this confirming the results in orthodontic and to determine the reliability of the TCP method in terms of the quantification of biofilm production.

5-5-The effectiveness of different bacterial species toward CRA, TCP and *icaAD* genes assays:-

The results showed CRA assay as Table (4-5 and 4-6) very little correlation [10 (21.3%) and 6 (12.8%) respectively] with corresponding methods *icaAD* genes [47(100%) and 47(100%) respectively], these results are in agreement with Arciola *et al.*, (2002); Cafiso *et al.*, (2004); Arciola *et al.*, (2005) when Compared between the CRA test results and PCR (*icaAD* genes) showed only [10(21.3%),6(12.8%)] isolates respectively in denture and orthodontic were positive results and revealed 37(78.7%) isolates in denture and 41(87.2%) isolates in orthodontic were forming bordeaux colonies (negative results) toward CRA, while they tested positive for the concomitant presence of the *icaAD* genes, with these results, isolates by CRA being false-negative when compared to PCR , furthermore , these isolates contained complete *IcaA* and/or *icaD* genes .On the other hand, the present study showed in denture with TCP results [44(93.6%)] high significant differences than CRA results [10(21.3%)] , the TCP method was found to be most sensitive, accurate and reproducible screening method for the detection of biofilm formation. However these results were in agreement with Mathur *et al.*, (2006) .No significant difference between *icaAD* gene and TCP results [47 (100%) and 44(93.6%) respectively] ,

Similar results have been reported by Arciola *et al.*, (2006). However, in orthodontic, no significant differences were found between CRA results [6(12.8%)] TCP results [11(23.4%)] , while high significant differences were found between *icaAD* gene results [47(100%)] and TCP results , these results were in agreement with Oliveira and Cunha, (2010), could be due to the fact that denture is made from polymethylmethacrylate resin (plastic) and the rough surface not only permit the bacteria attach to the surface but also to penetrate deep into the porous denture or appliance lead to be adhesion stronger than orthodontic . Based on the present results, the study is unable to recommend the CRA method for the detection of biofilm formation, in contrast, probably to recommend TCP method for detection of biofilm formation. But full accreditation on the *icaAD* genes method for detection of biofilm formation in denture and orthodontic bacterial species . Slime production depends on the presence of both/or *icaD* and *icaA*. Nevertheless, the reason for the absence of biofilm production in some *icaA* and *icaD* positive isolates in the present study may be the lack of *icaC* (Ziebuhr *et al.*, 1999). In other evidences, the biofilm accumulation is mediated by certain genes, such as *icaA*, *icaB*, *icaC*, *icaD* and *icaR*(Begun *et al.*, 2007; O’Gara *et al.*, 2007). The recent findings point to an important role of the *icaA* and *icaD* due to their ability to produce slime strongly in a high percentage of clinical isolates collected from patients with catheters associated infection (El-Mahallawy *et al.*, 2009) .

5-6-Type of bacteria have the ability to produce slime:-

The purpose of this study was to observe the ability of bacteria to produce slime and formation of biofilm, an important virulence factor, by bacterial isolates, among the

bacterial isolates in present study produce slime and form biofilm confirm this all bacterial isolates in present study when conducted tests *icaAD* gene the result are [47(100%) and 47(100%)] as Figure (4-5 and 4-6) . The results showed that these bacteria produce slime and forming biofilm in diverse environments from aquatic conditions to indwelling devices "vascular access ports/hemasites, scleral buckles, ureteral stents, urethral catheters and tracheoesophageal voice prostheses (Provox2)" , furthermore, these bacteria can produce biofilms on nonliving surfaces including polystyrene, glass, latex and silicone and on biological surfaces (Reed *et al.*, 1986 ; Reid *et al.*, 1992 ; Inbakandan *et al.*, 2010 ; Tićac *et al.*, 2010). These bacteria are: *Klebsiella pneumonia* in agreement with (Maki and Tambyah, 2001; Stewart and Costerton, 2001; Donlan and Costerton, 2002) , *Staphylococcus aureus* in agreement with (Leid *et al.*, 2002 ; Patel, 2005) , *Proteus mirabilis* in agreement with (Stickler *et al.*, 1993), *Proteus penneri* in agreement with (Rózalski *et al.*, 2007) , *Enterococcus faecalis* in agreement with (Baldassarri *et al.*, 2001 ; Sandoe *et al.*, 2003) , *Enterobacter cloacae* in agreement with (Kim *et al.*, 2012), *Enterococcus faecium* in agreement with (Van Wamel *et al.*, 2007) , *Bacillus cereus* in agreement with (Peng *et al.*, 2001), *Citrobacter freundii* in agreement with (Pereira *et al.*, 2010), *morganella morganii* in agreement with (Zubair *et al.*, 2011) , *Hafnia alvei* in agreement with (Vivas *et al.*, 2008), *Enterobacter aerogenes* in agreement with (Donlan & Costerton, 2002) , *Acinetobacter baumannii* in agreement with (Gaddy and Actis, 2009) , *Klebsiella oxytoca* in agreement with (Zubair *et al.*, 2011), *Lactococcus lactis* in agreement with (Zaidi *et al.*, 2011) , *Staphylococcus hominis* in agreement with (Krolasik *et al.*, 2010), *Staphylococcus epidermidis* in agreement with (Fey and Olson, 2010) , *Bacillus subtilis* in agreement with (Hamon and Lazazzera, 2001) , *Lactobacillus plantarum* in agreement with (Kubota *et al.*, 2008), *Streptococcus*

anginosus in agreement with (Petersen *et al.*, 2006) , *Escherichia fergusonii* in agreement with (Ingle *et al.*, 2011), *Pediococcus acidilactici* in agreement with (Kawarai *et al.*, 2007) , *Staphylococcus pasteurii* and *staphylococcus worneri* in agreement with (Marino *et al.*, 2011) and *Serratia marcescens* in agreement with (Rice *et al.*, 2005). *Enerobacter ludwigii* , *Klebsiella variicala*, *Chryseobacterium vietnamense* and *Enterobacter mori* are new species (Hoffmann *et al.*, 2005 ; Rosenblueth *et al.*, 2004 ; Li and Zhu , 2012 ; Zhu *et al.*, 2011). On the other hands information and research for *streptococcus equines* and *Proteus houserii* are very few, furthermore, in the present study these bacteria were tested on containment adherence gene (*icaAD* gene) this confirms these bacteria produces slime and formed biofilm .

5-7-Distribution of the bacterial species between denture and orthodontic :-

The presence of a denture or orthodontic in the oral mucosa alters the local environmental conditions to lead increase of bacteria formed biofilm causing infection and systematic disease (Hagg *et al.*, 2004 ; Daniluk *et al.*, 2006) . Dentures offer a reservoir for microorganisms associated with endocarditis, aspiration pneumonia, gastrointestinal infection and chronic obstructive pulmonary disease (Li *et al.*, 2000 ; Falah-Tafti *et al.*, 2008). Orthodontic treatment with fixed appliances leads to increase biofilm accumulation and elevated levels of cariogenic and periodontal bacteria ,furthermore, because orthodontic brackets make good oral hygiene difficult, resulting plaque accumulation and significantly increase risks of enamel demineralization or periodontal disease (Papaioannou *et al.*, 2007 ; Pandis *et*

al., 2008 ; Pellegrini *et al.*, 2009). In the present study there are 31(Three identified morphologically) species were isolated from denture and /or orthodontic.

5-8-Species isolated from denture and orthodontic:-

Klebsiella pneumonia isolate in both denture and orthodontic which is in agreement with (Goldberg *et al.*, 1997; Naranjo *et al.*, 2006). *klebsiellae* have become important pathogens in nosocomial infections can cause the *Klebsiella pneumonia* disease, human lungs inflammation and necrosis that sometimes produces a thick, bloody, mucoid sputum as currant jelly sputum (Chien-Ko *et al.*, 2002) *Klebsiella pneumonia* infections are mostly seen in people with a weakened immune system, this patient is believed to have impaired respiratory host defenses including persons with, liver disease, alcoholism, malignancy, diabetes , Chronic obstructive pulmonary diseases ,glucocorticoid therapy, renal failure and certain occupational exposures, many of these infections are obtained when a person is in the hospital for some other disease (Podschun *et al .*, 1998; Kabra *et al.*, 2001) common infection caused by *Klebsiella* bacteria outside the hospital is pneumonia (Schwaber and Carmeli , 2008).

Proteus mirabilis was isolated from which denture agreements with Goldberg *et al* 1997, Sumi *et al* (2002), Jass *et al* (2003), Tyrell *et al* (2003), Senpuku *et al* (2003). *Proteus mirabilis* infection can spread to other parts of the body (Coker *et al.*, 2000) and *Proteus penneri* isolate from stool (O'Hara *et al.*,2000 a and b). Furthermore ,these bacteria formed biofilm (Coker *et al.*, 2000; Rózalski *et al.*, 2007), could be entire through the speaking of person wearer denture or insertion

orthodontic Providing a favorable environment for the growth of bacteria , or oral fecal contamination .Isolated in orthodontic at the first time.

Enterococcus faecalis has been frequently found in root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases (Molander *et al.*,1998), resulting biofilm formation (Singh *et al.*, 2007) , perhaps that's causes may be done when the persons sick with these disease at the same time of those persons wearer these devices leading to transfer this bacteria and formed biofilm and adhesive.

Enterococcus faecium found in the oral cavity (Donelli *et al.*, 2004). Furthermore, the virulence factor of *E. faecium* is the enterococcal surface protein (Esp) , this protein allows the bacteria to aggregate and form biofilms (Willem *et al.*, 2007) , which may allow the bacteria to colonies the devices .

Bacillus cereus can spread easily to many types of foods such as plants, eggs, meat, and dairy products causing periodontal diseases and other more serious infections , furthermore, forming biofilm on various surfaces (Hoffmaster *et al.*, 2006; Oosthuizen *et al.*, 2002), all of these reasons confirm the adhesion of this bacteria in people with denture wearer or orthodontic insertion ,or the other way, may be the person suffers from periodontal diseases when this bacteria is found before insertion the devices ,after insertion, this bacteria formed biofilm and adhere on these devices .

Enterobacter mori is plant-pathogenic enterobacterium responsible for the bacterial wilt of mulberry plants (Zhu *et al.*, 2011). May be for this reasons, the

above plant, is the source of its existence in mouth . However, because this bacteria is new species there isn't any research confirms that the *Enterobacter mori* has the ability to form biofilm because of the lack of research dealing with this bacteria ,but only Zogaj *et al.*, (2003) says that genus *Enterobacter* spp. forming biofilm. On the other hands , the pre study recovered *Enterobacter mori* contain *icaAD* genes with rate 100% in denture and orthodontic offering a favorable circumstance for adhesion. This bacteria is recorded that isolated in denture and orthodontic at the first time.

Citrobacter freundii isolated from denture and orthodontic was in agreement with Daniluk *et al* (2006) when isolated it from denture. However , this bacteria habitat include the environment (soil, water, sewage), food and forming biofilm (Wang *et al.*, 2000; Pereira *et al.*, 2010).Therefore, it could entire into the mouth and adhesion on to orthodontic. the first recorded in orthodontic.

Enterobacter cloacae was isolated in both from denture and orthodontic , this results is in agreement with Naranjo *et al* (2006) and Daniluk *et al* (2006).Moreover, this strain is recovered at the first time in denture named "IRQBAS2" with ID number "HG003647".

5-9-species isolated from denture only :-

morganella morganii was isolated from denture which in agreement with Daniluk *et al* (2006).Moreover, this strain the first recovered in present study named "IRQBAS4" with number ID "HG003649"

Enterobacter aerogenes isolated from denture as recorded previously by Gomaa and Helal (2010).

Hafnia alvei causing skin infection, soft tissue infections such as sputum ,tracheal, bronchial aspirates, nasal smears, bronchoalveolar lavage fluid, mouth and throat infection (Janda *et al* .,2006) .most of these infections could occur when *Hafnia alvei* is coming from denture.

Proteus houserii found in manure, soil, polluted water, and in intestines of human and wide variety of animals (Garrity *et al.*, 2005), *Acinetobacter baumannii* isolated from soil and water samples in the environment (Yeom, *et al.*, 2013 a and b). Furthermore, isolat *A. baumannii* among service members of the Iraq and Afghanistan military operations: Operation Iraqi Freedom and Operation Enduring Freedom, respectively (Hujer *et al.*, 2006). These sources can play a role to export the bacteria to human mouth or oral fecal contamination .Isolates of these bacteria adhere in denture at the first time in the present study.

Klebsiella oxytoca can be found in a wide range of environments and this specie tends to colonize along the mucosa membranes of the colon and nasopharynx, urine and skin, however, they can colonize on all parts of the body (Ménard *et al* ., 2010). This bacteria isolated from orthodontic (Naranjo *et al.*, 2006) .Recorded adhere this bacteria to denture at the first time ,may be found in mouth and adhere to denture or transfer from nasopharynx to mouth for adhering.

Lactococcus lactis is widely used for industrial production of fermented dairy products such as milk, cheese, and yogurt (Tanous *et al.*, 2007). Therefore, these sources are good origin for this bacteria to transfer to adhere the denture and recorded this bacteria at the first time adhere to denture.

Chryseobacterium vietnamense strain GIMN1.005T isolated from a forest soil sample in Vietnam at first time in 2011 (Li and Zhu, 2011). However, from that time, this bacteria contain only one strain. But in the present study, an isolate was recovered from denture at the first time as new strain named "IRQBAS3" with ID number "HG003648".

Streptococcus equines was isolated from cow, human and horses (Hodge *et al.*, 1937; Hagan, 1988). May be source infected is oral fecal contamination. But this is the first isolation from the denture.

Klebsiella variicola was isolated in Mexico (2004) is a new species from plants (rice, maize, sugar cane and banana) and hospitals (Rosenblueth *et al.*, 2004) these sources could be the origin to isolate this bacteria at the first time from mouth adhere on denture

Staphylococcus hominis is commonly as a harmless commensal on human and animal skin (Kloos and Schleifer, 1975), may be this bacteria is transfer from skin into mouth and adhere. However, no previous study confirms the presence of this bacteria in denture.

5-10-Species isolated from orthodontic only:-

Staphylococcus aureus was isolated from orthodontic as with Al Groosh *et al.*, (2011) and Soomro *et al.*, (2012). *Staphylococcus epidermidis* was isolated from orthodontic as with Soomro *et al.*, (2012) . *Streptococcus anginosus* cultures have been taken from the mouth, throat and poor oral hygiene (Ruoff *et al.*, 1988 ; Yilmaz *et al.* , 2012). *Serratia marcescens* isolated from denture as the of result with Naranjo *et al.*, (2006).

Bacillus subtilis can contaminate food (Perez, 2000). *Lactobacillus plantarum* and *Pediococcus acidilactici* founds in dairy, meat, and much vegetable fermentations, (Barros *et al.*, 2001 ; De Vries *et al.*, 2006). However , these bacteria may be entire with food through the eating into the gap between the enamel surface and device and adhere. isolated these bacteria at first time in orthodontic.

