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ORIGINAL RESEARCH ARTICLE

Enumeration of Viridans Streptococci in Healthy Human Beings in Sagar, Madhya Pradesh.

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ABSTRACT

Dental plaque represents a complex community of different species of microorganisms which include a majority of streptococci and other microorganisms. Samples collected from 19 healthy individuals of different age groups from rural population of Sagar, Madhya Pradesh, yielded 80 isolates of genus Streptococcus of 17 different species including *Streptococcus agalactiae, S. cremoris, S. equinus, S. faecalis var malodoratus, S. faecium, Streptococcus grp* F, *Streptococcus grp* H, *Streptococcus grp* O, *Streptococcus grp* Q1, *S. lactis, S. milleri, S. mtior, S. mutans* sub sp. *sobrinus, S. pneumonae, S. pyogenes, S. sanguis I, S. uberis.* Out of these, most dominating species was found to be *S. faecium* followed by *S. milleri* and *S. cremoris*. Sixty five isolates were found α-haemolytic while remaining isolates showed no haemolysis (γ-haemolytic).

Keywords: Viridans, Streptococci, Dental plaque.

INTRODUCTION

The mouth provides a highly selective environment for microorganisms of more than 300 species [22]. They are found mostly in dental plaque, a film of food debris and dead cells embedded in polymer matrix of bacterial origin that covers the teeth surface. The majority of bacteria found in dental plaque are species of *Streptococcus* and are playing important role in various dental diseases such as caries and periodontitis [27]. Different species of streptococci have been reported from human oral cavity by various workers [13,21,31] and is well reported that unhygienic cleaning of teeth results in development of many opportunistic pathogenic species of *Streptococcus* in dental plaque[25] . These studies have indicated the need of local surveys to find out the diversity of microbial population particularly that of oral streptococci existing in dental plaque and that how many of them belong to species of viridans group.

Though the bacterial taxonomy is complicated but the data on numerical taxonomy have been compiled and developed as PIBwin computer based system [3]. This system has been found to be a good tool for identification of many prokaryotic microorganisms [12,15,19,20,26] . Matrices based on numerical taxonomy for identification of gram + ive, catalase negative aerobic cocci[10] have provided a workable basis for identification and differentiation of oral streptococci. The present paper reports the finding of a survey conducted for the enumeration of streptococci associated with dental plaque of people belonging to different age groups in Sagar, M.P.

MATERIALS AND METHODS

Collection of sample: A total of 19 samples were collected from the teeth scrapings of 5 females (aged between 12 to 26 years) and 14 males (aged between 12 - 55 years), representing 4 individuals in the age group I (1 - 15 years), 8 in age group II (16 - 30 years), 2 in age group III (31 - 45 years) and 6 in age group IV (above 45 years). The samples of dental plaque were collected in early morning before brushing and were obtained by rubbing the soft dental plaque with the help of sterile swab and immediately dipping in presterilized water vials. All the samples were brought to the laboratory and stored at 4°C, if not processed immediately for the isolation of streptococci.

Isolation of streptococci: For isolation of streptococci samples were shaken well and 0.1 ml of each was spread directly over Streptococcus Selection Agar media [14]. The plates were incubated at 37°±1°C under partial anaerobic

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condition for 48 hours. Morphologically different colonies appeared on spread plates were picked up and purified using diluted suspensions of the colonies [30] . Purified bacterial cultures were maintained on Todd Hewitt medium [29] at 4°C.

Morphological and Biochemical characterization:

For identification of isolated bacteria, these were grown on Streptococcus Selection Agar and colony characters were noted. The smear of each isolates was prepared and gram stained [24]. The Gram's staining reaction and morphological characteristics of the bacteria were studied microscopically. Catalase activity was determined by dipping the loop full of culture in H_2O_2 solution and appearance of bubbles was noted. Evolution of bubbles in H_2O_2 indicated catalase positive reaction. To study biochemical properties of the isolated bacteria a total of 54 tests were performed. These include fermentation of 30 carbohydrates (adonitol, D(+)arabinose, D(-)arabinose, cellobiose, dextrin, dulcitol, erythritol, fructose, galactose, glucose, inulin, inositol, lactose, maltose, mannitol, mannose, D(+)melezitose, D(+)mellibiose, methyl glucoside, methyl mannoside, methyl xyloside, D(+)raffinose,ribose, salicin, sorbitol, sorbose, sucrose, trehalose, D(+)xylose, L(-)xylose), activity of arginine dihydrolase, aesculin hydrolysis, starch hydrolysis, coagulase, DNase, β-galactosidase, gelatin liquification, lysine and ornithine decarboxylase, oxidase, phosphates, tryptophan deaminase, urease, and utilization of amylose, amygdalin, arbutin, citrate, glycerol, erythritol, acetyl glucosamine. In addition to these certain other biochemical tests including VP and indole tests, nitrate reduction and H_2S production were also studied. All tests were performed using standard protocols [4,14,24].

