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RESEARCH ARTICLE
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REVITALIZING SENIOR SMILES: INVESTIGATING ORAL PROBIOTICS FOR ENHANCED HEALTH

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Abstract

Recently, a group of live bacteria known as probiotics have been added to foods to utilise their benefits for human health. Probiotics are living bacteria present in the oral cavity, gut as well as intestine and Fthese are taken as food and oral supplements. More than 12.3% of the population consists of elderly people and suffer from various disorders due to constant change in the micro biota with body and mouth. Oral health issues are the common problems viz., Periodontitis, gingivitis, oral cancer, bad breath, tooth loss, tooth decay etc are the common dental problems. The increasing knowledge on oral diseases which are preventable by alteration of the oral biofilm has made way for the use of probiotic bacteria in dentistry. The beneficial effects of probiotics on oral cavity related with the maintenance of oral health especially in elderly people as these are the conventional option that can access easily. Probiotics work naturally against these colonising pathogens in the mouth through their mechanism of action. Probiotic strains like *Lactobacilli, Bifidiobacterium, Lactococci, Streptococci, Enterococci* etc are usually present in the oral cavity. Organism should possess several qualities to be a good probiotic strain. Probiotics deals with several oral disorders like dental caries, halitosis, periodontal diseases etc. There are many oral probiotic products that are currently available in the market, and there is a large demand for the products like them and also newer ones. The oral probiotic study helps to understand the effect of oral probiotics on oral health, risk factors, safety, usage, occurrence as well as its future applications.

Keywords: Oral Probiotics, Bacteria, Food, Caries.

INTRODUCTION

Probiotics are living organisms, or food products containing live microbes, that are beneficial to the health of the host [1]. The efficiency and safety of probiotics should be studied and proved efficiently. Probiotic effects are specific to the strains; hence each strain should be clinically tested separately for its effect on human health, and the effects exerted by one strain cannot be used to describe the other [2]. Oral probiotics are the organisms that are usually obtained in or isolated from oral cavity [3]. These organisms have various health benefits, especially on oral health. Since probiotics are live strains of bacteria which are non-pathogenic and have minimum to no side effects, one can easily rely on the probiotic therapy on curing certain oral and health disorders [4]. The mouth cavity is a large ecosystem with dynamic environmental changes and interactions between microorganisms that prevent the colonisation of pathogenic germs [5]. The varied oral microbiota can cause significant oral disorders like periodontitis and dental caries to manifest because of its imbalance [6]. Antimicrobial medication therapy and mechanical removal of bacterial plaques are the mainstays of conventional treatment for these disorders, albeit drug resistance may restrict their efficacy [7]. With the need for alternatives and adjuvants to regular therapies, probiotics may play an important role. Probiotics are live microbes, which when consumed in proper quantity, benefits human health in a tremendous way [8]. Due to the paucity of clinical data demonstrating the efficacy of probiotic-based therapy for oral disorders, they are currently not frequently employed for intestinal ailments [9]. Probiotics may outgrow harmful bacteria and boost the number of healthy bacteria in the mouth, which helps prevent and treat oral disorders [10]. Usually humans consume milk and other dairy products which contain numerous probiotic bacteria, hence there will be regular colonisation of probiotic species in the oral cavity. So far the effect of oral probiotics on dental caries, haliotis , gingivitis, cavity , oral cancer and other periodontal disorders are observed and implemented clinically [11]. Probiotic therapy refers to the administration of live organisms which is intended to enhance or restore the normal microflora, aiming to cure several disorders specially related to digestion, gastritis, oral illness and much more [12]. Since probiotic organisms are also a part of normal human microbiota, there won't be any side effects on the health .Elderly population who undergo natural process of senescence and regular change in the microbial compositions of the body cannot usually adapt themselves for the constant change in their body , their metabolism differs from that of a normal young adult , for this situation probiotic therapy comes to the rescue [13] .Commonly found probiotic strains in the oral cavity of an elderly person would be, strains like Lactobacillus, Bifidobacterium and Streptococcus, are likely to be able to occupy the oral cavity while the products are in active use: Streptococcus salivaris, lactic acid bacteria, Enterococcus, Lactococcus, Lactobacillus acidophilus, Bifidobacterium bifidis [14]. These are the major probiotic strains that colonise the oral cavity. Salivary and gingival crevicular fluid samples are usually used to know the microbial composition in oral cavity. A microorganism to be an oral probiotic should be able to adhere to colonize the surface of oral cavity. Microorganisms generally considered as probiotics do not have oral cavity as their natural habitat, studies shows that Lactobacilli are the residential oral flora which might plays vital role in microbial balance in the oral cavity [15]. The prevalence of Lactobacilli naturally in oral cavity is not proven since there is frequent consumption of milk products which in turn leads to short term establishment of colonies. A probiotic strain should have positive effects on people's health. It need to be risk-free and non-toxic to people [16]. They must exist as living cells. They must be able to live and function in the bodily environment. Under storage and field circumstances, they ought to be able to survive. Probiotics also have the capacity to colonise and stick to intestinal epithelia as well as survive passage through the digestive system. It ought to be able to sustain good viability [17]. It ought to eat the nutrients and substrates included in a typical diet. It need to be non-pathogenic and non-toxic. It must be able to influence the host in a positive way. It must be non-carcinogenic, secure for the host, and capable of producing lactate acid The host's immune system should be boosted by it [18].These probiotic strains act naturally as a therapy in preventing the normal oral diseases. Probiotic food products like yakult , yogurt , probiotic gums ,