Escherichia fergusonii is the infected open wounds in humans and may also cause bacteraemia or urinary tract infections (Mahapatra *et al.*,2005). As a first isolated from orthodontic, this bacteria may be cross-contamination from urinary tract infected into hand through urine and transfer into the mouth in gap between enamel and orthodontic through eating .

Staphylococcus pasteurii ,a poor data of the literature about epidemiology and natural habitat of *S. pasteurii*, and no systematic environmental surveys on this species have been published, five among the seven strains were from human

specimens (vomit, urine, and blood), while two of them were collected from mixed vegetables and goat's milk. Anyway, natural habitat of the organism actually remained uncertain (Bjorland *et al.*, 2005). This bacteria isolated at the first time in mouth ,may be found this bacteria in the vegetables and goat's milk are good origin to transefer and adhere into orthodontic

Staphylococcus worneri is found in the mouth (Ohara-Nemoto *et al.*, 2008) and they are able to produce biofilms on the surface of various materials, some of them of medical importance,(Gotz, 2002) .Therefore, all of these reasons confirm to adhere in orthodontic .

Enerobacter ludwigii a new species,(Hoffmann *et al.*, 2005). information on these bacteria are very few. Therefore, in the present study the presence of this bacteria adhere on the orthodontic the first recovered as a new strain named "IRQBAS1" with ID number "HG003648" .

The results showed the presence of some bacterial in denture and orthodontic ,on the other hands, some of these bacterial isolates are found only in denture and verse versa. Furthermore, since the presence of orthodontic or denture is to treat the persons' alteration of local environment, these devices provide favorable condition due to the inaccessibility of saliva and lack of mechanical cleaning by the tongue (Daniluk *et al.*, 2006). Hence, these devices act as reservoirs that harbor a mixed species of bacterial biofilm (biofilms on dental hard and soft tissues) following a variety of these organisms can cause of a potential respiratory infection and other

systematic diseases because the denture is made of acrylic resin ,acrylic denture is one of the main clinical problems (Ramage *et al* ., 2004). Surface deterioration of resin composites has been demonstrated by increased roughness, effects on filler particle exposure, and sometimes by a decreased microhardness of the materials upon exposure to biofilms *in vitro* (Beyth *et al.*, 2008). Moreover , orthodontic appliances severely hamper the efficacy of toothbrushing (Schatzle *et al.*, 2010), reducing the self-clearance by saliva (Ogaard *et al.*, 2008) , changing the composition of the oral flora (Badawi *et al.*, 2003) , increasing the amount of oral biofilm formed (Hagg *et al.*, 2004) ,colonizing of oral surfaces by cariogenic(Al Mulla *et al.*, 2009) and periodontopathogenic bacteria(Naranjo *et al.*, 2006) . Orthodontic composed of (A): adhesive; (B): bracket; (C): ligating with elastomeric ring; (D): ligating with steel ligature; (E): self-ligating bracket with the clip open; (F): self-ligating bracket with a closed clip; (G): arch wire; and (H): bonded retainer (Gameiro *et al.*, 2009 ; Pellegrini *et al.*, 2009 ;Van der Veen *et al.*, 2010). Adhesives, Composite resins for orthodontic bonding are in direct contact with the vulnerable enamel surface and their properties with respect to bacterial adhesion may be more important than other orthodontic materials, in general, excessive composite resin at the bracket-enamel-adhesive junction is prone to bacterial adhesion, especially since polymerization shrinkage may yield a gap with a width of up to 10 µm at the adhesive-enamel interface where bacteria find themselves protected against oral cleaning forces and antibacterial components of toothpastes and mouth rinses (Sukontapatipark *et al.*, 2001).

However , since in denture and orthodontic adhesive part made from the same article (Resin) and this article presence in mouth appropriate environment to form biofilm and adhesion of bacteria , on the other hand, this confirms the presence of

some bacterial species in denture such as *Staphylococcus aureus* , *Staphylococcus epidermidis* , *Bacillus subtilis* , *Streptococcus anginosus* (Budtz-Jorgensen *et al.*, 1983) ,in the present study this bacteria were isolated from orthodontic, but *Klebsiella oxytoca* isolated from denture other studies isolates from orthodontic (Naranjo *et al.*, 2006). However , this study confirms that too bacterial species which were isolated from these devices at the same time ,but these may depend on adhesion strength of bacterial species on these devices or may depend on oral hygiene or coincided with the presence of these bacteria with another mouth or systematic disease increases the presence of these bacteria .

5-11-Frequency of bacterial species from denture and orthodontic according to some factors:-

The results in this study showed that some factors are closely related with people who wear denture and the presence of some species .However, observed in denture (Table 4-7), that the presence of bacteria in individuals aged 35> (elderly people) more than the aged < 35, this relationship between elderly person with denture and bacteria ,due to the decrease in immune function associated with ageing (Tada *et al.*, 2006), Furthermore, Saltzman and Peterson, (1987) reported that changes in the oral bacterial flora occur in individuals aged >70 years, leading to opportunistic infections associated with a decrease in immune function .On the other hands , there was a statistically significant relationship between bacterial presence and lack denture with cleanliness, this result is in agreement with De Visschere *et al.*, (2006) and no statistically significant relationship was between bacterial presence and date denture wearer , dental caries, gingivitis ,tonsillitis and smoking cigarettes in the present

study may be do to sample collected from persons without severing of this disease and no cigarettes smoking, Furthermore, the presence of bacteria in denture not depending on date of denture wearer.

In orthodontic the results showed the presence of bacteria with adolescent aged <18 more than >18 (Table 4-8) , Furthermore , statistically significant relationship was between the increase bacterial on orthodontic and gingivitis , on the other hands , no significant difference in date of orthodontic insertion , this result is in agreement with Ristic *et al.*, (2007). However statistically significant relationship between the increase of bacteria with time of orthodontic washing in day , could be due to the method of brushing teeth is incorrect leading to increase bacterial species in mouth. No statistically significant relationship was found between increase bacteria with dental caries ,tonsillitis and cigarettes smoking, because all sample were collected tack from non- smoking adolescent and without severing of this disease.

Conclusions and Recommendations

6-1-Conclusions:-

- Identification of bacterial species in denture and/or orthodontic which not found in previous studies of these two sources.
- Identification of bacterial species in dentures which is not found in orthodontic and the vice versa is right.
- Identification of new four bacterial strains as a first recording internationally with European nucleotide archive (ENA).
- The study showed that the best test to determine the ability of adherence is the genetic test.
- *Klebsiella pneumoniae* has the higher frequency in dentures and orthodontic among the other bacterial species.

6-2-Recommendations:-

- Use the PCR nucleotide sequences as the best test for diagnosing the bacterial species among the other assays .Since , different bacterial species were recognized in the present study .
- Use the genetic method for determining the ability of bacteria to adherence .
- other study should be accomplished to determine the sensitivity of the bacteria under the study toward antibiotics ,toothpastes and other medical solution .

- Several studies should be taken to show the direct relationship between the adherent bacteria from denture and orthodontic with lower respiratory and digestive system diseases.
- More studies should be performed from the same sources but with yeast , fungi and viruses.

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Appendix

❖ Appendix-1:-Alignment and concatenating of bacterial species obtained from the present study after sequencing data and reference strain by GeneBank using "CLUSTALW"

Bacillus-cereus	TACC-CCACCGACTTCGGGTGTTACAAACTCTC-GGGGTGTGACGGGCGG 48
Streptococcus-equinus	TACC-TCACCGACTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Bacillus-cereus-B1T	TACC-CCACCGACTTCGGGTGTTACAAACTCTC-GGGGTGTGACGGGCGG 48
Streptococcus-equinus-KLDS3.06	TACC-TCACCGACTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Enterococcus-faecalis	TACC-TCACCGACTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Lactococcus-lactis	TAGG-CAACCTACTTCGGGTACTCCCAACTCCC-GTGGTGTGACGGGCGG 48
Enterococcus-faecium	TACC-TCACCGACTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Enterococcus-faecalis-KLDS4.03	TACC-TCACCGACTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Lactococcus-lactis-LCT	TAGG-CAACCTACTTCGGGTACTCCCAACTCCC-GTGGTGTGACGGGCGG 48
Enterococcus-faecium-IB1T	TACC-TCACCGACTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Pediococcus-acidilactici	TACC-CCACCGGCTTGGGTGTTACAAACTCTC-ATGGTGTGACGGGCGG 48
Proteus-mirabilis	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Lactobacillus-plantarum	TACC-CCACCGACTTGGGTGTTACAAACTCTC-ATGGTGTGACGGGCGG 48
Morganella-morganii	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Bacillus-subtilis	TACC-TCACCGACTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Hafnia-alvei	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Chryseobacterium-vietnamense	TACGGTCACCGACTTCAGGTACCCAGACTTCC-ATGGTGTGACGGGCGG 49
Streptococcus-anginosus	TACC-TCACCGACTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Bacillus-subtilis-N11T	TACC-TCACCGACTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Hafnia-alvei-E117T	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-aureus	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Escherichia-fergusonii	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Enterobacter-mori-R3-3T	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-aureus-NBRC1271	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Escherichia-fergusonii-G7T	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Enterobacter-ludwigii-NRCG12T	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-epidermidis-BBN	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Enterobacter-cloacae-478T	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-warneri	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Klebsiella-variicola	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Citrobacter-freundii	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-warneri-FUA2075	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Klebsiella-variicola-7T	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Enterobacter-mori	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-epidermidis	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Enterobacter-cloacae	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Serratia-marcescens-A4T	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-pasteuri	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Acinetobacter-baumanni	TAGG-CTAGCTACTTCTGGTGAACAAACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-pasteuri-C1P01T	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Acinetobacter-baumanni-DSM300	TAGG-CTAGCTACTTCTGGTGAACAAACTCCC-ATGGTGTGACGGGCGG 48
Klebsiella-pneumoniae	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-hominis-S9-624T	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Klebsiella-pneumoniae-SDM45T	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Serratia-marcescens	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Staphylococcus-hominis	TACT-CCACCGGCTTCGGGTGTTACAAACTCTC-GTGGTGTGACGGGCGG 48
Citrobacter-Freundii-MRB070408	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Proteus-penneri	TAAG-CTACCTACTTCTTTTGCAACCCACTCCC-ATGGTGTGACGGGCGG 48
Morganella-morganii-MFS05T	TAAG-CTACCTACTTCTTTTGCAACCCACTCCCCATGGTGTGACGGGCGG 49

Enterobacter-ludwigii	TAAG-CTACCTACTTCTTTTGCAACCACTCCC-ATGGTGTGACGGCGG	48
Lactobacillus-plantarum-KLDS1.	TACC-CCACCGACTTGGGTGTACAAACTCTC-ATGGGTGACGGCGG	48
Proteus-penneri-Z2T	TAAG-CTACCTACTTCTTTTGCAACCACTCCC-ATGGTGTGACGGCGG	48
Chryseobacterium-vietnamense-G	TACGGTCAACCGACTTCAGGTACCCAGACTTCC-ATGGCTTGACGGCGG	48
Streptococcus-anginosus-CO2T	TACC-TCACCGACTTCGGGTGTACAAACTCTC-GTGGTGTGACGGCGG	48
Pediococcus-acidilactici-L169T	TACC-CCACCGGCTTGGGTGTACAAACTCTC-ATGGTGTGACGGCGG	48
Proteus-mirabilis-ALK419T	TAAG-CTACCTACTTCTTTTGCAACCACTCCC-ATGGTGTGACGGCGG	48
	** * * * *	
Bacillus-cereus	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Streptococcus-equinus	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Bacillus-cereus-B1T	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Streptococcus-equinus-KLDS3.06	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Enterococcus-faecalis	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Lactococcus-lactis	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Enterococcus-faecium	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Enterococcus-faecalis-KLDS4.03	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Lactococcus-lactis-LCT	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Enterococcus-faecium-1B1T	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Pediococcus-acidilactici	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Proteus-mirabilis	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Lactobacillus-plantarum	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Morganella-morganii	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Bacillus-subtilis	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Hafnia-alvei	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Chryseobacterium-vietnamense	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Streptococcus-anginosus	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Bacillus-subtilis-N11T	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Hafnia-alvei-E117T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Staphylococcus-aureus	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Escherichia-fergusonii	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Enterobacter-mori-R3-3T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Staphylococcus-aureus-NBRC1271	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Escherichia-fergusonii-G7T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Enterobacter-ludwigii-NRCG12T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Staphylococcus-epidermidis-BBN	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Enterobacter-cloacae-478T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Staphylococcus-warneri	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Klebsiella-variicola	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Citrobacter-freundii	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Staphylococcus-warneri-FUA2075	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Klebsiella-variicola-7T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Enterobacter-mori	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Staphylococcus-epidermidis	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Enterobacter-cloacae	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Serratia-marcescens-A4T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Staphylococcus-pasteuri	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Acinetobacter-baummanni	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Staphylococcus-pasteuri-C1P01T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Acinetobacter-baummanni-DSM300	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Klebsiella-pneumoniae	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Staphylococcus-hominis-S9-624T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Klebsiella-pneumoniae-SDM45T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Serratia-marcescens	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Staphylococcus-hominis	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Citrobacter-freundii-MRB070408	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Proteus-penneri	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Morganella-morganii-MFS05T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Enterobacter-ludwigii	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Lactobacillus-plantarum-KLDS1.	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Proteus-penneri-Z2T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97
Chryseobacterium-vietnamense-G	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Streptococcus-anginosus-CO2T	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Pediococcus-acidilactici-L169T	TGTGTACAAGGCCCGGGAACGTATTACC CGCGGCATG-CTGATCCGCGAT	97
Proteus-mirabilis-ALK419T	TGTGTACAAGGCCCGGGAACGTATTACC CGGTAGCATT-CTGATCTACGAT	97