Characterization of haemolytic properties:

Haemolytic activity of each test organism was studied [16]. For this blood agar medium was prepared using blood agar base (Hi Media) by supplementing 7% defibrinated sterile sheep blood (v/v). The organisms were then streaked over the solidified medium and incubated under partial anaerobic conditions. Appearance of greenish zones around the colonies were observed and the isolates were classified as per their haemolytic properties [7].

Identification:

Identification of streptococci was done on the basis of their morphological, biochemical and haemolytic properties using PIBwin computer kit [3].The properties of each test bacteria were matched with the available matrix of Gram Positive Anaerobic Cocci[10] and the ID score to the nearest species were noted in all the cases.

RESULTS AND DISCUSSION

Streptococcus selection agar is a sensitive enrichment medium for the selective isolation of Streptococci from the specimens expected to have numerous microorganisms and hence, it is used in the present study for isolation of streptococci from dental plaque samples. All 80 *Streptococcus* isolates have been obtained from 19 dental plaque samples during present study. The results of isolation were shown in (**Table 1**). The number of streptococci found to be maximum in samples obtained from the persons belonging to the age group II i.e., 16-30 years old followed by age group III, 31-45 years of age.

On the basis of morphological characteristics, absence of catalase activity and haemolytic properties, all the isolates were identified as *Streptococcus* and are grouped in species of this genus based on their maximum ID score to the nearest species using PIBwin software. Following Feltham and Sneath (1982) only 5 isolates i.e., DPS043, DPS061, DPS032, DPS038 and DPS019 could be confirmed to the species level as their ID score was found to be greater than 0.999 (**Table 2**). On the basis of maximum ID score to the nearest species the test isolates were classified in to 17 species and the number of isolates grouped in each species is given in parentheses. These species include, *S. agalactiae (1), S. cremoris(7), S. equinus(2), S. faecalis(4), S. faecium(19), S. group F(1), S. group H(3), S. group O(1), S. group Q1(5), S. lactis(1), S. milleri(9), S. mitior(16), S. mutans(3), S. pneumonae(1), S. pyogenes(1), S. sanguis I(5), S. uberis(1).*

Among 80 isolates 65 isolates were found alpha haemolytic 15 were found to be non haemolytic streptococci (**Table 2**). These groups are pioneer in the colonization of the human oral cavity and comprise a large part of the commensal microbiota of the dental plaque.Streptococci of alpha as well as non haemolytic nature in "viridans group"was reviewed and classified earlier [7]. The identification of oral streptococci is a difficult task due to lack of uniformity in their cultural and biochemical characteristics [2,5,9,18,23] .

Mature dental plaque comprises more than 50% of streptococci [28]. Presence of different percentages of streptococcal species (i.e., *S. anginosus, S. gordonii, S. mitis, S mitis2, S. mutans S. oralis, S. sanguis, S. salivarius, S. vestibularis*) in samples from cheek, tounge, pharynx, supragingival

plaque, subgingival plaque of the mouth[11]. In the present study *Streptococcus faecium* was recorded as most frequent taxa among isolated streptococci and also showed its presence in clinical isolates with 95% of frequency [9].

In the present study seven isolates (DPS003, DPS013, DPS018, DPS020, DPS039 and DPS049) have been grouped in *Streptococcus cremoris* (ID score 0.37657 - 0.95599). Identification of these isolates could not be confirmed as *S. cremoris* as none of these showed ID score > 0.999 following Feltham and Sneath (1982). 4 streptococcal isolates (i.e., DPS019, DPS101, DPS027 and DPS025) have been grouped in species *S. faecalis.* One of them showed ID score 0.99924 and hence it has been confirmed as *S. faecalis.* PIBwin ID score of other three isolates was found to be in range of 0.74580-0.99409. In all 19 isolates have been grouped as *S. faecium*, out of these only 4 could be assigned to this species (ID score 0.99961-0.99989) while remaining 15 isolates were found closely related to *S. faecium* with PIBwin ID score in the range of 0.35477-0.99817. In all five isolates are grouped together in Streptococcus group Q1. Out of these three showed ID score in the range of 0.99696-0.99677 thus their identity is confirmed. The ID score of other 2 isolates was found to be only 0.68586 and 0.36385 for this species. Among the isolated streptococci, only 9 isolates have been grouped as *S. milleri* which showed ID score in the range of 0.64567-0.99624 for this taxon. 16 isolates have been grouped as *S. mitior* they showed ID score in the range of 0.48990-0.99247 for this species. Among the isolated streptococci, only one isolate showed its resemblance with *S. agalactiae* (ID score 0.72913), Streptococcus group F (ID score 0.75122), Streptococcus group O (ID score 0.91725), *Streptococcus lactis* (ID score 0.92992), *Streptococcus pneumonae* (ID score 0.55814), *Streptococcus pyogenes* (ID score 0.98156), *Streptococcus sanguis* (ID score 0.74188). The identification studies using PIBwin matrices indicated a great variation in ID scores to the nearest taxa in which they are grouped and that many of the isolated streptococci could not achieve desired ID score for confirmed identification in which they are grouped. This indicates that oral streptococci possess a wide difference in their biochemical properties.