probiotic toothpastes , probiotic suspension are administered in order to enhance the probiotic population in the oral cavity [19].The mode of action of probiotics include production of bacteriocin and short chain fatty acid, decreasing the gut pH, competition for the nutrient for the stimulation of mucosal barrier function and immunomodulation [20]. Probiotics usually function by competing with the pathogenic strains of bacteria in turn preventing the resulting oral disorders [21]. This study aims at isolating the probiotic strains from the oral cavity to study the percentage of prevalence of oral probiotics in elderly people.

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MATERIALS AND METHODS

Screening and Isolation:

Samples were collected from oral cavity by swabbing through the surface of mouth. Samples were collected in closed sterile swab sticks, swiped through cavity of elderly people. Older adults over the age of 50 make up the subjects for the collection of samples. These swab sticks were filled with LB broth to maintain the cells alive until plating was complete, after which the swab sticks were chilled. On De Man Rogosa & Sharpe (MRS) agar, plating is carried out. The samples were serially diluted, and the plating is done for the dilution 10^{-5} and 10^{-6}

Maintenance and preparation of bacterial isolates:

Isolates (As-1, As-2, As-3, As-4, As-5, As-6, As-7, As-8, As-9, As-10, As-11, As-12, As-13, As-14, As-15, As-16, As-17, As-18, As-19, As-20, As-21, As-22, As-23, As-24, As-25.) from isolated culture plates were sub cultured in 2ml M17 broth and incubated at 37˚C for 24hrs. Purity was checked by streaking on MRS agar (HI media) & incubated for 24hrs at 37℃. Further single colonies of cultures were subcultured in M17 broth for further tests.

Phenotypic Identification:

Colony morphology, cell morphology, arrangements, gram staining was done for phonotypic characterisation. Colony shape, size, colour & consistency were observed.

Gram staining:

Gram staining was used to categorise the Gram reaction, and microscopy was used to examine the morphology. Whether the isolates are gramme positive or negative, and whether they are rods, cocci, or coccobacilli.

The procedure of Gram staining mentioned in brief as follows:

1. A thin smear of samples was prepared by heat fixing.
2. Smear was saturated with crystal violet and let it for 30 seconds to 1 minute before washing with purified water.
3. Flooded with the gram’s iodine for a minute and washed using water.
4. Decolourized using 95% Ethanol for 10-20 seconds and washed using water.
5. Counter stained with safranin and let it for 1 minute and rinse with water.
6. Air dried, blot dried and observed using microscope.

Biochemical characterization

Biochemical tests were done to identify the unknown bacteria. Biochemical tests performed in this study were catalase test, citrate utilization test, methyl red test, Voges-Proskauer test, indole test, triple sugar iron test, glucose fermentation test by using standard protocols. Milk coagulation test was done to confirm whether the bacteria are probiotic stains.

1. Catalase test

Principle: This test shows the presence of catalase which is an enzyme that catalyses the release of oxygen from hydrogen peroxide (H2O2). It is used to differentiate between bacteria that produces an enzyme catalase.

2. Citrate utilization test

Principle: The ability of an organism to utilise citrate as a source of energy is evaluated using the citrate utilisation test. The medium includes inorganic ammonium salt as a source of nitrogen and Simon's citrate as a source of carbon. Simon's citrate agar is the testing media.

3. Methyl red test

Principle: The ability of bacteria to use glucose and transform it into a stable acid, such as lactic acid, acetic acid, or formic acid, is assessed using the methyl red test.