Bacillus-cereus	TACTAGCGATTCCGACTTCATGTAGGCAAGTTGCAGCCTACAATCCGAAC	147
Streptococcus-equinus	TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Bacillus-cereus-B1T	TACTAGCGATTCCGACTTCATGTAGGCAAGTTGCAGCCTACAATCCGAAC	147
Streptococcus-equinus-KLDS3.06	TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Enterococcus-faecalis	TACTAGCGATTCCGCTTCATGCAGGCGAGTTGCAGCCTGCAATCCGAAC	147
Lactococcus-lactis	TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Enterococcus-faecium	TACTAGCGATTCCGCTTCATGCAGGCGAGTTGCAGCCTGCAATCCGAAC	147
Enterococcus-faecium-KLDS4.03	TACTAGCGATTCCGCTTCATGCAGGCGAGTTGCAGCCTGCAATCCGAAC	147
Lactococcus-lactis-LCT	TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Enterococcus-faecium-IB1T	TACTAGCGATTCCGCTTCATGCAGGCGAGTTGCAGCCTGCAATCCGAAC	147
Pediococcus-acidilactici	TACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Proteus-mirabilis	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Lactobacillus-plantarum	TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Morganella-morganii	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Bacillus-subtilis	TACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGAAC	147
Hafnia-alvei	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Chryseobacterium-vietnamense	TACTAGCGATTCCGACTTCATAGAGTCGAGTTGCAGACTCCAATCCGAAC	149
Streptococcus-anginosus	TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Bacillus-subtilis-N11T	TACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGAAC	147
Hafnia-alvei-E117T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-aureus	TACTAGCGATTCCGACTTCATGTAGTCGAGTTGCAGACTGCGATCCGAAC	147
Escherichia-fergusonii	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Enterobacter-mori-R3-3T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-aureus-NBRC1271	TACTAGCGATTCCGACTTCATGTAGTCGAGTTGCAGACTACAATCCGAAC	147
Escherichia-fergusonii-G7T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Enterobacter-ludwigii-NRCG12T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-epidermidis-BBN	TACTAGCGATTCCGACTTCATATAGTCGAGTTGCAGACTCCAATCCGAAC	147
Enterobacter-cloacae-478T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-warneri	TACTAGCGATTCCGACTTCATGTAGTCGAGTTGCAGACTCCAATCCGAAC	147
Klebsiella-variicola	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Citrobacter-freundii	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-warneri-FUA2075	TACTAGCGATTCCGACTTCATGTAGTCGAGTTGCAGACTACAATCCGAAC	147
Klebsiella-variicola-7T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Enterobacter-mori	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-epidermidis	TACTAGCGATTCCGACTTCATATAGTCGAGTTGCAGACTCCAATCCGAAC	147
Enterobacter-cloacae	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Serratia-marcescens-A4T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-pasteuri	TACTAGCGATTCCGACTTCATGTAGTCGAGTTGCAGACTACAATCCGAAC	147
Acinetobacter-baumannii	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-pasteuri-C1P01T	TACTAGCGATTCCGACTTCATGTAGTCGAGTTGCAGACTACAATCCGAAC	147
Acinetobacter-baumannii-DSM300	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Klebsiella-pneumoniae	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-hominis-S9-624T	TACTAGCGATTCCGACTTCATGTAGTCGAGTTGCAGACTACAATCCGAAC	147
Klebsiella-pneumoniae-SDM45T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Serratia-marcescens	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Staphylococcus-hominis	TACTAGCGATTCCGACTTCATGTAGTCGAGTTGCAGACTACAATCCGAAC	147
Citrobacter-freundii-MRB070408	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Proteus-penneri	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Morganella-morganii-MFS05T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	148
Enterobacter-ludwigii	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Lactobacillus-plantarum-KLDS1.	TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Proteus-penneri-Z2T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147
Chryseobacterium-vietnamense-G	TACTAGCGATTCCGACTTCATAGAGTCGAGTTGCAGACTCCAATCCGAAC	149
Streptococcus-anginosus-CO2T	TACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Pediococcus-acidilactici-L169T	TACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTACAATCCGAAC	147
Proteus-mirabilis-ALK419T	TACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC	147

Bacillus-cereus	T-GAAAACGGTTTTATGAAATAGCTCCACCTCGGGCTTTGCGAGCTCTT	196
Streptococcus-equinus	T-GAGATTGGCTTTAAGAGATTGCTTCCGCTACCCGCTCGGACTCGGACTCGT	196
Bacillus-cereus-B1T	T-GAAAACGGTTTTATGAAATAGCTCCACCTCGGGCTTTGCGAGCTCTT	196
Streptococcus-equinus-KLDS3.06	T-GAGATTGGCTTTAAGAGATTGCTTCCGCTACCCGCTCGGACTCGGACTCGT	196
Enterococcus-faecalis	T-GAGAGAAGCTTTAAGAGATTGATGACCTCCGGGTCTAGGACTCGT	196
Lactococcus-lactis	T-GAGAATGGTTTTAAGAGATTAGCTAAACATCAGCTGCTCGGACTCGT	196
Enterococcus-faecium	T-GAGAGAAGCTTTAAGAGATTGATGACCTCCGGGTCTAGGACTCGT	196
Enterococcus-faecium-KLDS4.03	T-GAGAGAAGCTTTAAGAGATTGATGACCTCCGGGTCTAGGACTCGT	196
Lactococcus-lactis-LCT	T-GAGAATGGTTTTAAGAGATTAGCTAAACATCAGCTGCTCGGACTCGT	196

<i>Tetrorococcus-faecium</i> -IT1	T-GAGAGAAAGCTTTAAAGAGATTGTCATGACGCTTCGGCGTTAGCGACATCGT	196
<i>Pediococcus-acidilactici</i>	T-GAGAAATGGTTTAAAGAGATTGCTGAACCTTCGGCGTTTCGCAACTCGT	197
<i>Proteus-mirabilis</i>	T-ACGACGACTTTATAGAGTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	198
<i>Lactobacillus-plantarum</i>	T-GAGAAATGGCTTAAAGAGATTGACTTACTTCGCGAGTTCGCAACTCGT	199
<i>Morganella-morganii</i>	T-ACAGCGTACTTTATAGATTCGCGCTTGACCTTCGGGGTCGCTTCCTT	200
<i>Lactobacillus-subtilis</i>	TGAGAACAGA-TTTGTGGGATTGGCTTAACCTTCGCGCTTCGCTGCGCTT	201
<i>Bacillus-alvei</i>	T-ACGACATACTTTATAGAGTCGCGCTTGCCTTCGCGAGTTCGCTTCCTT	202
<i>Streptococcus-vietnamense</i>	TGA-GACCGGCTTTC-GAGATTGTCATCGCTACGCTTGTAGTCGGCTT	203
<i>Streptococcus-anginosus</i>	TGA-GACTGGCTTCAGAGATTAGCTTGCATCCAGCGTTCGCACTCGT	204
<i>Bacillus-luxii</i> -N11T	TGAGAACAGA-TTTGTGGATTGGCTTAACCTTCGCGCTTCGCTGCGCTT	205
<i>Bacillus-alvei</i> -E117T	T-ACGACATACTTTATAGAGTCGCGCTTGCCTTCGCGAGTTCGCTTCCTT	206
<i>Staphylococcus-aureus</i>	T-GAGAACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	207
<i>Escherichia-fergusonii</i>	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	208
<i>Enterobacter-mori</i> -R3-3T	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	209
<i>Staphylococcus-aureus</i> -NRCL271	T-ACGACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	210
<i>Escherichia-fergusonii</i> -C7T	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	211
<i>Enterobacter-ludwigii</i> -NRG612T	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	212
<i>Staphylococcus-epidermidis</i> -BBN	T-GAGAACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	213
<i>Enterobacter-cl</i> -rae-478T	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	214
<i>Staphylococcus-warneri</i>	T-GAGAACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	215
<i>Klebsiella-variella</i>	T-ACGACATACTTTATAGAGTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	216
<i>Citrobacter-freundii</i>	T-ACGACATACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	217
<i>Staphylococcus-warneri</i> -FUA2075	T-GAGAACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	218
<i>Klebsiella-variicola</i> -7T	T-ACGACATACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	219
<i>Enterobacter-mori</i>	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	220
<i>Staphylococcus-epidermidis</i>	T-GAGAACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	221
<i>Enterobacter-cloacae</i>	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	222
<i>Serratia-marcescens</i> -A4T	T-ACGAGTACTTTATAGGTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	223
<i>Staphylococcus-pasteuri</i>	T-GAGAACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	224
<i>Acinetobacter-baumanni</i>	T-ACGATCGGCTTTTATGGATTAGCATGACCTGCTGTAGTGAACACTT	225
<i>Staphylococcus-pasteuri</i> -C1P01T	T-GAGAACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	226
<i>Acinetobacter-baumanni</i> -DSM300	T-AAGATCGGCTTTTATGAGTATGACATGCTGCTGCGAGGTTCGCTTCCTT	227
<i>Leibishia-pneumoniae</i>	T-ACGACATACTTTATAGAGTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	228
<i>Staphylococcus-hominis</i> -S9-624T	T-GAGAACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	229
<i>Klebsiella-pneumoniae</i> -SDM45T	T-ACGACATACTTTATAGAGTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	230
<i>Serratia-marcescens</i>	T-ACGAGTACTTTATAGGTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	231
<i>Staphylococcus-hominis</i>	T-GAGAACAACTTTATGGGATTTCGTGACCTTCGCGGTTTCGCTGCGCTT	232
<i>Citrobacter-freundii</i> -MRB070408	T-ACGACATACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	233
<i>Proteus-penneri</i>	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	234
<i>Morganella-morganii</i> -MFS05T	T-ACGAGTACTTTATAGGTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	235
<i>Enterobacter-ludwigii</i>	T-ACGACGCACTTTATAGGTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	236
<i>Lactobacillus-plantarum</i> -KLD51-	T-GAGAAATGGCTTAAAGAGATTGACTTACTTCGCGAGTTCGCAACTCGT	237
<i>Enterobacter-penneri</i> -22T	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	238
<i>Streptococcus-anginosus</i> -C02T	T-GAGAGTGGCTTCAGAGATTAGCTTGCCTTCGCGAGGTTCGCAACTCGT	239
<i>Pediococcus-acidilactici</i> -L169T	T-GAGAAATGGTTTAAAGAGATTGACTTAACTTCGCGGTTTCGCAACTCGT	240
<i>Proteus-mirabilis</i> -ALK419T	T-ACGACGCACTTTATGAGTTCGCGCTTGCCTTCGCGAGGTTCGCTTCCTT	241

Bacillus-subtilis-N11T	TGT-TCTGTCCATTGTAGCACGTGTGTAGCCCAAGTATAAGGGGCATGA	245
Hafnia-alvei-E117T	TGTATATG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Staphylococcus-aureus	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Escherichia-fergusonii	TGTATGCG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Enterobacter-mori-R3-3T	TGTATGCG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGGCATGA	245
Staphylococcus-aureus-NBRC1271	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Escherichia-fergusonii-G7T	TGTATGCG-CCATTGTAGCACGTGTGTAGCCCTGGTCTGAAGGGCCATGA	245
Enterobacter-ludwigii-NRCG12T	TGTATGCG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGGCATGA	245
Staphylococcus-epidermidis-BBN	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Enterobacter-cloacae-478T	TGTATGCG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Staphylococcus-warneri	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Klebsiella-varicola	TGTATATG-CCATTGTAGCACGTGTGTAGCCCTGGTCTGAAGGGCCATGA	245
Citrobacter-freundii	TGTATATG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Staphylococcus-warneri-FUA2075	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Klebsiella-varicola-7T	TGTATATG-CCATTGTAGCACGTGTGTAGCCCTGGTCTGAAGGGCCATGA	245
Enterobacter-mori	TGTATGCG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Staphylococcus-epidermidis	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Enterobacter-cloacae	TGTATGCG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Serratia-marcescens-A4T	TGTATACG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Staphylococcus-pasteuri	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Acinetobacter-baumanni	TGTACCGA-CCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATGA	245
Staphylococcus-pasteuri-ClP01T	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Acinetobacter-baumanni-DSM300	TGTACCGA-CCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATGA	245
Klebsiella-pneumoniae	TGTATATG-CCATTGTAGCACGTGTGTAGCCCTGGTCTGAAGGGCCATGA	245
Staphylococcus-hominis-S9-624T	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Klebsiella-pneumoniae-SDM45T	TGTATATG-CCATTGTAGCACGTGTGTAGCCCTGGTCTGAAGGGCCATGA	245
Serratia-marcescens	TGTATACG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Staphylococcus-hominis	TGTATTGT-CCATTGTAGCACGTGTGTAGCCCAAAATCATAAGGGGCATGA	245
Citrobacter-freundii-MRB070408	TGTATATG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Proteus-penneri	TGTATCTG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Morganella-morganii-MFS05T	TGTATACG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	246
Enterobacter-ludwigii	TGTATGCG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Lactobacillus-plantarum-KLDS1.	TGTACCAT-CCATTGTAGCACGTGTGTAGCCCAAGTATAAGGGGCATGA	245
Proteus-penneri-22T	TGTATCTG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
Chryseobacterium-vietnamense-G	TGTACCGG-CCATTGTATTACGTGTGTAGCCCAAGGCGTAAGGGGCATGA	246
Streptococcus-anginosus-CO2T	TGTACCAAG-CCATTGTAGCACGTGTGTAGCCCAAGTATAAGGGGCATGA	245
Pediococcus-acidilactici-L169T	TGTACCAT-CCATTGTAGCACGTGTGTAGCCCAAGTATAAGGGGCATGA	245
Proteus-mirabilis-ALK419T	TGTATCTG-CCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGA	245
*** ***** *		
Bacillus-cereus	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCAAC	294
Streptococcus-equinus	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTATACCCGGCAGTCTCG	294
Bacillus-cereus-B1T	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCAAC	294
Streptococcus-equinus-KLDS3.06	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTATACCCGGCAGTCTCG	294
Enterococcus-faecalis	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCTCG	294
Lactococcus-lactis	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTATACCCGGCAGTCTCG	294
Enterococcus-faecium	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCTCG	294
Enterococcus-faecalis-KLDS4.03	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCTCG	294
Lactococcus-lactis-LCT	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCTCG	294
Pediococcus-faecium-IB1T	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCTCG	294
Pediococcus-acidilactici	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCTCG	294
Proteus-mirabilis	TGACTTGACGTCATCCCCACCTTCCTC-CGGTTTATACCCGGCAGTCTCC	294
Lactobacillus-plantarum	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCTCG	294
Morganella-morganii	TGACTTGACGTCATCCCCACCTTCCTC-CGGTTTATACCCGGCAGTCTCC	294
Bacillus-subtilis	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCAAC	294
Hafnia-alvei	TGACTTGACGTCATCCCCACCTTCCTC-CGGTTTATACCCGGCAGTCTCC	294
Chryseobacterium-vietnamense	TGATTTGACGTCATCCCCACCTTCCTCTACTTGTG-GTAGGGCAGTCTCA	295
Streptococcus-anginosus	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCTCG	294
Bacillus-subtilis-N11T	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCAAC	294
Hafnia-alvei-E117T	TGACTTGACGTCATCCCCACCTTCCTC-CAGTTTATACCCGGCAGTCTCC	294
Staphylococcus-aureus	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCAAC	294
Escherichia-fergusonii	TGACTTGACGTCATCCCCACCTTCCTC-CAGTTTATACCTGGCAGTCTCC	294
Enterobacter-mori-R3-3T	TGACTTGACGTCATCCCCACCTTCCTC-CAGTTTATACCTGGCAGTCTCC	294
Staphylococcus-aureus-NBRC1271	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCAAC	294
Escherichia-fergusonii-G7T	TGACTTGACGTCATCCCCACCTTCCTC-CAGTTTATACCTGGCAGTCTCC	294
Enterobacter-ludwigii-NRCG12T	TGACTTGACGTCATCCCCACCTTCCTC-CAGTTTATACCTGGCAGTCTCC	294
Staphylococcus-epidermidis-BBN	TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTACCCGGCAGTCAAC	294

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Enterobacter-cloacae-478T      TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Staphylococcus-warneri        TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTGACACGGCAGTCAAC 294
Klebsiella-variicola          TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Citrobacter-freundii          TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Staphylococcus-warneri-FUA2075 TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTGACACGGCAGTCAAC 294
Klebsiella-variicola-7T      TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Enterobacter-mori             TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Staphylococcus-epidermidis    TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTGACACGGCAGTCAAC 294
Enterobacter-cloacae         TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Serratia-marcescens-A4T      TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Staphylococcus-pasteuri      TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTGACACGGCAGTCAAC 294
Acinetobacter-baumannii      TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTGTCACTGGCAGTATCC 294
Staphylococcus-pasteuri-C1P01T TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTGACACGGCAGTCAAC 294
Acinetobacter-baumannii-DSM300 TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTGTCACTGGCAGTATCC 294
Klebsiella-pneumoniae        TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Staphylococcus-hominis-S9-624T TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTGACACGGCAGTCAAC 294
Klebsiella-pneumoniae-SDM45T TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Serratia-marcescens          TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Staphylococcus-hominis       TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTGACACGGCAGTCAAC 294
Citrobacter-freundii-MRB070408 TGACTTTGACGTCATCCCCACCTTCCTC-CAGTTTATCACTGGCAGTCTCC 294
Proteus-penneri              TGACTTTGACGTCATCCCCACCTTCCTC-CGGTTTATCACTGGCAGTCTCC 294
Morganella-morganii-MFS05T   TGACTTTGACGTCATCCCCACCTTCCTC-CGGTTTATCACTGGCAGTCTCC 295
Lactobacillus-plantarum-KLDS1. TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTGTGACACGGCAGTCTCA 294
Proteus-penneri-Z2T          TGACTTTGACGTCATCCCCACCTTCCTC-CGGTTTATCACTGGCAGTCTCC 294
Chryseobacterium-vietnamense-G TGATTTGACGTCATCCCCACCTTCCTCTCTATTCG-GTAGGCGAGTCTCA 295
Streptococcus-anginosus-CO2T TGATTTGACGTCATCCCCACCTTCCTC-CGGTTTATTAACCGGAGTCTCG 294
Pediococcus-acidilactici-L169T TGATTTGACGTCGTCACCTTCCTC-CGGTTTGTGACACGGCAGTCTCA 294
Proteus-mirabilis-ALK419T    TGACTTTGACGTCATCCCCACCTTCCTC-CGGTTTATCACTGGCAGTCTCC 294
*** ***** * ** *****

Bacillus-cereus               TTAAAGTGCCCA--ACTAAAT--GA--TGGAACATAAAATCAAGGGTTGC 338
CTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAACAAATAGGGGTTGC 338
Bacillus-cereus-B1T           TTAAAGTGCCCA--ACTAAAT--GA--TGGCAACTAAAATCAAGGGTTGC 338
Streptococcus-equinus-KLDS3.06 CTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAACAAATAGGGGTTGC 338
Enterococcus-faecalis        CTAGAGTGCCCA--ACTAAAT--GA--TGGCAACTAACAAATAGGGGTTGC 338
Lactococcus-lactis           TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAACAAATAGGGGTTGC 338
Enterococcus-faecium         CTAGAGTGCCCA--ACTAAAT--GA--TGGCAACTAACAAATAGGGGTTGC 338
Enterococcus-faecalis-KLDS4.03 CTAGAGTGCCCA--ACTAAAT--GA--TGGCAACTAACAAATAGGGGTTGC 338
Lactococcus-lactis-LCT       TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAACAAATAGGGGTTGC 338
Enterococcus-faecium-IB1T    CTAGAGTGCCCA--ACTAAAT--GA--TGGCAACTAACAAATAGGGGTTGC 338
Pediococcus-acidilactici     CTAGAGTGCCCA--ACTGAAT--GC--TGGCAACTAGTAATAAGGGGTTGC 338
Proteus-mirabilis           TTTGAGTTCCCG--CCATCAC--CGCGTGCCCAACAAAGGATAAGGGGTTGC 340
Lactobacillus-plantarum      CCAGAGTGCCCA--ACTTAAT--GC--TGGCAACTAGTAATAAGGGGTTGC 338
Morganella-morganii         TTTGAGTTCCCG--CCATCAC--CGCGTGCCCAACAAAGGATAAGGGGTTGC 340
Bacillus-subtilis            TTAGAGTGCCCA--ACTGAAT--GC--TGGCAACTAAGATAAAGGGGTTGC 338
Hafnia-alvei                 TTTGAGTTCCCG--CCATCAC--CGCGTGCCCAACAAAGGATAAGGGGTTGC 340
Chryseobacterium-vietnamense CTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAGTACAGGGGTTGC 339
Streptococcus-anginosus     CTAGAGTGCCCA--ACTCAAT--GA--TGGCAACTAACAAATAGGGGTTGC 338
Bacillus-subtilis-N11T      TTAGAGTGCCCA--ACTGAAT--GC--TGGCAACTAAGATAAAGGGGTTGC 338
Hafnia-alvei-E117T          TTTGAGTTCCCG--CCATCAC--CGCGTGCCCAACAAAGGATAAGGGGTTGC 340
Staphylococcus-aureus       TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAAGCTTAAGGGGTTGC 338
Escherichia-fergusonii      TTTGAGTTCCCG--GCCGAC--CGC--TGGCAACAAAGGATAAGGGGTTGC 339
Enterobacter-mori-R3-3T     TTTGAGTTCCCG--GCCGAC--CGC--TGGCAACAAAGGATAAGGGGTTGC 339
Staphylococcus-aureus-NBRC1271 TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAAGCTTAAGGGGTTGC 338
Escherichia-fergusonii-G7T TTTGAGTTCCCG--GCCGAC--CGC--TGGCAACAAAGGATAAGGGGTTGC 339
Enterobacter-ludwigii-NRCG12T TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAAGCTTAAGGGGTTGC 338
Staphylococcus-epidermidis-BBN TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAAGCTTAAGGGGTTGC 338
Enterobacter-cloacae-478T   TTTGAGTTCCCG--GCCTAAC--CGC--TGGCAACAAAGGATAAGGGGTTGC 339
Staphylococcus-warneri      TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAAGCTTAAGGGGTTGC 338
Klebsiella-variicola        TTTGAGTTCCCG--GCCTAAC--CGC--TGGCAACAAAGGATAAGGGGTTGC 339
Citrobacter-freundii        TTTGAGTTCCCG--GCCGAC--CGC--TGGCAACTAAGCTTAAGGGGTTGC 339
Staphylococcus-warneri-FUA2075 TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAAGCTTAAGGGGTTGC 339
Klebsiella-variicola-7T     TTTGAGTTCCCG--GCCTAAC--CGC--TGGCAACAAAGGATAAGGGGTTGC 339
Enterobacter-mori           TTTGAGTTCCCG--GCCGAC--CGC--TGGCAACAAAGGATAAGGGGTTGC 339
Staphylococcus-epidermidis TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAAGCTTAAGGGGTTGC 338
Enterobacter-cloacae        TTTGAGTTCCCG--GCCTAAC--CGC--TGGCAACAAAGGATAAGGGGTTGC 339

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Serratia-marcescens-A4T	TTTGAGTTCCTCCG--GCCGAAC-CGC--TGGCAACAAGGATAAGGGTTGC	339
Staphylococcus-pasteuri	TTAGAGTGCCCA--ACTTAA--TGA--TGGCAACTAAGCTTAAGGGTTGC	338
Acinetobacter-baumanni	TTAAAGTTCCTCA--TCCGAAA-TGC--TGGCAAGTAAGGATAAGGGTTGC	339
Staphylococcus-pasteuri-ClP01T	TTAGAGTGCCCA--ACTTAA--TGA--TGGCAACTAAGCTTAAGGGTTGC	338
Acinetobacter-baumanni-DSM300	TTAAAGTTCCTCA--TCCGAAA-TGC--TGGCAAGTAAGGATAAGGGTTGC	339
Klebsiella-pneumoniae	TTTGAGTTCCTCCG--GCCGGAC-CGC--TGGCAACAAGGATAAGGGTTGC	339
Staphylococcus-hominis-S9-624T	TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAAGCTTAAGGGTTGC	338
Klebsiella-pneumoniae-SDM45T	TTTGAGTTCCTCCG--GCCGGAC-CGC--TGGCAACAAGGATAAGGGTTGC	339
Serratia-marcescens	TTTGAGTTCCTCCG--GCCGAAC-CGC--TGGCAACAAGGATAAGGGTTGC	339
Staphylococcus-hominis	TTAGAGTGCCCA--ACTTAAT--GA--TGGCAACTAAGCTTAAGGGTTGC	338
Citrobacter-freundii-MRB070408	TTTGAGTTCCTCCG--GCCGAAC-CGC--TGGCAACAAGGATAAGGGTTGC	339
Proteus-penneri	TTTGAGTTCCTCCCA--CCATTACGTGC--TGGCAACAAGGATAAGGGTTGC	340
Morganella-morganii-MFS05T	TTTGAGTTCCTCCG--CCATCACGCGC--TGGCAACAAGGATAAGGGTTGC	341
Enterobacter-ludwigii	TTTGAGTTCCTCCG--GCCATAAC-CGC--TGGCAACAAGGATAAGGGTTGC	339
Lactobacillus-plantarum-KLDS1.	CCAGAGTGCCCA--AC-TTA-ATGC--TGGCAACTGATAATAAGGGTTGC	338
Proteus-penneri-Z2T	TTTGAGTTCCTCA--CCATTACGTGC--TGGCAACAAGGATAAGGGTTGC	340
Chryseobacterium-vietnamense-G	CTAGAGTCCCCA--ACTTAAT--GA--TGGCAACTAGGTACAGGGTTGC	339
Streptococcus-anginosus-CO2T	CTAGAGTGCCCA--ACTCAAT--GA--TGGCAACTAACATAAGGGTTGC	338
Pediococcus-acidilactici-L169T	CTAGAGTGCCCA--ACTGAAT--GC--TGGCAACTAGTATAAGGGTTGC	338
Proteus-mirabilis-ALK419T	TTTGAGTTCCTCCACCATTACGT--GC--TGGCAACAAGGATAAGGGTTGC	340
	*** **	
Bacillus-cereus	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Streptococcus-equinus	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Bacillus-cereus-B1T	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Streptococcus-equinus-KLDS3.06	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Enterococcus-faecalis	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Lactococcus-lactis	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Enterococcus-faecium	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Enterococcus-faecalis-KLDS4.03	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Lactococcus-lactis-LCT	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Enterococcus-faecium-IB1T	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Pediococcus-acidilactici	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Proteus-mirabilis	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	390
Lactobacillus-plantarum	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Morganella-morganii	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	390
Bacillus-subtilis	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Hafnia-alvei	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	390
Chryseobacterium-vietnamense	GCTCGTTGCGAGGACTTAACCTAACACCTCAGCGCACGAGCTGACGACAAC	389
Streptococcus-anginosus	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Bacillus-subtilis-N11T	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Hafnia-alvei-E117T	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	390
Staphylococcus-aureus	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Escherichia-fergusonii	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Enterobacter-mori-R3-3T	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAAC	388
Staphylococcus-aureus-NBRK1271	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Escherichia-fergusonii-G7T	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Enterobacter-ludwigii-NRKG12T	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAAC	388
Staphylococcus-epidermidis-BBN	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Enterobacter-cloacae-478T	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Staphylococcus-warneri	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Klebsiella-variicola	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Citrobacter-freundii	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Staphylococcus-warneri-FUA2075	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Klebsiella-variicola-7T	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Enterobacter-mori	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Staphylococcus-epidermidis	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Enterobacter-cloacae	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Serratia-marcescens-A4T	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Staphylococcus-pasteuri	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Acinetobacter-baumanni	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAGC	389
Staphylococcus-pasteuri-ClP01T	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Acinetobacter-baumanni-DSM300	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAGC	389
Klebsiella-pneumoniae	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Staphylococcus-hominis-S9-624T	GCTCGTTGCGGGACTTAACCCAACATCTCAGCACACGAGCTGACGACAAC	388
Klebsiella-pneumoniae-SDM45T	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389
Serratia-marcescens	GCTCGTTGCGGGACTTAACCCAACATTTTCACAAACAGAGCTGACGACAGC	389

Staphylococcus-hominis	GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAAC	388
Citrobacter-freundii-MRB070408	GCTCGTTGCGGGACTTAACCCAACATTTTCAACAACAGAGCTGACGACAGC	389
Proteus-penneri	GCTCGTTGCGGGACTTAACCCAACATTTTCAACAACAGAGCTGACGACAGC	390
Morganella-morganii-MFS05T	GCTCGTTGCGGGACTTAACCCAACATTTTCAACAACAGAGCTGACGACAGC	391
Enterobacter-ludwigii	GCTCGTTGCGGGACTTAACCCAACATTTTCAACAACAGAGCTGACGACAGC	389
Lactobacillus-plantarum-KLDS1.	GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAAC	388
Proteus-penneri-Z2T	GCTCGTTGCGGGACTTAACCCAACATTTTCAACAACAGAGCTGACGACAGC	390
Chryseobacterium-vietnamense-G	GCTCGTTGCGGGACTTAACCTACACCTTCACGGCAGAGCTGACGACAAC	389
Streptococcus-anginosus-CO2T	GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAAC	388
Pediococcus-acidilactici-L169T	GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAAC	388
Proteus-mirabilis-ALK419T	GCTCGTTGCGGGACTTAACCCAACATTTTCAACAACAGAGCTGACGACAGC	390