In the present study 7 isolates of *Streptococcus cremoris* have been isolated from samples of dental plaque. This species is basically found in dairy products and is considered to be non pathogenic strain. *Streptococcus milleri* has been reported to forms a part of the normal flora of the mouth, gastrointestinal tract, and genitourinary tract and are often found to be associated with purulent infections (Gossling, 1988; Whitworth, 1990) and also isolated from deep-seated abscesses in the brain, thorax, and abdomen (Unsworth, 1983). In the present study only three isolates have been found to possess similarity with *Streptococcus mutans.* This species is reported as cariogenic and was frequently isolated from human dental plaque [13,31]. In our study only four strains of *Streptococcus faecalis* were obtained, earliar, *faecalis* strains were isolated from subgingival biofilm samples associated with refractory periodontitis and periodontitis [1,6] . Strains of *Streptococcus pyogenes* are known to affect its host in many ways and thus are the causes of a large range of diseases (Cunningham, 2001). In our study only a single isolate of *S. pyogenes* was obtained. Three major pathogenic species i.e. *S. mutans*, *S. faecalis*, and *S. faecium* which are responsible in causing dental diseases were obtained in the present study though the samples were originated from the healthy individuals. The results of present study suggest the need of many surveys to evaluate the incidence of pathogenic streptococci in healthy individuals.

Table 1: Prevalence of the isolated Streptococci as per age groups of the sampled persons

Group	Age	No. of samples	No. of isolates	No. of isolates%/ per person
I	1 to 15	4	15	3.75
II	16 to 30	8	38	9.5
III	31 to 45	2	11	5.5
IV	Above 45	5	16	2.66
Total		19	80	

Table 2: Identification of Streptococci based on their PIBwin ID score

S No	Strain designation	PIBwin ID Score
GROUP 1: *Streptococcus agalactiae*		
1	*Streptococcus agalactiae DPS002*	0.72913
GROUP 2: *Streptococcus cremoris*		
1	*Streptococcus cremoris DPS049*	0.95599
2	*Streptococcus cremoris DPS020*	0.95422
3	*Streptococcus cremoris DPS003*	0.94386
4	*Streptococcus cremoris DPS018*	0.82716
5	*Streptococcus cremoris DPS083*	0.73307
6	*Streptococcus cremoris DPS012*	0.68147
7	*Streptococcus cremoris DPS039*	0.37657
2	*Streptococcus grp Q1 DPS013*	0.99528
3	*Streptococcus grp Q1 DPS047*	0.97696
4	*Streptococcus grp Q1 DPS028*	0.68586
5	*Streptococcus grp Q1 DPS064*	0.36385
GROUP 10: *Streptococcus lactis*		
1	*Streptococcus lactis DPS100*	0.92992
GROUP 11: *Streptococcus milleri'*		
1	*Streptococcus milleri' DPS102*	0.99624
2	*Streptococcus milleri' DPS071*	0.99346
3	*Streptococcus milleri' DPS046*	0.96805