4. Voges – Provoskauer test

Principle:

The Voges-Proskauer (VP) test is used to determine if a microorganism produces acetyl methyl carbinol from the fermentation of glucose. Acetyl methyl carbinol is converted to diacetyl in the presence of naphthol, a strong alkali (40 percent KOH), and outside air.

5. Indole Test

The indole test is used to determine if bacteria have the ability to break down the medium-accumulating amino acid tryptophane into indole.

Principle: Tryptophan is an amino acid that can be deaminated and hydrolysed by bacteria that generate the tryptophanase enzyme. Tryptophan is reductively deaminated to create indole, which is then transformed to indole pyruvic acid. Tryptophanase catalyses the deamination process, which removes the amine group from the tryptophan molecule. The reaction's end products are indole, pyruvic acid, ammonium, and energy. Pyridoxal phosphate is required as a coenzyme.

6.Glucose fermentation test

Principle: This experiment uses purple broth to measure the fermentation reactions of pure cultures of microorganisms. It assesses an organism's capacity to ferment glucose and transform the by-product of glycolysis, pyruvic acid, into gaseous substances. This is a standard test for identifying Gram-negative enteric bacteria, of which only some are gas-producing, but all are glucose fermenters.

Genomic DNA Isolation:

The DNA was isolated using standard protocols.

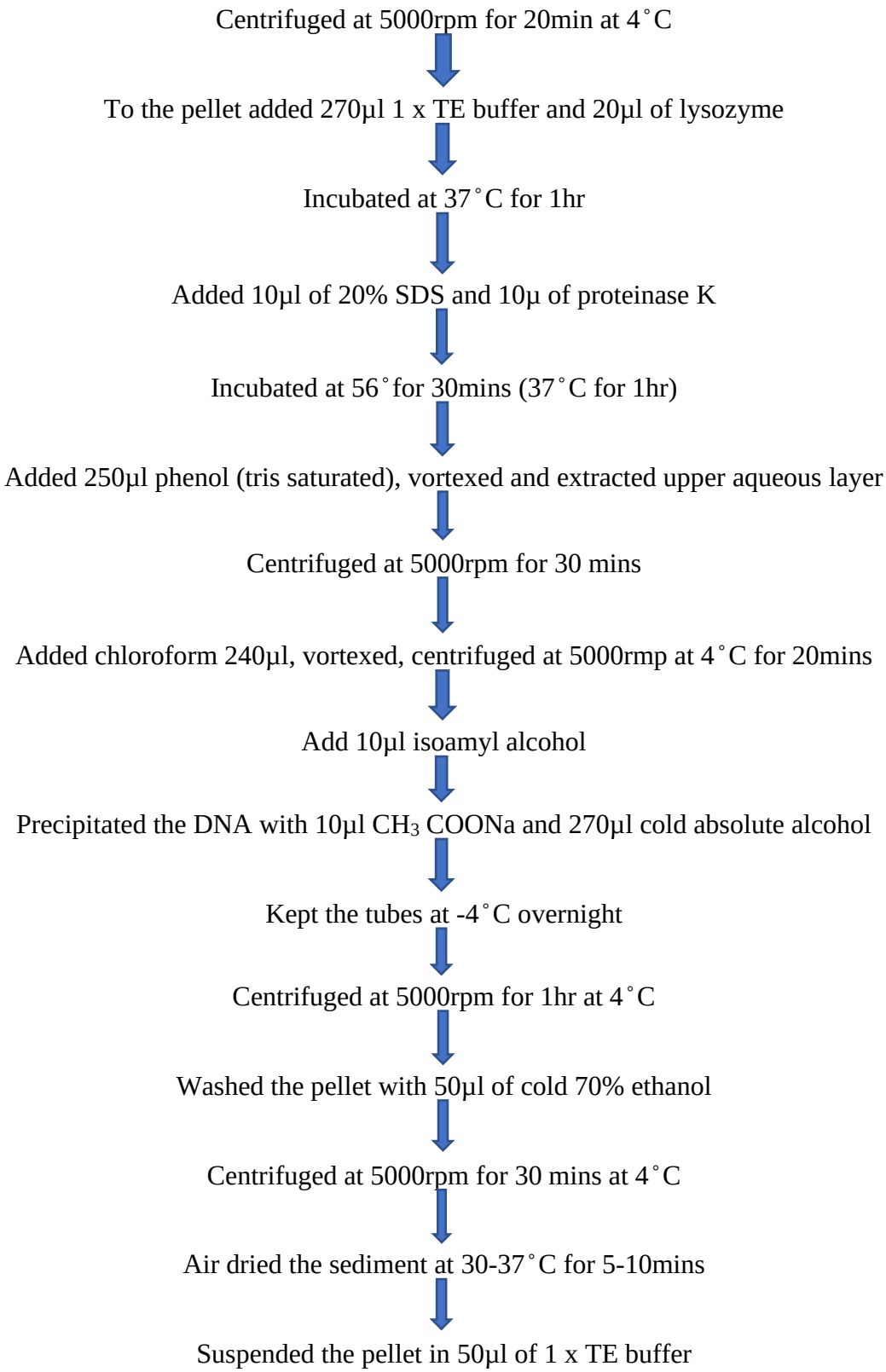
Isolation of DNA:

1ml of overnight culture in ependroff tubes

Centrifuged at 5000rpm for 20min at 4˚C

Discarded the supernatant

Added 270µl 1 x TE buffer and dissolved the pellet in vortex



Requirements:

1. TI buffer (acts as a buffer in electrophoretic tank)
2. Ethidium bromide (a fluorescent dye used for staining nucleic acid)
3. Agarose (act as a matrix for separation of nucleic acid)
4. DNA loading dye (track the DNA in electrophoresis gel)
5. Bromophenol blue
6. Xylene cyanol

7. Glycerol
8. Distilled water
9. Electrophoresis apparatus
10. Micropipettes
11. Micro tips
12. Conical flask
13. Oven
14. Stirrer

Preparation of solutions:

1. Ethidium bromide (EtBr)

a. Ethidium bromide (EtBr) 10mg
b. Distilled water 1ml

2. Agarose gel 0.8%

i. Agarose 0.8g
ii. 1 x TAE 100 ml
iii. EtBr 3µl

3. DNA Loading Dye

i. Bromophenol blue 0.25%
ii. Xylene cyanol 0.25%
iii. Glycerol 30%

The dye was prepared in distilled water & stored at 4˚C

Gel-electrophoresis:

Procedure:

1. Preparation of agarose gel:

1) Removed the conical flask from the oven while wearing hand gloves, and waited until your palms are comfortable before handling it.
2) Dissolve the agarose in 1 x TAE buffer in the conical flask.
3) Placed the conical flask in the oven and heated until the solution turns clear.
4) Poured the agarose solution into the gel casting tray after cleaning it with ethanol, taping the open sides with cello tape, and setting up the equipment.
5) Allowed to set for 15 to 20 minutes.

2. Preparation of DNA samples

- Mixed the DNA sample (pellet) in 50µl of TE buffer in an Eppendorf

3. Loading of DNA samples

- Using a micropipette, combined 3 l of gel loading dye with 5 l of DNA sample.
- Be careful when removing the comb from the gel that has solidified to prevent damage to the wells.
- Added TAE buffer to the tank.
- Filled the electrophoresis tank with agarose gel.
- The wells will be positioned with the cathode in mind.
- Carefully pipette-loaded the samples into the gel.
- Ran the samples until it has covered half of the gel's surface.
- Removed the electrophoresis tank's gel and examine.

Observation & Results: -

Agarose is used to separate DNA fragments, and its concentration is 0.8 percent. To assess the DNA's purity, agarose gel electrophoresis was conducted.

PCR AMPLIFICATION:
Requirements:
Buffer (10x), working (1x)
MgCl2 1.5mM
dNTPs 200 - 250µM
Primer 50-100PM
Template 50-100Pg
Taq polymerase 1U-3U
Autoclaved water

PCR reaction mixture [25µl]

10X std. Taq. Reaction buffer	2.5µl
10mM dNTPs	0.5 µl
Taq. DNA polymerase	0.125 µl
10µM Forward Primer(27F)	0.5 µl
10µM Reverse Primer (1494r)	0.5 µl
Nuclease free water	20.875µl

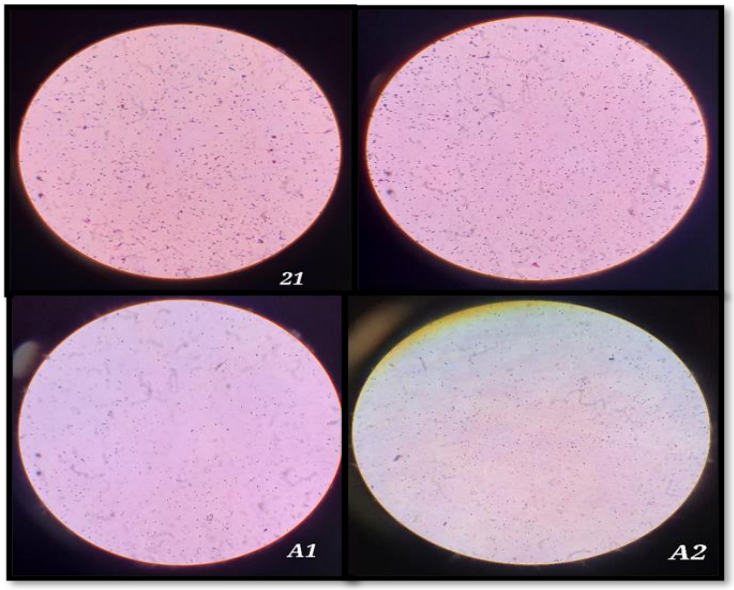
PCR Programme
Lead temperature - 104ºC
95º C 5 min
94ºC 1 min - Denaturation
55º C 1min - Annealing
72º C 2 min - Extension
72º C 5 min - Final extension