Bacillus-cereus	CATGCACCACCTGTCACTCTGCTCCC--GAAGGA-AAAGC--CCTATCTC	433
Streptococcus-equinus	CATGCACCACCTGTCAACCGATGTTCC--GAAG-A-AACTT--CCTATCTC	432
Bacillus-cereus-B1T	CATGCACCACCTGTCACTCTGCTCCC--GAAGGA-AAAGC--CCTATCTC	433
Streptococcus-equinus-KLDS3.06	CATGCACCACCTGTCAACCGATGTTCC--GAAG-A-AACTT--CCTATCTC	432
Enterococcus-faecalis	CATGCACCACCTGTCACTTTGTCCCC--GAAGGG-AAAGC--TCTATCTC	433
Lactococcus-lactis	CATGCACCACCTGT-ATCCCGTGTTCC--CGAAGG-AACTT--CCTATCTC	432
Enterococcus-faecium	CATGCACCACCTGTCACTTTGTCCCC--GAAGGG-AAAGC--TCTATCTC	433
Enterococcus-faecium-KLDS4.03	CATGCACCACCTGTCACTTTGTCCCC--GAAGGG-AAAGC--TCTATCTC	433
Lactococcus-lactis-LCT	CATGCACCACCTGT-ATCCCGTGTTCC--CGAAGG-AACTT--CCTATCTC	432
Enterococcus-faecium-IB1T	CATGCACCACCTGTCACTTTGTCCCC--GAAGGG-AAAGC--TCTATCTC	433
Pediococcus-acidilactici	CATGCACCACCTGTCACTCTGCTCCCC--GAAGGG-AAAGC--CTAATCTC	433
Proteus-mirabilis	CATGCAGCACCTGTCTCAGGTTTCCC--GAAGGC-ACTCC--TCTATCTC	435
Lactobacillus-plantarum	CATGCACCACCTGTATCCATGTCCCC--GAAGGG-AAGCT--TCTATCTC	433
Morganella-morganii	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--AGCATCTC	435
Bacillus-subtilis	CATGCACCACCTGTCACTCTGCCCCC--GAAGGG-GAGCT--CCTATCTC	433
Hafnia-alvei	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCTATCTC	435
Chryseobacterium-vietnamense	CATGCAGCACCT-TGAAAAATGT-CC--GAAGA--AAAGT--C-TATTTCT	430
Streptococcus-anginosus	CATGCACCACCTGTCAACCGATGTTCC--GAAGA-A-AACTT--CCTATCTC	432
Bacillus-subtilis-N11T	CATGCACCACCTGTCACTCTGCCCCC--GAAGGG-GAGCT--CCTATCTC	433
Hafnia-alvei-E117T	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--GCTATCTC	435
Staphylococcus-aureus	CATGCACCACCTGTCACTTTGTCCCC--GAAGGG-GAAGGC-TCTATCTC	435
Escherichia-fergusonii	CATGCAGCACCTGTCTCAGGTTTCCC--GAAGGC-ACATTCT-TC-ATCTC	434
Enterobacter-mori-R3-3T	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-AC-CAA-TCCATCTC	434
Staphylococcus-aureus-NBRC1271	CATGCACCACCTGTCACTTTGTCCCC--GAAGGG-GAAGGC-TCTATCTC	435
Escherichia-fergusonii-G7T	CATGCAGCACCTGTCTCAGGTTTCCC--GAAGGC-ACATTCT-TC-ATCTC	434
Enterobacter-ludwigii-NR0G12T	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA-GC-ATCTC	434
Staphylococcus-epidermidis-BBN	CATGCACCACCTGTCACTCTGTCCCC--GAAGGG-GAAAAAC-TCTATCTC	435
Enterobacter-cloacae-478T	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCCATCTC	434
Staphylococcus-warneri	CATGCACCACCTGTCACTTTGTCCCCGAAGGGG-AAAGC--TCTATCTC	435
Klebsiella-variicola	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCCATCTC	434
Citrobacter-freundii	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--AGCATCTC	434
Staphylococcus-warneri-FUA2075	CATGCACCACCTGTCACTTTGTCCCCGAAGGGG-AAAGC--TCTATCTC	435
Klebsiella-variicola-7T	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCCATCTC	434
Enterobacter-mori	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCCATCTC	434
Staphylococcus-epidermidis	CATGCACCACCTGTCACTCTGTCCCC--GAAGGG-GAAAAAC-TCTATCTC	435
Enterobacter-cloacae	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCCATCTC	434
Serratia-marcescens-A4T	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCCATCTC	434
Staphylococcus-pasteuri	CATGCACCACCTGTCACTTTGTCCCC--GAAGGGGAAAGC--TCTATCTC	435
Acinetobacter-baumanni	CATGCAGCACCTGT-ATCTAGATTCCC--GAAGGC-ACCAA--TCCATCTC	434
Staphylococcus-pasteuri-C1P01T	CATGCACCACCTGTCACTTTGTCCCC--GAAGGGGAAAGC--TCTATCTC	435
Acinetobacter-baumanni-DSM300	CATGCAGCACCTGT-ATCTAGATTCCC--GAAGGC-ACCAA--AGCATCTC	434
Klebsiella-pneumoniae	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCCATCTC	434
Staphylococcus-hominis-S9-624T	CATGCACCACCTGTCACTTTGTCCCC--CGAAGG-GGAAACTTCTATCTC	435
Klebsiella-pneumoniae-SDM45T	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCCATCTC	434
Serratia-marcescens	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--TCCATCTC	434
Staphylococcus-hominis	CATGCACCACCTGTCACTTTGTCCCC--CGAAGG-GGAAACTTCTATCTC	435
Citrobacter-freundii-MRB070408	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--AGCATCTC	434
Proteus-penneri	CATGCAGCACCTGTCTCAGGTTTCCC--GAAGGC-ACTCC--TCTATCTC	435
Morganella-morganii-MFS05T	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--AGCATCTC	436
Enterobacter-ludwigii	CATGCAGCACCTGTCTCAGAGTTCCC--GAAGGC-ACCAA--AGCATCTC	434
Lactobacillus-plantarum-KLDS1.	CATGCACCACCTGTATCCATGTCCCC--GAAGGG-AAGCT--CTAATCTC	433
Proteus-penneri-Z2T	CATGCAGCACCTGTCTCAGGTTTCCC--GAAGGC-ACTCC--TCTATCTC	435
Chryseobacterium-vietnamense-G	CATGCAGCACCT-TGAAAAATGT-CC--GAAGA--AAAGT--C-TATTTCT	430
Streptococcus-anginosus-CO2T	CATGCACCACCTGTCAACCGATGTTCC--GAAGA--AACTT--CCTATCTC	432

Pedococcus-acidilactici-L169T	CATGCACCACGTGTCATCTGTGCCCC--GAAGGG-AACGC--CTAATCTC	433
Proteus-mirabilis-ALK419T	CATGCAGCAGCTGTCTCAGCGTTCC--GAAGGC-ACTCC--TCTATCTC	435

Bacillus-cereus	T-AGGGTTGTCAA-AG--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Streptococcus-equis	T-AGGAATAGCATCGG--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Bacillus-cereus-B1T	T-AGGGTTGTCAA-AG--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Streptococcus-equis-KLDS3.06	T-AGGAATAGCATCGG--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Enterococcus-faecalis	T-AG-AGTGGTCAAAG--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Lactococcus-lactis	T-AGGAATAGCAGCAG--TATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Enterococcus-faecium	T-AG-AGTGGTCAAAG--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Enterococcus-faecalis-KLDS4.03	T-AG-AGTGGTCAAAG--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Lactococcus-lactis-LCT	T-AGGAATAGCAGCAG--TATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Enterococcus-faecium-IB1T	T-AG-AGTGGTCAAAG--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Pedococcus-acidilactici	TTAGG--TTGGCAGAA--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Proteus-mirabilis	TAAAGGATTGCGT---GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	481
Lactobacillus-plantarum	T--TAGATTGTCATAG--TATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Morganella-morganii	TGCTAAGTTCTC-TGG--ATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	481
Bacillus-subtilis	TAG--GATTGTC-AGAG-GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Hafnia-alvei	TAGCAAATTTCT-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	481
Chryseobacterium-vietnamense	TAA--ACCTGTC-ATTTCCATTTAAGCCTTGGTAAGGTTCTTCGCGTTG	477
Streptococcus-anginosus	TAG--AAATAGC-ATCGGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Bacillus-subtilis-N11T	TAG--GATTGTC-AGAG-GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Hafnia-alvei-E117T	TAGCAAATTTCT-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	481
Staphylococcus-aureus	TAGAGTTGTCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Escherichia-fergusonii	TGAAAACTTCCG-TG--GATGTCAAGACCAGTAAGGTTCTTCGCGTTG	480
Enterobacter-mori-R3-3T	TGGAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Staphylococcus-aureus-NBRC1271	TAGAGTTGTCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Escherichia-fergusonii-G7T	TGAAAACTTCCG-TG--GATGTCAAGACCAGTAAGGTTCTTCGCGTTG	480
Enterobacter-ludwigii-NRCG12T	TGCTAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Staphylococcus-epidermidis-BBN	TAGAGGGGTCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Enterobacter-cloacae-478T	TGGAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Staphylococcus-warneri	TAGAGCGGTCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Klebsiella-variicola	TGGAAGTTCTG-TG--GATGTCAAGACCAGTAAGGTTCTTCGCGTTG	480
Citrobacter-freundii	TGCTAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Staphylococcus-warneri-FUA2075	TAGAGCGGTCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Klebsiella-variicola-7T	TGGAAGTTCTG-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Enterobacter-mori	TGGAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Staphylococcus-epidermidis	TAGAGGGGTCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Enterobacter-cloacae	TGGAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Serratia-marcescens-A4T	TGGAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Staphylococcus-pasteuri	TAGAGCGGTCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Acinetobacter-baumannii	TGGAAGTTTCT-AG--TATGTCAAGGCCAGTAAGGTTCTTCGCGTTG	480
Staphylococcus-pasteuri-C1P01T	TAGAGCGGTCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Acinetobacter-baumannii-DSM300	TGGAAGTTTCT-AG--TATGTCAAGGCCAGTAAGGTTCTTCGCGTTG	480
Klebsiella-pneumoniae	TGGAAGTTCTG-TG--GATGTCAAGACCAGTAAGGTTCTTCGCGTTG	480
Staphylococcus-hominis-S9-624T	TAGAAGGTCFCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Klebsiella-pneumoniae-SDM45T	TGGAAGTTCTG-TG--GATGTCAAGACCAGTAAGGTTCTTCGCGTTG	480
Serratia-marcescens	TGGAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Staphylococcus-hominis	TAGAAGGTCFCAA-AG--GATGTCAAGATTGGTAAGGTTCTTCGCGTTG	481
Citrobacter-freundii-MRB070408	TGCTAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Proteus-penneri	TAAAGGATTGCG-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	481
Morganella-morganii-MFS05T	TGCTAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	482
Enterobacter-ludwigii	TGCTAAGTTCTC-TG--GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	480
Lactobacillus-plantarum-KLDS1.	TTA--GATTGTCATAGT--ATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Proteus-penneri-Z2T	TAAAGGATTGCG-TGG--ATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	481
Chryseobacterium-vietnamense-G	TAAACCTGTCAATTC--CCATTTAAGCCTTGGTAAGGTTCTTCGCGTTG	477
Streptococcus-anginosus-CO2T	TAGAAATAGCATCGG--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Pedococcus-acidilactici-L169T	TTAGG--TTGGCAGAA--GATGTCAAGACCTGGTAAGGTTCTTCGCGTTG	479
Proteus-mirabilis-ALK419T	TAAAGGATTGCGT---GATGTCAAGAGTAGTAAGGTTCTTCGCGTTG	481
	* * * * *	
Bacillus-cereus	CTTCAAATTAACACCATGCTCCACCGTGGTGGGGCCCCCGTCAATTC	529
Streptococcus-equis	CTTCGAATTAACACCATGCTCCACCGTGGTGGGGCCCCCGTCAATTC	529
Bacillus-cereus-B1T	CTTCAAATTAACACCATGCTCCACCGTGGTGGGGCCCCCGTCAATTC	529
Streptococcus-equis-KLDS3.06	CTTCGAATTAACACCATGCTCCACCGTGGTGGGGCCCCCGTCAATTC	529
Enterococcus-faecalis	CTTCGAATTAACACCATGCTCCACCGTGGTGGGGCCCCCGTCAATTC	529

Lactococcus-lactis CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Enterococcus-faecium CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Enterococcus-faecalis-KLDS4.03 CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Lactococcus-lactis-LCT CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Enterococcus-faecium-IB1T CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Pediococcus-acidilactici CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Proteus-mirabilis CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Lactobacillus-plantarum CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Morganella-morganii CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Bacillus-subtilis CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Hafnia-alvei CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Chryseobacterium-vietnamense CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 527
Streptococcus-anginosus CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Bacillus-subtilis-N11T CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Hafnia-alvei-E117T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Staphylococcus-aureus CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Escherichia-fergusonii CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Enterobacter-mori-R3-3T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Staphylococcus-aureus-NBRC1271 CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Escherichia-fergusonii-G7T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Enterobacter-ludwigii-NRCCG12T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Staphylococcus-epidermidis-BBN CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Enterobacter-cloacae-478T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Staphylococcus-warneri CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Klebsiella-variicola CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Citrobacter-freundii CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Staphylococcus-warneri-FUA2075 CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Klebsiella-variicola-7T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Enterobacter-mori CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Staphylococcus-epidermidis CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Enterobacter-cloacae CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Serratia-marcescens-A4T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Staphylococcus-pasteuri CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Acinetobacter-baummanni CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Staphylococcus-pasteuri-C1P01T CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Acinetobacter-baummanni-DSM300 CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Klebsiella-pneumoniae CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Staphylococcus-hominis-S9-624T CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Klebsiella-pneumoniae-SDM45T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Serratia-marcescens CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Staphylococcus-hominis CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Citrobacter-freundii-MRB070408 CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Proteus-penneri CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Morganella-morganii-MFS05T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 532
Enterobacter-ludwigii CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 530
Lactobacillus-plantarum-KLDS1. CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Proteus-penneri-Z2T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531
Chryseobacterium-vietnamense-G CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 527
Streptococcus-anginosus-CO2T CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Pediococcus-acidilactici-L169T CATCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 529
Proteus-mirabilis-ALK419T CTTCGAATTA AACACACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC 531

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Bacillus-cereus CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Streptococcus-equinus CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Bacillus-cereus-B1T CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Streptococcus-equinus-KLDS3.06 CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Enterococcus-faecalis CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Lactococcus-lactis CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Enterococcus-faecium CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Enterococcus-faecalis-KLDS4.03 CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Lactococcus-lactis-LCT CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Enterococcus-faecium-IB1T CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Pediococcus-acidilactici TTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Proteus-mirabilis ATTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 581
Lactobacillus-plantarum CTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 579
Morganella-morganii ATTTTGAGTTTTCAGCCTTGGCGCGTACTCCCAAGCGGAGTGCTTAATGC 581