GROUP 3 : *Streptococcus equinus*		
1	*Streptococcus equinus DPS024*	0.86192
2	*Streptococcus equinus DPS031*	0.70486
GROUP 4: *Streptococcus faecalis*		
1	*Streptococcus faecalis var malodoratus DPS019*	0.99924
2	*Streptococcus faecalis var malodoratus DPS101*	0.99409
3	*Streptococcus faecalis DPS027*	0.85617
4	*Streptococcus faecalis DPS045*	0.74580
GROUP 5: *Streptococcus faecium*		
1	*Streptococcus faecium DPS043*	0.99989
2	*Streptococcus faecium DPS061*	0.99972
3	*Streptococcus faecium DPS032*	0.99961
4	*Streptococcus faecium DPS038*	0.99961
5	*Streptococcus faecium DPS026*	0.99817
6	*Streptococcus faecium DPS053*	0.99677
7	*Streptococcus faecium DPS035*	0.99447
8	*Streptococcus faecium DPS010*	0.98413
9	*Streptococcus faecium DPS055*	0.97447
10	*Streptococcus faecium DPS037*	0.97146
11	*Streptococcus faecium DPS065*	0.95391
12	*Streptococcus faecium DPS054*	0.93927
13	Streptococcus faecium DPS058	0.92374
14	*Streptococcus faecium DPS052*	0.84074
15	*Streptococcus faecium DPS015*	0.82751
16	*Streptococcus faecium DPS041*	0.80120
17	*Streptococcus faecium DPS016*	0.67525
18	*Streptococcus faecium DPS017*	0.56321
19	*Streptococcus faecium DPS075*	0.35477
GROUP 6: *Streptococcus grp* F		
1	*Streptococcus grp F DPS073*	0.75122
GROUP 7: *Streptococcus grp* H		
1	*Streptococcus grp H DPS090*	0.73406
2	*Streptococcus grp H DPS025*	0.63476
3	*Streptococcus grp H DPS009*	0.34996
GROUP 8: *Streptococcus grp* O		
1	*Streptococcus grp O DPS094*	0.91725
GROUP 9: *Streptococcus grp* Q1		
1	*Streptococcus grp Q1 DPS074*	0.99677
4	*Streptococcus milleri' DPS004*	0.95383
5	*Streptococcus milleri' DPS066*	0.87051
6	*Streptococcus milleri' DPS040*	0.83734
7	*Streptococcus milleri' DPS051*	0.78093
8	*Streptococcus milleri' DPS036*	0.75929
9	*Streptococcus milleri' DPS014*	0.64567
GROUP 12: *Streptococcus mitior'*		
1	*Streptococcus mitior' DPS021*	0.99247
2	*Streptococcus mitior' DPS082*	0.99002
3	*Streptococcus mitior' DPS033*	0.92324
4	*Streptococcus mitior' DPS048*	0.90296
5	*Streptococcus mitior' DPS022*	0.86479
6	*Streptococcus mitior' DPS030*	0.81792
7	*Streptococcus mitior' DPS081*	0.79538
8	*Streptococcus mitior' DPS095*	0.72955
9	*Streptococcus mitior' DPS034*	0.63581
10	*Streptococcus mitior' DPS092*	0.61711
11	*Streptococcus mitior' DPS001*	0.60043
12	*Streptococcus mitior' DPS006*	0.56433
13	*Streptococcus mitior' DPS011*	0.52670
14	*Streptococcus mitior' DPS005*	0.52670
15	*Streptococcus mitior' DPS089*	0.49688
16	*Streptococcus mitior' DPS008*	0.48990
GROUP 13: *Streptococcus mutans*		
1	*Streptococcus mutans subsp sobrinus' DPS069*	0.98757
2	*Streptococcus mutans subsp sobrinus' DPS070*	0.89075
3	*Streptococcus mutans subsp sobrinus' DPS059*	0.54998
GROUP 14: *Streptococcus pneumonae*		
1	*Streptococcus pneumonae DPS076*	0.55814
GROUP 15: *Streptococcus pyogenes*		
1	*Streptococcus pyogenesDPS044*	0.98156
GROUP 16: *Streptococcus sanguis*		
1	*Streptococcus sanguis I DPS007*	0.74988
2	*Streptococcus sanguis I DPS072*	0.64441
3	*Streptococcus sanguis I DPS 068*	0.57898
4	*Streptococcus sanguis I DPS023*	0.53345
5	*Streptococcus sanguis I DPS029*	0.46449
GROUP 17: *Streptococcus uberis*		
1	*Streptococcus uberis DPS067*	0.59199

Table 3: Occurrence of different species of streptococci from the dental plaque.

Streptococcus species	No. of isolates	Frequency obtained
S. agalactiae	1	1.25
S. cremoris	7	**7.5**
S. equinus	2	2.5
S. faecalis	4	5
S. faecium	19	23.75
S. group F	1	1.25
S. group H	3	1.25
S .group O	1	3.75
S .group Q1	5	6.25
S .lactis	1	1.25
S. milleri	9	11.25
S. mitior	16	20
S. mutans	3	3.75
S. pneumonae	1	1.25
S. pyogenes	1	1.25
S. sanguis I	5	7.5
S. uberis	1	1.25
Total	80	

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