RESULTS
Colony Morphology
The aim was to characterise all the 25 isolates, which were isolated from oral samples. The samples were collected from different healthy individuals aged above 50, then revived on fresh M17 broth tubes and incubated at 37℃ for 24 hours and streaked on the MRS agar to check the purity of colonies. They were designated as (As-1, As-2, As-3, As-4, As-5, As-6, As-7, As-8, As-9, As-10, As-11, As-12, As-13, As-14, As-15, As-16, As-17, As-18, As-19, As-20, As-21, As-22, As-23, As-24, As-25).

For plating MRS agar is used, the samples were serially diluted and then spread on the agar. Here 10^{-4} & 10^{-5} dilutions were taken and then spread plate method is followed. Then these colonies were preserved by streaking quadrants on MRS agar (Figure 1)

Fig 1. Serially diluted samples were Spread plated Selected cultures were Quadrant streaked

Microscopic characterization:
Microscopic characters of the isolates were found out to be gram positive cocci, gram positive rods, gram negative cocci, gram negative rods.



A B

Fig 2. Gram's-stained cultures under 100x magnification

Biochemical characterization
1.Catalase test:

Fig 3. Catalase +ve Result of As-18 and Catalase -ve Result of As -1

2.Indole test:

Fig 4. Indole -ve Result of As-1 and Indole -ve Result of As-9

3.MR test:

Fig 5. MR positive result of As-1 and MR negative result of As-7

4.VP test:

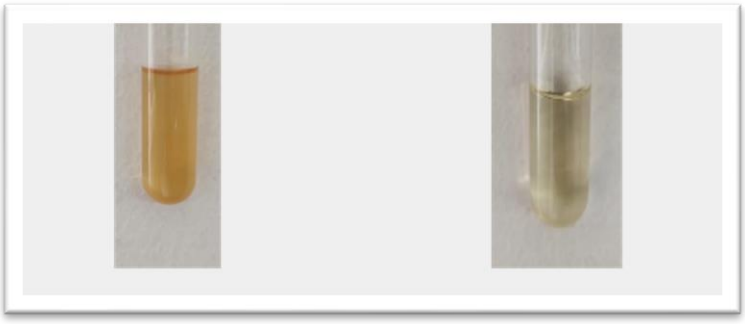


Fig 6. VP positive result of As-1 & VP negative result of As-2

5.Citrate utilization test:

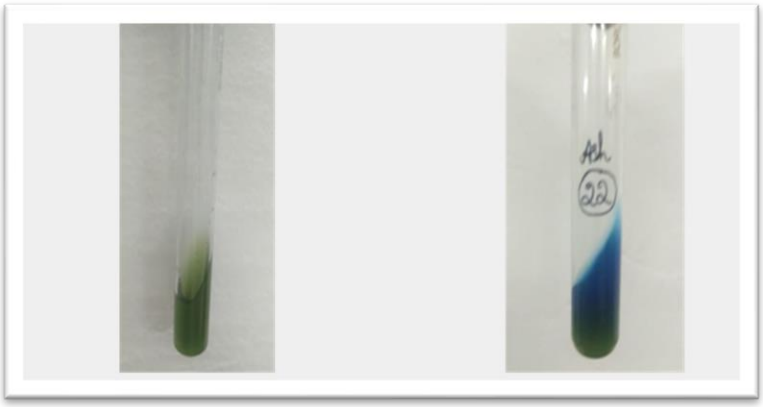


Fig 7. Citrate -ve Result of As-12 & Citrate +ve Result of As-22

6. Glucose fermentation test

Fig 8. Glucose fermentation +ve Result of As-1 & Glucose fermentation -ve Result of As-10

Microscopic characterization:

Out of 25 isolates, 7 are gram negative,18 is gram positive,14 are cocci shape and 11 are rod shape strains.