Bacillus-subtilis CTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGC 579
Hafnia-alvei ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 581
Chryseobacterium-vietnamense CTTTGAGTTTCAAACTTGCGGTTCGTACTCCCCAGGTGGCTAACTTATAC 577
Streptococcus-anginosus CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 579
Bacillus-subtilis-N11T CTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGC 579
Hafnia-alvei-E117T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 581
Staphylococcus-aureus CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Escherichia-fergusonii ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Enterobacter-mori-R3-3T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Staphylococcus-aureus-NBRC1271 CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Escherichia-fergusonii-G7T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Enterobacter-ludwigii-NRCG12T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Staphylococcus-epidermidis-BBN CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Enterobacter-cloacae-478T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Staphylococcus-warneri CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Klebsiella-variicola ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Citrobacter-freundii ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Staphylococcus-warneri-FUA2075 CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Klebsiella-variicola-7T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Enterobacter-mori ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Staphylococcus-epidermidis CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Enterobacter-cloacae ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Serratia-marcescens-A4T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTAATGC 580
Staphylococcus-pasteuri CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Acinetobacter-baumannii ATTTGAGTTTAACTCTTGCGACCGTACTCCCCAGGCGGTGCGATTATACGC 580
Staphylococcus-pasteuri-ClP01T CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Acinetobacter-baumannii-DSM300 ATTTGAGTTTAACTCTTGCGACCGTACTCCCCAGGCGGTGCGATTATACGC 580
Klebsiella-pneumoniae ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Staphylococcus-hominis-S9-624T CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Klebsiella-pneumoniae-SDM45T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Serratia-marcescens ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Staphylococcus-hominis CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 581
Citrobacter-freundii-MRB070408 ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Proteus-penneri ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 581
Morganella-morganii-MFS05T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTAATGC 582
Enterobacter-ludwigii ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 580
Lactobacillus-plantarum-KLDS1. CTTTGAGTTTCAAGCTTGCGGCCGTACTCCCCAGGCGGAATGCTTAATGC 579
Proteus-penneri-22T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 581
Chryseobacterium-vietnamense-G CTTTGAGTTTCAAACTTGCGTTCGTACTCCCCAGGTGGCTAACTTATAC 577
Streptococcus-anginosus-CO2T CTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGC 579
Pediococcus-acidilactici-L169T TTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGATTACTTAATGC 579
Proteus-mirabilis-ALK419T ATTTGAGTTTAAACCTTGCGGCCGTACTCCCCAGGCGGTGCGATTAAACGC 581

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Bacillus-cereus GTTAACTTCAGCA-----CTAAGGGCGGAAACCCCTTAACACTTAG- 621
Streptococcus-equinus GTTAGCTGCGCA-----CTAAGCCCGGAAAGGGCTTAACACTTAG- 621
Bacillus-cereus-B1T GTTAACTTCAGCA-----CTAAGGGCGGAAACCCCTTAACACTTAG- 621
Streptococcus-equinus-KLDS3.06 GTTAGCTGCGCA-----CTAAGCCCGGAAAGGGCTTAACACTTAG- 621
Enterococcus-faecalis GTTTCGTCAGCA-----CTGAAGGCGGAAACCCCTCAACACTTAG- 621
Lactococcus-lactis GTTAGCTGCGATA-----C-AGAGAAGCTTATAGCTCCCTACACTTAG- 620
Enterococcus-faecium GTTTCGTCAGCA-----CTGAAGGCGGAAACCCCTCAACACTTAG- 621
Enterococcus-faecalis-KLDS4.03 GTTTCGTCAGCA-----CTGAAGGCGGAAACCCCTCAACACTTAG- 621
Lactococcus-lactis-LCT GTTAGCTGCGATA-----C-AGAGAAGCTTATAGCTCCCTACACTTAG- 620
Enterococcus-faecium-IB1T GTTTCGTCAGCA-----CTGAAGGCGGAAACCCCTCAACACTTAG- 621
Pediococcus-acidilactici GTTAGCTGCGCA-----CTGAAGGCGGAAACCCCTCAACACTTAG- 621
Proteus-mirabilis GTTAGCTCCAGAA-----GCCACGGTTCAAGCA-CAACCTCTAAA- 622
Lactobacillus-plantarum GTTAGCTGCGCA-----CTGAAGGCGGAAACCCCTCAACACTTAG- 621
Morganella-morganii GTTAGCTCCGAAGCCACGCTCAAGGGCACAA--CCTCCAA-----G- 622
Bacillus-subtilis GTTAGCTGCGCA-----CTAAGGGCGGAAACCCCTTAACACTTAG- 621
Hafnia-alvei GTTAGCTCCGAAGCCACGCTCAAGGGCACAA--CCTCCAA-----G- 622
Chryseobacterium-vietnamense TTTGCTTAGTCT-----CTGAATCC--GAA--ACCCAAAACGAG- 615
Streptococcus-anginosus GTTAGCTGCGCA-----CTAAGTCCCGGAAAGGACCTAACACTTAG- 621
Bacillus-subtilis-N11T GTTAGCTGCGCA-----CTAAGGGCGGAAACCCCTTAACACTTAG- 621
Hafnia-alvei-E117T GTTAGCTCCGAAGCCACGCTCAAGGGCACAA--CCTCCAA-----G- 622
Staphylococcus-aureus GTTAGCTGCGCA-----CTAAGGGCGGAAACCCCTTAACACTTAG- 623
Escherichia-fergusonii GTTAGCTCCGAAGCCACGCTCAAGGGCACAA--CCTCCAA-----G- 621
Enterobacter-mori-R3-3T GTTAGCTCCGAAGCCACGCTCAAGGGCACAA--CCTCCAA-----G- 621

Staphylococcus-aureus-NBRC1271	GTAGCTGCAGCA-----CTAAGGGGCGGAAACCCCTAACACTTAG- 623
Escherichia-fergusonii-G7T	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Enterobacter-ludwigii-NRCG12T	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Staphylococcus-epidermidis-BBN	GTAGCTGCAGCA-----CTAAGGGGCGGAAACCCCTAACACTTAG- 623
Enterobacter-cloacae-478T	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Staphylococcus-warneri	GTAGCTGCAGCA-----CTAAGGGGCGGAAACCCCTAACACTTAG- 619
Klebsiella-variicola	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Citrobacter-freundii	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Staphylococcus-warneri-FUA2075	GTAGCTGCAGCA-----CTAAGGGGCGGAA--CCCTCTAAC--AC- 619
Klebsiella-variicola-7T	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Enterobacter-mori	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Staphylococcus-epidermidis	GTAGCTGCAGCA-----CTAAGGGGCGGAAACCCCTAACACTTAG- 623
Enterobacter-cloacae	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Serratia-marcescens-A4T	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Staphylococcus-pasteuri	GTAGCTGCAGCA-----CTAAGGGGCGGAAACCCCTAACACTTAG- 623
Acinetobacter-baumanni	GTAGCTGCGCA-----CTAAGG--CTCAAAGGGCCCAACGGCTAG- 621
Staphylococcus-pasteuri-ClP01T	GTAGCTGCAGCA-----CTAAGGGGCGGAAACCCCTAACACTTAG- 623
Acinetobacter-baumanni-DSM300	GTAGCTGCGCA-----CTAAGG--CTCAAAGGGCCCAACGGCTAG- 621
Klebsiella-pneumoniae	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Staphylococcus-hominis-S9-624T	GTAGCTGCAGCA-----CTAAGGGGCGGAA--CCCTCTAAC--AC- 619
Klebsiella-pneumoniae-SDM45T	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Serratia-marcescens	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Staphylococcus-hominis	GTAGCTGCAGCA-----CTAAGGGGCGGAA--CCCTCTAAC--AC- 619
Citrobacter-freundii-MRB070408	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Proteus-penneri	GTAGCTCCGGAAGCCACGGTTCAGGGGCACAA--CCTCTAAA----- 622
Morganella-morganii-MFS05T	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 623
Enterobacter-ludwigii	GTAGCTCCGGAAGCCACGCCTCAAGGGGCACAA--CCTCCAA-----G- 621
Lactobacillus-plantarum-KLDS1.	GTAGCTGCAGCA-----CTAAGGGGCGGAAACCCCTCAACACTTAG- 622
Proteus-penneri-Z2T	GTAGCTCCAGAA-----GCCACGGTTCAGACCA--CAACCTCTAA- 621
Chryseobacterium-vietnamense-G	TTTCGCTTAGTCT-----CTGAATCC--GAAA--ATCAAAAACAG- 615
Streptococcus-anginosus-CO2T	GTAGCTGCGCA-----CTAAGTCCCGGAAAGGACCTCAACACTTAG- 621
Pediococcus-acidilactici-L169T	GTAGCTGCAGCA-----CTAAGGGGCGGAAACCCCTCAACACTTAG- 621
Proteus-mirabilis-ALK419T	GTAGCTCCAGAA-----GCCACGGTTCAGACCA--CAACCTCTAAA- 622
	* * * *
Bacillus-cereus	-CACTCA---TCGTTTACGGCGTGAATACCAAGGGTATCTAATCCTGTT 666
Streptococcus-equinus	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Bacillus-cereus-B1T	-CACTCA---TCGTTTACGGCGTGAATACCAAGGGTATCTAATCCTGTT 666
Streptococcus-equinus-KLDS3.06	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Enterococcus-faecalis	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Lactococcus-lactis	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 665
Enterococcus-faecium	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Enterococcus-faecalis-KLDS4.03	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Lactococcus-lactis-LCT	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 665
Enterococcus-faecium-IB1T	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Pediococcus-acidilactici	-TAATCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Proteus-mirabilis	-TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 667
Lactobacillus-plantarum	-CATTCA---TCGTTTACGGCTATGGACTACCAAGGGTATCTAATCCTGTT 666
Morganella-morganii	-TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 667
Bacillus-subtilis	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Hafnia-alvei	-TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 667
Chryseobacterium-vietnamense	-TTAGCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Streptococcus-anginosus	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Bacillus-subtilis-M11T	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Hafnia-alvei-E117T	-TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 667
Staphylococcus-aureus	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Escherichia-fergusonii	-TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Enterobacter-mori-R3-3T	-TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Staphylococcus-aureus-NBRC1271	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Escherichia-fergusonii-G7T	-TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Enterobacter-ludwigii-NRCG12T	-TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Staphylococcus-epidermidis-BBN	-CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Enterobacter-cloacae-478T	-TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Staphylococcus-warneri	-TTAGCACTCATCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Klebsiella-variicola	-TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Citrobacter-freundii	-TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Staphylococcus-warneri-FUA2075	-TTAGCACTCATCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668

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Klebsiella-variicola-7T -TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Enterobacter-mori -TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Staphylococcus-epidermidis -CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Enterobacter-cloacae -TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Serratia-marcescens-A4T -TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Staphylococcus-pasteuri -CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Acinetobacter-baumanni -TAGACA---TCGTTTACGGCATGGACTACCAAGGGTATCTAATCCTGTT 666
Staphylococcus-pasteuri-ClP01T -CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Acinetobacter-baumanni-DSM300 -TAGACA---TCGTTTACGGCATGGACTACCAAGGGTATCTAATCCTGTT 666
Klebsiella-pneumoniae -TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Staphylococcus-hominis-S9-624T -TTAGCACTCATCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Klebsiella-pneumoniae-SDM45T -TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Serratia-marcescens -TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Staphylococcus-hominis -TTAGCACTCATCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Citrobacter-freundii-MRB070408 -TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Proteus-penneri -TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 667
Morganella-morganii-MFS05T -TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 668
Enterobacter-ludwigii -TCGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Lactobacillus-plantarum-KLDS1. -ATT--CA---TCGTTTACGGTATGGACTACCAAGGGTATCTAATCCTGTT 666
Proteus-penneri-Z2T -ATCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 667
Chryseobacterium-vietnamense-G -TTAGACA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 660
Streptococcus-anginosus-CO2T -CACTCA---TCGTTTACGGCGTGGACTACCAAGGGTATCTAATCCTGTT 666
Pediococcus-acidilactici-L169T -TAATCA---TCGTTTACGGCATGGACTACCAAGGGTATCTAATCCTGTT 666
Proteus-mirabilis-ALK419T -TCGACA---TCGTTTACAGCGTGGACTACCAAGGGTATCTAATCCTGTT 667
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Bacillus-cereus TGCTCCCCACG-CTTTCGCGCCTCAGTGTCAAGTTACAGACCAGAAAAGTC 715
Streptococcus-equinus TGCTCCCCACG-CTTTCGAGCCTCAGCGTCAAGTTACAGACCAGAGA-GCC 714
Bacillus-cereus-B1T TGCTCCCCACG-CTTTCGCGCCTCAGTGTCAAGTTACAGACCAGAAAAGTC 715
Streptococcus-equinus-KLDS3.06 TGCTCCCCACG-CTTTCGAGCCTCAGCGTCAAGTTACAGACCAGAGA-GCC 714
Enterococcus-faecalis TGCTCCCCACG-CTTTCGAGCCTCAGCGTCAAGTTACAGACCAGAGA-GCC 714
Lactococcus-lactis TGCTCCCCACG-CTTTCGAGCCTCAGTGTCAAGTTACAGGCCAGAGA-GCC 713
Enterococcus-faecium TGCTCCCCACG-CTTTCGAGCCTCAGCGTCAAGTTACAGACCAGAGA-GCC 714
Enterococcus-faecalis-KLDS4.03 TGCTCCCCACG-CTTTCGAGCCTCAGCGTCAAGTTACAGACCAGAGA-GCC 714
Lactococcus-lactis-LCT TGCTCCCCACG-CTTTCGAGCCTCAGCGTCAAGTTACAGGCCAGAGA-GCC 713
Enterococcus-faecium-IB1T TGCTCCCCACG-CTTTCGAGCCTCAGCGTCAAGTTACAGACCAGAGA-GCC 714
Pediococcus-acidilactici CGCTACCCATG-CTTTCGAGCCTCAGCGTCAAGTTACAGACCAGAGA-GCC 714
Proteus-mirabilis TGCTCCCCACG-CTTTCGACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 715
Lactobacillus-plantarum TGCTACCCATA-CTTTCGAGCCTCAGCGTCAAGTTACAGACCAGAGA-GCC 714
Morganella-morganii TGCTCCCCACG-CTTTCGACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 715
Bacillus-subtilis CGCTCCCCACG-CTTTCGCTCCTCAGCGTCAAGTTACAGACCAGAGA-GTC 714
Hafnia-alvei TGCTCCCCACG-CTTTCGACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 715
Chryseobacterium-vietnamense CGCTCCCCACG-CTTTCGCTCCATCAGCGTCAAGTCTTGTCTAGTAA-CCT 708
Streptococcus-anginosus CGCTCCCCACG-CTTTCGAGCCTCAGCGTCAAGTTACAGACCAGAGA-GCC 714
Bacillus-subtilis-N11T CGCTCCCCACG-CTTTCGCTCCTCAGCGTCAAGTTACAGACCAGAGA-GTC 714
Hafnia-alvei-E117T TGCTCCCCACG-CTTTCGACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 715
Staphylococcus-aureus TGATCCCCACG-CTTTCGCACATCAGCGTCAAGTCTTTGTCACGGGG-GCC 716
Escherichia-fergusonii TGCTCCCCACG-CTTTCGACCTCAGCGTCAAGTCTTCGTCACGGGG-GCC 714
Enterobacter-mori-R3-3T TGCTCCCCACG-CTTTCGCACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 714
Staphylococcus-aureus-NBRCl271 TGATCCCCACG-CTTTCGCACATCAGCGTCAAGTTACAGACCAGAAA-GTC 716
Escherichia-fergusonii-67T TGCTCCCCACG-CTTTCGCACCTCAGCGTCAAGTCTTCGTCACGGGG-GCC 714
Enterobacter-ludwigii-NRCG12T TGATCCCCACG-CTTTCGCACATCAGCGTCAAGTTACAGACCAGAAA-GTC 716
Staphylococcus-epidermidis-BBN TGCTCCCCACG-CTTTCGCACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 714
Enterobacter-cloacae-478T TGATCCCCACG-CTTTCGCACATCAGCGTCAAGTTACAGACCAGAAA-GTC 716
Staphylococcus-warneri TGCTCCCCACG-CTTTCGCACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 714
Klebsiella-variicola TGCTCCCCACG-CTTTCGCACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 714
Citrobacter-freundii TGATCCCCACG-CTTTCGCACATCAGCGTCAAGTCTTTGTCACGGGG-GCC 716
Staphylococcus-warneri-FUA2075 TGATCCCCACG-CTTTCGCACATCAGCGTCAAGTCTTTGTCACGGGG-GCC 716
Klebsiella-variicola-7T TGCTCCCCACG-CTTTCGCACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 714
Enterobacter-mori TGCTCCCCACG-CTTTCGCACCTCAGCGTCAAGTCTTTGTCACGGGG-GCC 714
Staphylococcus-epidermidis TGATCCCCACG-CTTTCGCACATCAGCGTCAAGTTACAGACCAGAAA-GTC 716
Enterobacter-cloacae TGCTCCCCACG-CTTTCGCACCTCAGCGTCAAGTCTTCGTCACGGGG-GCC 714
Serratia-marcescens-A4T TGATCCCCACG-CTTTCGCACATCAGCGTCAAGTTACAGACCAGAAA-GTC 716
Staphylococcus-pasteuri TGCTCCCCACG-CTTTCGCACATCAGCGTCAAGTCTTTGTCACGGGG-GCC 716
Acinetobacter-baumanni TGCTCCCCATG-CTTTCGTACCTCAGCGTCAAGTATTAGGCCAGATG-GCT 714
Staphylococcus-pasteuri-ClP01T TGATCCCCACG-CTTTCGCACATCAGCGTCAAGTATTACAGACCAGAAA-GTC 716
Acinetobacter-baumanni-DSM300 TGCTCCCCATG-CTTTCGTACCTCAGCGTCAAGTATTAGGCCAGATG-GCT 714