Isolates	Gram staining	Shape	Catalase	Indole	MR	VP	Citrate	Glucose fermentation
As-1	+	cocci	-	-	+	+	+	+
As-2	-	cocci	-	-	+	-	+	+
As-3	+	cocci	-	-	+	-	+	-
As-4	+	cocci	-	-	+	-	+	-
As-5	+	cocci	-	-	+	+	+	+
As-6	+	cocci	-	-	+	-	+	-
As-7	-	cocci	-	-	-	-	+	+
As-8	+	cocci	-	-	+	-	+	+
As-9	+	cocci	-	+	+	-	-	+
As-10	+	cocci	-	+	+	+	-	+
As-11	+	rod	-	+	+	+	-	+
As-12	-	cocci	-	+	+	+	-	+
As-13	+	rod	-	-	-	_	+	-
As-14	+	rod	-	-	+	+	+	+
As-15	+	rod	-	-	+	+	+	+
As-16	-	cocci	-	-	+	-	+	+
As-17	-	cocci	-	-	+	+	+	+
As-18	-	cocci	+	-	+	+	+	+
As-19	+	rod	-	+	+	-	-	-
As-20	-	rod	-	+	+	-	-	+
As-21	+	rod	-	+	-	-	+	+
As-22	+	rod	-	-	+	+	+	+
As-23	+	rod	-	-	+	+	+	+
As-24	+	rod	-	+	+	+	+	+
As-25	+	rod	-	-	+	+	+	+

Table 4.5: Biochemical characterisation of isolates

Biochemical characterization:

Catalase - Out of 25 isolates, 1 showed positive and 24 showed negative.

Indole - Out of 25 isolates,8 showed positive and 17 shows negative.

MR - Out of 25 isolates, 22 isolates showed positive, and 3 isolates showed negative.

VP – Out of **25** isolates,13 shows positive and 12 showed negative.

Citrate – Out of 25 isolates, 20 showed positive and 5 showed negative.

Glucose fermentation – Out of 25 isolates, 24 showed positive and only one showed negative.

Genomic DNA Extraction:
DNA isolation was done according to conventional procedure. The nanodrop absorbance ratio was measured after the DNA was isolated using standard protocol. The extracted DNA was quantified for the qualitative analysis and 16S- rRNA. DNA bands were visible on 0.8% agarose gel.

Table 4.6: DNA purity of samples.

Samples	Concentration ng/µl	260/230
As-1	225.266	1.90
As-2	472.426	1.71
As-3	538.960	1.77
As-4	133.159	2.03
As-5	210.246	1.77

16s rRNA sequencing:
The genomic DNA was extracted from all the 25 isolates of oral samples and subjected for the molecular analysis of PCR. DNA was analysed both qualitatively and quantitatively. The DNA after the amplification it was subjected for the agarose gel electrophoresis with 0.8% of the agarose gel, which displayed sharp single band and confirmed the selected and isolated E. coli bacteria from the samples.
The reaction conditions for the 16S r RNA for Universal primer pairs using the primers for the PCR and steps are annealing temperature was set to different condition of the gradient, and Pre-heating temperature was 95° C for 5 minutes, Denaturation was 95° C for 50 seconds, and annealing for 55° C with primer annealing was 1.30 seconds, Extension temperature were 25° C for 5 mins, Final Extension 4° C for the 5 minutes with run of 35 cycles.

Primer used:

27F	**AGA GTT TGA TCC TGG CTC AG**
1492R	**CGG TTA CCT TGT TAC GAC TT**

Basic Local Alignment Search Tool (BLAST) results:
The sequence of amplified PCR was obtained from Barcode Biosciences (Bangalore, India). These sequences were used as a query sequence for the identification of respective resistant gene. It was performed using Basic Local Alignment Search Tool (BLAST). For every sequence the most unique sequence against BLAST protein (blastp) of protein database were considered.

i)FASTA sequence of As-23:

>0622_512_003_PCR_A-23_16SF_F01.ab1
CCCGTAGATGAGTGCTAGGAGTTGGAGGCCTTCCAGCGCCGTTGCGCTTGCTGGCAGCT
AACGCGTTAAATCACTCCGCC
TGGGGAGTACGACCGCAAGGTTAGAAACTCAAATGAAATTGACGGGGGCCCGAAAGC
GGTGGAGATGTGGTTTATTCGAT
GCACGCGAAGAACCTTACTGGTCTTGACATCCTTTAACTTTCTAGAGAGGAGCTTTGCC
TTCGGGAACTGAGAGACAGGT
GCTGCATGGCTGTCGTCAGCTCGTGTTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTATTGTTTGTTGCC
ATCGTTTAGGTGGGCAATTTAGAGGAAATGGCGGTGGATAACCGGAAGGAGGGGGGG
GATGACGTCAATCATCATGCCCC
TTATGAACTGGGCTACACACGTGCTACAATGGGATATACAACGAGAAGCGACCTCGCG
AGAGCAAGCGGATCTCTTAAAG
CTTGTCTCAGTTCGGATTGCAGGCTGCAACTCGACTGCATGAAGCCGGAATCGCTAGTA
ATCGCAGATCAGCATGCTACG

GTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTGTGTTGCAC
CAGAAGTAGGTGAGTTAACCT
TCTTGAAGGCGCTCACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACGAGGGTAA
CCGGCCCTGGTTGATCAAGGT
TTTTCACGTGTAAGAGCGAGTCGTTGACAGAAGGGAGCCGCCTCCGACCTAGGAGTTC
CCTCTAAATTTTGACGATTTTC
ACCGTTACACGTGAGATTCCAGCCTTCCCTTGACAAGACTTCAATCCTGCCAGTTCCAA
GAGACCCTCCGCCGGTAGGCC
GGGGGATTTTCCATCTCGACTGTGAACACCCCGCCGGCTTGCAGTAGCCCCATATATTC
GGAGAAACGGTTGACACCTCC
TTGATACCGGGTCTGCGACTGATTACCTGCTCTCTCTGAGAAACTGAGCGACGTTATAC
TGCCTGTCTCTCGAGAAGTTA
CGCGAAGCTTTTCACACGATG

>0622_512_004_PCR_A-23_16SR_F01.ab1

CTCCAGGCGGTCTGTTTAATGCGTTAGCTCCGGAACTGACGCGCTCGAGGCCTCCCTCC
TTATCGACATCCGTTACGGCG
TGGACTAACCAGGGAATCTTCCTGGTTGCTTCCAACGTTTCGCACCTGAGCGGCAGTCT
TTGGACCAGAGAGCCGCCTCC
CCCACGGGAGTCCTCCCAAATTTTACCCATTTCCCCGGTTACCCTGGAAATCCACCCCC
CCCCTTCTGACTCCAAGCTGC
CCAGTTCCAATGGACCCTCCCCGGTGGACCCGGGGATTTCCCCTTCAAATTGACAAACC
GGCCGGGGGGTCCTTTACCCCC
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ACCCGTGCTTTTTCGGCGGGAA
ACCGCCATCGGAGAGGCTATTACTCTCATCCTCGTCCTCCCCGATGAAAGTGCTTTACC
AACCCAAGGACTTCTTCCCAT
CCGCCGGATGGGTGCGTCCAGATTGCCCCCATTGGGCAATAATCCCCTATGGTGGCTTC
CGGAAGAATCTGGACCGTGTC
TCAATTCCAATGTGGGTGGACATCCCTCCAGAGCAGCTAATGATCGTCGCCTATGTGAA
GCGTTAACCTACCTACTAACT
AATCGCATCTGGGCACATCTGATGGCATGAAGCCCGAAAGGTCCTTTACTTTGGACTTG
CTACGTTATGCAGTGTTATCT
ACCGTTTCCAGCAGTTATCCACCCTCCATCACGCTAGTGTACGCACTACATTACTCACC
CGTTCGTCGCTCGTCAGCCGA
GTAGTCATGCTCTCTGTGCTAACAGCTCCGACTGCATGAGTTAGGCTGCCGTCGCGGTC
ATGCCGGAGGCAGCATCAGAC
TCTACGTGAACCACGCCTCAAGGGTCTAGCGTGTACCCCCATAAGATGTGCAGGAAAC
GGGTTGGACCCTCTCAGGATAC
CGCGGATCGAGGCGACCTGGATTAGCCTGTCTCTTCTCTTGAGAGAACCAGAAAGGTC
GCAGGATGTATTCCTGAACTGG
TTTTCTGCTATGGAAAGAGGGTTTACGCGCGGAGAGCGTTCCACACACCGGGGTGGCC
AACTTGCCTATGCAAATTCCAG
GTTCGCCAAGAAGGGTGGAACAAT

The resulting hits of BLAST results viz., E-value were < 0.0, Query Coverage was found to be 61% for ***Klebsiella varicola***, the Percentage of identity were found to be 88.47 % similarity with existing NCBI database.

ii)FASTA sequence of As-15:

>0622_512_001_PCR_A-15_16SF_E01.ab1

ATAACGATGAGTGCTAAGTGTTGGAGGGTTTCCAGCGCCTTCAGTGCTGCAGCTAACGC
ATTAAGCACTCCGCCTGGGGA

GTACGACCGCAAGGTTGAAACTCAAATGGAATTGACGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGAAGCA
ACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTCCC
CTTCGGGGGCAAAGTGACAGG
TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
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CCCGAAGTCGGTGAGGTAACC
TTTTTGAAGCCGCCCGCCTAAGGTGGAATAGATAATGGGGGTGAATCCTAAAAAAGGG
TAACCGGCCCGTTTTCCCCACG
GCCTTTTTAAGCCTTAAAAAGTAAACAAAAAAACTAAAAACCCCCTTTCCCCCCATAGG
GTTTTCCCAAAAATTTTTGCG
CTTTTCCCGGAAAAAAGGGGAATTCACCTTTTTTGTTTGTGACCCCATGTCCCGCAGTTT
CAAAGGGAGCCTCCCCGGGG
GGGGGGGGGGGGGTTTTCACTCGAGATAGAAAAAAACAGACGGGGTGTCTGTGTTTCC
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ATACGAGGAAGAAGTATCTTTT
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GATAACCACTAGAAGTGGGTATG
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The resulting hits of BLAST results viz., E-value were < 0.0, Query Coverage was found to be 55% for ***Enterococcus faecium,*** the Percentage of identity were found to be 97.44% similarity with existing NCBI database.

iii)FASTA sequence of As-09

>0522_656_001_PCR_BK_16SF_G01.ab1
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GGCGCCCCCGTGTTAGCGGGG
AGAGGAGATAAAAGAAAAAAGAGAGGGTTTTTTCTGGAGAAAAAGAAAAA

The resulting hits of BLAST results viz., E-value were < 0.0, Query Coverage was found to be 95% for ***Klebsiella pneumoniae***, the Percentage of identity were found to be 99.57 % similarity with existing NCBI database.

Discussion

A total of 25 isolates were obtained for this investigation from the oral cavities of aged healthy individuals. These isolates were subsequently processed, and the following microorganisms were discovered and validated using biochemical assay*: Klebsiella pneumoniae, Klebsiella variicola, Enterococcus faecium, lactic acid bacteria, and streptococcus.*

Like this, 36 *streptococcus* and 19 *lactobacillus* strains were developed from Terai et al 2015.'s collection of 896 isolates. The purpose of the study was to identify and isolate probiotic strains from healthy adults over the age of 50's oral cavity. Plaque patient samples were probably collected by Jiang et al 2018 similar research on probiotics in the oral cavity. Gram staining was carried out to determine the morphology and staining characteristics of the organisms. To determine the sort of organism, biochemical tests were conducted. Various tests were conducted, including those for catalase, indole, methyl red, vouges Proskauer, citrate utilisation, etc.
Similarly, the goal of Forhada et al 2016.'s investigation was to find a probiotic strain of organism that could produce lactic acid on MRS agar. Direct microscopy was used to look at the cultural and morphological traits of bacteria.

To validate the strain of organism present in a healthy oral cavity and determine the frequency of probiotic species in the geriatric oral profile, DNA isolation was used in this study. After PCR amplification, a gel electrophoresis is conducted to capture the band pictures.

CONCLUSIONS

Probiotic organisms are the live strains of bacteria found in normal flora which have decreases or no pathogenicity. These organisms have positive effect on oral health. Probiotic therapy is a new conventional method used in the maintenance of oral health. This is due to the capability of probiotic strain to compete with other disease-causing organisms by colonizing in the oral cavity.

These uses of probiotics are studied to implement it in the treatment of many oral diseases especially in geriatric population. Some exclusive probiotic strains found in normal micro flora of mouth can be isolated in developing and designing a desired product. Many probiotic products have a high demand in the current market. Already many established probiotic products like chewing gums, mouthwash, toothpaste, tablets etc are available in the market. Similar products with increased quality or new probiotic products might have a large scope in this world where there is rapid change in food habits and lifestyle. However, it required further scientific study and clinical trials to know more about the strain, their effect, usage, safety, potentials and applications as probiotics in oral health, which can conveniently be prescribed to elderly people as a probiotic therapy to cure the oral disorders despite

of the presence of other systemic diseases like cardiovascular disorders, diabetes etc. Since oral health plays a vital role in the wellbeing of an individual, development of oral probiotic therapy can be a drastic investment in the field of oral health.

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