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Lactobacillus-plantarum-KLDS1.	GCCTTCGCCACTGGTGTCTTCCATATATCTACGCAATTTCCACGCTACAC	764
Proteus-penneri-22T	GCCTTCGCCACCGTATTCTCCACATATCTACGCAATTTCCACGCTACAC	765
Chryseobacterium-vietnamense-G	GCCTTCGCAATTTGGTGTCTTAAGTAATATCTATGCAATTTCCACGCTACAC	758
Streptococcus-anginosus-C02T	GCTTTTCGCCACCGTGTCTCCATATATCTACGCAATTTCCACGCTACAC	764
Pediococcus-acidilactici-L169T	GCCTTCGCCACTGGTGTCTTCCATATATCTACGCAATTTCCACGCTACAC	764
Proteus-mirabilis-ALK419T	GCCTTCGCCACCGTATTCTCCACATCTCTACGCAATTTCCACGCTACAC	765
	*** **	
Bacillus-cereus	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCAATG	814
Streptococcus-equinus	ATGGAATTCACCTTCCC-CCTTCTGCACCTCAAGTCTTAACAGTTTCCA-A	812
Bacillus-cereus-B1T	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCAATG	814
Streptococcus-equinus-KLDS3.06	ATGGAATTCACCTTCCC-CCTTCTGCACCTCAAGTCTTAACAGTTTCCA-A	812
Enterococcus-faecalis	ATGGAATTCACCTTCCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCAATG	813
Lactococcus-lactis	ATGGAATTCACCTTCCC-TCTCCTGCACCTCAAGTCTACCAGTTTCCAATG	812
Enterococcus-faecium	ATGGAATTCACCTTCCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCAATG	813
Enterococcus-faecalis-KLDS4.03	ATGGAATTCACCTTCCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCAATG	813
Lactococcus-lactis-LCT	ATGGAATTCACCTTCCC-TCTCCTGCACCTCAAGTCTACCAGTTTCCAATG	812
Enterococcus-faecium-IB1T	ATGGAATTCACCTTCCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCAATG	813
Pediococcus-acidilactici	ATGGAATTCACCTGTCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCAATG	813
Proteus-mirabilis	ATGGAATTCACCCCC-TCTACAAGACTCTAGCCATCCAGATTTCCAGATG	814
Lactobacillus-plantarum	ATGGAATTCACCTGTCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCGATG	813
Morganella-morganii	ATGGAATTCACCCCC-TCTACAAGACTCTAGCTGACCATATCAGATG	814
Bacillus-subtilis	GTGGAATTCACCTTCCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCAATG	813
Hafnia-alvei	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCAAATG	814
Chryseobacterium-vietnamense	TACTTATTCAGCTACT-TCAACAACACTCAAGCTTGCCAGTATCAATGG	807
Streptococcus-anginosus	ATGGAATTCACCTTCCC-CCTTCTGCACCTCAAGTTAAACAGTTTCCAAG	813
Bacillus-subtilis-N11T	GTGGAATTCACCTTCCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	813
Hafnia-alvei-E117T	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCAAATG	814
Staphylococcus-aureus	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Escherichia-fergusonii	CTGGAATTCACCCCC-TCTACGAGACTCAAGCTTGCCAGTATCAGATG	813
Enterobacter-mori-R3-3T	CTGGAATTCACCCCCCTCTACAAGACTCTAGCTTGCCAGTTTCCAATG	814
Staphylococcus-aureus-NBRC1271	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Escherichia-fergusonii-G7T	CTGGAATTCACCCCC-TCTACGAGACTCAAGCTTGCCAGTATCAGATG	813
Enterobacter-ludwigii-NRCC12T	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Staphylococcus-epidermidis-BBN	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Enterobacter-cloacae-478T	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Staphylococcus-warneri	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Klebsiella-variicola	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Citrobacter-freundii	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Staphylococcus-warneri-FUA2075	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Klebsiella-variicola-7T	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Enterobacter-mori	CTGGAATTCACCCCCCTCTACAAGACTCTAGCTTGCCAGTTTCCAATG	814
Staphylococcus-epidermidis	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Enterobacter-cloacae	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Serratia-marcescens-A4T	CTGGAATTCACCCCC-TCTACGAGACTCTAGCTTGCCAGTTTCCAATG	813
Staphylococcus-pasteuri	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Acinetobacter-baumannii	CTGGAATTCACCATCC-TCTCCCATCTCTAGCTTGCCAGTTTCCAATG	813
Staphylococcus-pasteuri-C1P01T	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Acinetobacter-baumannii-DSM300	CTGGAATTCACCATCC-TCTCCCATCTCTAGCTTGCCAGTTTCCAATG	813
Klebsiella-pneumoniae	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Staphylococcus-hominis-S9-624T	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Klebsiella-pneumoniae-SDM45T	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Serratia-marcescens	CTGGAATTCACCCCC-TCTACGAGACTCTAGCTTGCCAGTTTCCAATG	813
Staphylococcus-hominis	ATGGAATTCACCTTTCC-TCTTCTGCACCTCAAGTTTCCAGTTTCCAATG	815
Citrobacter-freundii-MRB070408	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Proteus-penneri	ATGGAATTCACCCCC-TCTACAAGACTCTAGCCATCCAGATTTCCAGATG	814
Morganella-morganii-MFS05T	ATGGAATTCACCCCC-TCTACAAGACTCTAGCTGACCATATCAGATG	816
Enterobacter-ludwigii	CTGGAATTCACCCCC-TCTACAAGACTCTAGCTTGCCAGTTTCCAATG	813
Lactobacillus-plantarum-KLDS1.	ATGGAATTCACCTGTCC-TCTTCTGCACCTCAAGTTTCCCAGTTTCCGATG	813
Proteus-penneri-22T	ATGGAATTCACCCCC-TCTACAAGACTCTAGCCAACAGTTTCCAGATG	814
Chryseobacterium-vietnamense-G	TACTTATTCAGCTACT-TCAACAACACTCAAGCTTGCCAGTATCAATGG	807
Streptococcus-anginosus-C02T	ATGGAATTCACCTTCCC-CCTTCTGCACCTCAAGTTAAACAGTTTCCAAG	813
Pediococcus-acidilactici-L169T	ATGGAATTCACCTGTCC-TCTTCTGCACCTCAAGTCTCCCAGTTTCCAATG	813
Proteus-mirabilis-ALK419T	ATGGAATTCACCCCC-TCTACAAGACTCTAGCCAACAGTTTCCAATG	814
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Bacillus-cereus	ACCTTCACCGTTGAGCGTGGGCTTTACATCAGACTTAAGAAACACC	864

Bacillus-cereus	TGCGGCGGGTTACGCC-ATAAATCCGGATA	896
Streptococcus-equinus	TGCGGCGGGTTACGCC-ATAAATCCGGACA	894
Bacillus-cereus-BIT	TGCGGCGGGTTACGCC-ATAAATCCGGATA	896
Streptococcus-equinus-KLDS3.06	TGCGGCGGTTTACGCC-ATAAATCCGGACA	894
Enterococcus-faecalis	TGCGGCGGTTTACGCC-ATAAATCCGGACA	895
Lactococcus-lactis	TGCGGCGGTTTACGCC-ATAAATCCGGACA	893
Enterococcus-faecium	TGCGGCGGTTTACGCC-ATAAATCCGGACA	895
Enterococcus-faecalis-KLDS4.03	TGCGGCGGTTTACGCC-ATAAATCCGGACA	895
Lactococcus-lactis-LCT	TGCGGCGGTTTACGCC-ATAAATCCGGACA	893
Enterococcus-faecium-IRIT	TGCGGCGGTTTACGCC-ATAAATCCGGACA	895

Pediococcus-acidilactici	TGCGCTCGCTTTACGCCC-AATAAATCCGATA	895
Proteus-mirabilis	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
Lactobacillus-plantarum	TGCGCTCGCTTTACGCCC-AATAAATCCGGACA	895
Morganella-morganii	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
Bacillus-subtilis	TGCGAGCCCTTTACGCCC-AATAATTCGGACA	895
Hafnia-alvei	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
Chryseobacterium-vietnamense	TACGGACCCTTTAAACCC-AATAAATCCGGATA	899
Streptococcus-anginosus	TGCGCTCGCTTTACGCCC-AATAAATCCGGACA	884
Bacillus-subtilis-N11T	TGCGAGCCCTTTACGCCC-AATAATTCGGACA	895
Hafnia-alvei-E117T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
Staphylococcus-aureus	TACGCGCCTTTACGCCC-AATAATTCGGATA	897
Escherichia-fergusonii	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Enterobacter-mori-R3-3T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
Staphylococcus-aureus-NBRC1271	TACGGCGCCTTTACGCCC-AATAATTCGGATA	897
Escherichia-fergusonii-G7T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Enterobacter-ludwigii-NRCG12T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	894
Staphylococcus-epidermidis-BBN	TACGCGGCCTTTACGCCC-AATAATTCGGATA	897
Enterobacter-cloacae-478T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Staphylococcus-warneri	TACGCGGCCTTTACGCCC-AATAATTCGGATA	897
Klebsiella-variicola	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Citrobacter-freundii	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Staphylococcus-warneri-FUA2075	TACGCGGCCTTTACGCCC-AATAATTCGGATA	897
Klebsiella-variicola-7T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Enterobacter-mori	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
Staphylococcus-epidermidis	TACGCGGCCTTTACGCCC-AATAATTCGGATA	897
Enterobacter-cloacae	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Serratia-marcescens-A4T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Staphylococcus-pasteuri	TACGCGCCTTTACGCCC-AATAATTCGGATA	897
Acinetobacter-baumannii	TACGCGCCTTTACGCCC-AGTAATTCGGATA	895
Staphylococcus-pasteuri-C1P01T	TACGCGCCTTTACGCCC-AGTAATTCGGATA	895
Acinetobacter-baumannii-DSM300	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Klebsiella-pneumoniae	TACGCGGCCTTTACGCCC-AATAATTCGGATA	897
Staphylococcus-hominis-S9-624T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Klebsiella-pneumoniae-SDM45T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Serratia-marcescens	TACGCGGCCTTTACGCCC-AATAATTCGGATA	897
Staphylococcus-hominis	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	895
Citrobacter-freundii-MRB070408	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
Proteus-penneri	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
Morganella-morganii-MFS05T	TGCGTGGCCTTTACGCCCCAGTAATTCGGATA	899
Enterobacter-ludwigii	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	894
Lactobacillus-plantarum-KLDS1.	TGCGCTCGCTTTACGCCC-AATAAATCCGGACA	895
Proteus-penneri-Z2T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
Chryseobacterium-vietnamense-G	TACGGACCCTTTAAACCC-AATAAATCCGGATA	889
Streptococcus-anginosus-CO2T	TGCGCTCGCTTTACGCCC-AATAAATCCGGACA	894
Pediococcus-acidilactici-L169T	TGCGCTCGCTTTACGCCC-AATAAATCCGGATA	895
Proteus-mirabilis-ALK419T	TGCGTGGCCTTTACGCCC-AGTAATTCGGATA	896
	* * * * *	

❖ Appendix-2

A-1- Enterobacter ludwigii partial 16S rDNA gene, strain IRQBAS1.(GeneBank).



Enterobacter ludwigii partial 16S rDNA gene, strain IRQBAS1.

Gen Bank: HG003646.1

[FASTAGraphics](#)

[Go to:](#)

LOCUS	HG003646	1443 bp	DNA	linear	BCT 27-JUN-2013
DEFINITION	Enterobacter ludwigii partial 16S rRNA gene, strain IRQBAS1.				
ACCESSION	HG003646				
VERSION	HG003646.1 GI:511630606				
KEYWORDS	.				
SOURCE	Enterobacter ludwigii				
ORGANISM	Enterobacter ludwigii Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;Enterobacteriaceae; Enterobacter.				
REFERENCE	1				
AUTHORS	Abd Al-Abbas,M.J. and Hussein,K.A.				
TITLE	Genetic study of bacteria from denture and orthopedic tools				
JOURNAL	Unpublished				
REFERENCE	2 (bases 1 to 1443)				
AUTHORS	Abd Al-Abbas,M.				
TITLE	Direct Submission				
JOURNAL	Submitted (30-APR-2013) Biology, University of Basrah, Iraq/Basrah/University of Basrah/Biology Departmet, 61004, IRAQ				
FEATURES	Location/Qualifiers				
source	1..1443 /organism="Enterobacter ludwigii"				

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        /strain="IRQBAS1 = NRCG12"
        /isolation_source="orthopedic tools"
        /db_xref="taxon:299767"
gene      <1..>1443
          /gene="16S rRNA"
rRNA     <1..>1443
          /gene="16S rRNA"
          /product="16S ribosomal RNA"

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ORIGIN

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61  cgagtggcgg acgggtgagt aatgtctcgg aaactgcctg atggaggggg ataactactg
121 gaaacggtag ctaataccgc ataacgtcgc aagaccaaag agggggacct tcgggcctct
181 tgccatcaga tgtgccca ga tgggattagc tagtaggtgg ggtaacggct cacctaggcg
241 acgatcccta gctggctcga gaggatgacc agccacactg gaaactgagac acggtccaga
301 ctccctacggg aggcagcagt ggggaatatt gcacaatggg cgcaagcctg atgcagccat
361 gccgcgtgta tgaagaagcc ctctcgggtt taaagtactt tcagcgggga ggaagcgcat
421 aaggttaata accttgtcga ttgacgttac ccgcagaaga agcaccggct aactccgtgc
481 cagcagccgc ggtaatacgg aggggtgcaag cgttaatcgg aattactggg cgtaaagcgc
541 acgcaggcgg totgtcaagt cggtatgtga atcccgggct caacctggga actgcattcg
601 aaactggcag gctagagtct ttagaggggg ggtagaattc caggtgtagc ggtgaaatgc
661 gtagagatct ggaggaatac cggtaggcga ggcggccccc tggacaaaga ctgacgtcga
721 ggtgcgaaag cgtggggagc aaacaggatt agataccctg gtagtccacg ccgtaaacga
781 tgtcgacttg gaggttgtgc ccttgaggcg tggcttccgg agctaacgga ttaagtcgac
841 gcgcctgggga gtacggccgc aagggttaaaa ctcaaatagaa ttgacggggg cccgcacaag
901 cggtaggagca tgtggtttaa ttcatgcaaa ccgcaagaac cttacctact cttgacatcc
961 agagaactta gcagagatgc tttggtgcct tcgggaactc tgagacaggt gctgcatggc
1021 tgtcgtcagc tcgtgttgtg aaatgttggg ttaagtcccc caacgagcgc aaccttatac
1081 ctttgttgcc agcggtttag ccgggaactc aaaggagact gccagtata aactggagga
1141 aggtggggat gacgtcaagt catcatggcc cttacgagta gggctacaca cgtgctacaa
1201 tggcgcatac aaagagaagc gacctcgcga gagcaagcgg acctcataaa gtgcgtcgta
1261 gtccggattg gagtctgcaa ctgcactcca tgaagtcgga atcgctagta atcgtggatc
1321 agaatgccac ggtgaatacg ttcccgggcc ttgtacacac cgcccgtcac accatgggag
1381 tgggttgcaa aagaagtagg tagcttaacc ttccgggagg cgcttaccac tttgtgattg
1441 ctg

```

//

**B-1- *Enterobacter cloacae* partial 16SrDNA gene, strain IRQBAS2.
(GeneBank)**



***Enterobacter cloacae* partial 16SrDNA gene, strain IRQBAS2.**

GenBank: HG003647.1

[FASTAGraphics](#)

[Go to:](#)

LOCUS HG003647 1430 bp DNA linear BCT 27-JUN-2013
DEFINITION *Enterobacter cloacae* partial 16S rRNA gene, strain IRQBAS2.
ACCESSION HG003647
VERSION HG003647.1 GI:511630607
KEYWORDS .
SOURCE *Enterobacter cloacae*
ORGANISM [Enterobacter cloacae](#)
Bacteria; Proteobacteria; Gammaproteobacteria;
Enterobacteriales;Enterobacteriaceae; Enterobacter; Enterobacter
cloacae complex.
REFERENCE 1
AUTHORS Abd Al-Abbas,M.J. and Hussein,K.A.
TITLE Genetic study of bacteria from denture and orthopedic tools
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1430)
AUTHORS Abd Al-Abbas,M.
TITLE Direct Submission
JOURNAL Submitted (30-APR-2013) Biology, University of Basrah,
Iraq/Basrah/University of Basrah/Biology Departmet, 61004, IRAQ
FEATURES Location/Qualifiers
source 1..1430
/organism="Enterobacter cloacae"

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        /mol_type="genomic DNA"
        /strain="IRQBAS2 = Nr. 3"
        /isolation_source="orthopedic tools"
        /db_xref="taxon:550"
gene      <1..>1430
          /gene="16S rRNA"
rRNA     <1..>1430
          /gene="16S rRNA"
          /product="16S ribosomal RNA"

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ORIGIN

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    1  cacatgcaag tcgaacggta acaggaagca gcttgctgct tcgctgacga gtggcggacg
   61  ggtgagtaat gtctgggaaa ctgcctgatg gagggggata actactggaa acggtagcta
  121  ataccgcata acgtcgcaag accaaagagg gggaccttcg ggcctcttgc catcggatgt
  181  gcccagatgg gattagctag taggtggggg aacgggtcac ctaggcgacg atccctagct
  241  ggtctgagag gatgaccagc cacactggaa ctgagacacg gtccagactc ctacgggagg
  301  cagcagtggt gaatatgtca caatgggctc aagcctgatg cagccatgcc gcgtgtatga
  361  agaaggcctt cgggttgtaa agtactttca gcggggagga aggcgacagg gttaataacc
  421  ctgtcgattg acgttacccg cagaagaagc accggctaac tccgtgccag cagccgcggt
  481  aatacggagg gtgcaagcgt taatcggaat tactgggcgt aaagcgacag caggcggctc
  541  gtcaagtcgg atgtgaaatc cccgggctca acctgggaac tgcattcgaa actggcaggc
  601  tagagtcttg tagagggggg tagaattcca ggtgtagcgg tgaatgctg agagatctgg
  661  aggaataccg gtggcgaagg cggccccctg gacaaagact gacgtccagg tgcgaaagcg
  721  tggggagcaa acaggattag ataccctggt agtccacgcc gtaaacgatg tcgacttgga
  781  ggttggtgcc ttgaggcgtg gcttccggag ctaacgcgtt aagtcgacgg cctggggagt
  841  acggccgcaa ggttaaaact caaatgaatt gacgggggcc cgcacaagcg gtggagcatg
  901  tgggtttaatt cgatgcaacg cgaagaacct tacctactct tgacatccag agaactttag
  961  agagatgctt tgggtgccttc gggaaactctg agacaggtgc tgcattggct tcgtcagctc
 1021  gtgttgtgaa atgttgggtt aagtcgccga acgagcgcaa cccttatcct ttgttgccag
 1081  cgtgccggcc gggaaactca aggagactgc cagtgataaa ctggagggaag gtggggatga
 1141  cgtcaagtca tcatggccct tacgagtagg gctacacacg tgctacaagt gcgcatacaa
 1201  agagaagcga cctcgcgaga gcaagcggac ctcataaagt gcgtcgtagt ccggaattgga
 1261  gtctgcaact cgactccatg aagtcggaat cgctagtaat cgtggatcag aatgccacgg
 1321  tgaatacgtt cccgggcctt gtacacaccg cccgtcacac catgggagtg ggttgcaaaa
 1381  gaagtaggta gcttaacctt cgggagggcg cttaccactt tgatagtgtc

```

//

C-1- *Chryseobacterium vietnamense* partial *16SrDNA* gene, strain **IRQBAS3**. (GeneBank).



Chryseobacterium vietnamense partial *16S rDNA* gene, strain **IRQBAS3**.

GenBank: HG003648.1

[FASTAGraphics](#)

[Go to:](#)

```

LOCUS      HG003648 1356 bp      DNA      linear      BCT 27-JUN-2013
DEFINITION Chryseobacterium vietnamense partial 16S rRNA gene, strain IRQBAS3.
ACCESSION  HG003648
VERSION    HG003648.1  GI:511630629
KEYWORDS   .
SOURCE     Chryseobacterium vietnamense
  ORGANISM Chryseobacterium vietnamense
            Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales;
            Flavobacteriaceae; Chryseobacterium.

REFERENCE  1
  AUTHORS  Abd Al-Abbas,M.J. and Hussein,K.A.
  TITLE    Genetic study of bacteria from denture and orthopedic tools
  JOURNAL  Unpublished
REFERENCE  2 (bases 1 to 1356)
  AUTHORS  Abd Al-Abbas,M.
  TITLE    Direct Submission
  JOURNAL  Submitted (30-APR-2013) Biology, University of Basrah,
            Iraq/Basrah/University of Basrah/Biology Departmet, 61004, IRAQ

FEATURES             Location/Qualifiers
     source            1..1356
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                       /mol_type="genomic DNA"
                       /strain="IRQBAS3 = GIMN1.005"
                       /isolation_source="denture tools"
                       /db_xref="taxon:866785"
     gene              <1..>1356
  
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/ gene="16S rRNA"
rRNA <1..>1356
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/ product="16S ribosomal RNA"

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ORIGIN

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61  attaatatccc cataatatat tggatggcat catctggtat tgaanaactcc ggtggataga
121 gatggggcagc cgcaagatta gatagtgggt gaggtaacgg ctcaccaagt ctacgatctt
181 tagggggcct gagaggggtga tccccacac tggtactgag acacggacca gactcctacg
241 ggaggcagca gtgaggaata ttggacaatg ggtgagagcc tgaatccagc atcccgcgtg
301 aaggacgacg gccctatggg ttgtaaactt cttttgtata gggataaacc tagatacgtg
361 tatctagctg aaggctactat acgaataagc accggctaac tccgtgccag cagccgcggt
421 aatacggagg gtgcaagcgt tatccggatt tattgggttt aaaggggccg taggctgatt
481 tgtaagtcag tgggtaaaac tcacagctta actgtgaaac tgccattgat actgcaagtc
541 ttgagtgttg ttgaagtagc tgggaataagt agtgtagcgg tgaatgcat agatattact
601 tagaacacca attgcgaagg caggttacta agcaacaact gacgctgatg gacgaaagcg
661 tgggggagcga acaggattag ataccctggt agtccacgcc gtaaaccgat ctaactcgtt
721 tttgggtttt cggattcaga gactaagcga aagtgataag ttagccacct ggggagtacg
781 aacgcaagtt tgaaactcaa aggaattgac gggggcccg ccaagcggtg gattatgtgg
841 tttaattcga tgatacgca ggaaccttac caaggcttaa atgggaaatg acaggtttag
901 aaatagactt ttcttcggac atttttcaag gtgctgcatg gttgtcgta gctcgtgccg
961 tgaggtgtta ggttaagtcc tgcaacgagc gcaaccctg tcactagtgt ccatcattaa
1021 gttggggact ctagtgagac tgccacgca agtagagagg aagtgggga tgacgtcaaa
1081 tcatcacggc ccttacgcct tgggccacac acgtaataca atggccggtg cagagggcag
1141 ctacacagtg atgtgatgca aatctcgaaa gccgggtctca gttcggattg gagtctgcaa
1201 ctcgactcta tgaagctgga atcgctagta atcgcgcatc agccatggcg cgtggaatac
1261 gttcccggtc cttgtacaca ccgccgtca agccatggaa gtctggggta cctgaagtcg
1321 gtgaccgtaa caggagctgc gtaggtaagc atgcccg

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//

D-1- *Morganella morganii* partial 16S rDNA gene, strain IRQBAS4
(GeneBank).



***Morganella morganii* partial 16S rDNA gene, strain IRQBAS4**

GenBank: HG003649.1

[FASTAGraphics](#)

[Go to:](#)

LOCUS HG003649 1451 bp DNA linear BCT 27-JUN-2013
DEFINITION *Morganella morganii* partial 16S rRNA gene, strain IRQBAS4.
ACCESSION HG003649
VERSION HG003649.1 GI:511630630
KEYWORDS .
SOURCE *Morganella morganii*
ORGANISM *Morganella morganii*
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; *Morganella*.
REFERENCE 1
AUTHORS Abd Al-Abbas, M.J. and Hussein, K.A.
TITLE Genetic study of bacteria from denture and orthopedic tools
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1451)
AUTHORS Abd Al-Abbas, M.
TITLE Direct Submission
JOURNAL Submitted (30-APR-2013) Biology, University of Basrah,
Iraq/Basrah/University of Basrah/Biology Department, 61004, IRAQ
FEATURES Location/Qualifiers
source 1..1451
/organism="Morganella morganii"
/mol_type="genomic DNA"
/strain="IRQBAS4 = MFS05"
/isolation_source="denture tools"
/db_xref="taxon:582"
gene <1..>1451
/gene="16S rRNA"

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rRNA          <1..>1451
               /gene="16S rRNA"
               /product="16S ribosomal RNA"

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ORIGIN

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   61 ctgctgacga cggcgggacg ggtgagtaat gtatggggat ctgcctgatg gcgggggata
  121 actactggaa acggtagcta ataccgcata atgtcttcgg accaaagcgg gggaccttcg
  181 ggccctcgcg catcagatga acccatatgg gattagcttg taggtgaggt aacggctcac
  241 ctaggcgacg atccctagct ggtctgagag gatgatcagc cacctggga ctgagacacg
  301 gccagactc ctacgggagg cagcagtggg gaatatgtga caatgggcgc aagcctgatg
  361 cagccatgcc cgtgttatga agaaggcctt cgggttgtaa agtactttca gtcgggagga
  421 aggtggtaag gttaataacc ttatcaattg acgttaccga cagaagaagc accggctaac
  481 tcctgtccag cagccgcggt aatacggagg gtgcaagcgt taatcggaat tactgggcgt
  541 aaagcgcacg caggcgggtg attgagtcag atgtgaaatc cccgggctta acccggaat
  601 tgcattctgat actgttcagc tagagtcttg tagagggggg tagaattcca tgtgtagcgg
  661 tgaaatgcgt agagatgtgg aggaataacc gtggcgaaag cggccccctg gacaaagact
  721 gacgctcagc tgcgaaaagc tggggagcaa acaggattag ataccctggt agtccacgct
  781 gtaaacgatg tcgacttgga ggttgtgccc ttgaggcgtg gcttccggag ctaacgcgtt
  841 aagtcgacgc cctggggagt acggccgcaa ggttaaaact caaatgaatt gacggggggc
  901 cgcacaagcg gtggagcatg tggtttaatt cgatgcaacg cgaagaacct tactactct
  961 tgacatccag agaacttagc agagatgctt tgggtgcctc gggaaactctg agacaggcgc
 1021 tgcatggctg tcgtcagctc gtgttgtgaa atgttggggt aagtcgccga acgagcgcaa
 1081 cccctatcct ttgttgccag cgcgtgatgg cgggaactca aaggagactg ccggtgataa
 1141 accggaggaa ggtggggatg acgtcaagtc atcatggccc ttacgagtag ggctacacac
 1201 gtgctacaat ggcgtataca aagggaagcg accccgcgag ggcaagcgga actcataaag
 1261 tacgtcgtag tccggattgg agtctgcaac tcgactccat gaagtcggaa tcgctagtaa
 1321 tcgtagatca gaatgctacg gtgaatacgt tcccgggcct tgtacacacc gcccgtcaca
 1381 ccattgggagt gggttgcaaa agaagtaggt agcttaacct tcgggagggc gcttaccact
 1441 tgataattgt g

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