In vitro susceptibility of viridans streptococci to leaf extracts of *Mangifera Indica*

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Received: 22 December 2006 / Final revision: 15 March 2007 / Accepted: 23 April 2007

Abstract The susceptibility of Viridans streptococci to leaf extracts of *Mangifera indica* was studied on 53 clinical isolates from 39 patients. All the isolates were found to be susceptible to both water and methanol extract of *M. indica* leaves. Minimum inhibitory concentration (MIC) of methanol extract for all isolates were <50 mg/mL and MIC of water extract for all isolates were <200 mg/mL. Methanol extract was found to have better action against Viridans streptococci than water extract.

Keywords Viridans streptococci · *Mangifera indica*, · *Streptococcus mutans* · Leaf extract

Introduction

Association of Streptococci with dental caries is well established¹. Among the human oral micro flora, *S. mutans* is considered as one of the most important cariogenic species^{2,3}. Dental caries is the outcome of multiple complex processes involving factors like diet, microorganisms, trace elements, saliva, genetic predisposition and tooth morphology. It is highly prevalent in India, which is influenced by the lack of dental awareness among the public. Epidemiological studies show that 66.2% of urban and 47.8% of rural population is affected by dental caries⁴ and 88% of children of age group 6–11 suffer from the same⁵.

Regarding treatment needs, 63.6% of children require treatment for various reasons in accordance with dental caries prevalence of different age groups⁶. The suppression of the bacteria, especially *S. mutans* by antimicrobial agents is consequently of medical importance. The increased use of antibiotics both for caries prophylaxis and treatment could ultimately lead to the development of antibiotic resistance. Resistance to Cefuroxime, Penicillin, Tetracycline and mercury has been reported for *S. mutans*⁷. The threat of other pathogens acquiring this resistance is always present. An example is the transfer of penicillin resistant

Table 1 Table showing the frequency of isolated organisms.

Organism	No	%
<i>S.mutans</i>	31	58
<i>S.salivarius</i>	9	16.98
<i>S.mitior</i>	9	16.98
<i>S.milleri</i>	2	3.7
<i>S.sanguis</i>	2	3.7

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Table 2 MICs of methanol and water extract for each isolate.

Isolate No:	Patient	Isolate	MIC (mg/mL) Methanol extract	MIC (mg/mL) Water extract
1	P1	<i>S.mutans</i>	50	200
2	P2	<i>S.mutans</i>	50	200
3	P2	<i>S.milleri</i>	50	200
4	P3	<i>S.mutans</i>	50	200
5	P4	<i>S.mutans</i>	25	200
6	P5	<i>S.mutans</i>	25	200
7	P5	<i>S.salivarius</i>	25	100
8	P6	<i>S.mutans</i>	25	200
9	P7	<i>S.mutans</i>	25	200
10	P8	<i>S.mutans</i>	25	100
11	P8	<i>S.mitior</i>	25	200
12	P9	<i>S.mutans</i>	50	100
13	P10	<i>S.mutans</i>	50	200
14	P11	<i>S.mutans</i>	50	200
15	P11	<i>S.mitior</i>	25	100
16	P11	<i>S.salivarius</i>	25	200
17	P12	<i>S.salivarius</i>	50	100
18	P13	<i>S.salivarius</i>	25	100
19	P13	<i>S.mutans</i>	50	200
20	P14	<i>S.mutans</i>	50	100
21	P14	<i>S.sanguis</i>	50	200
22	P15	<i>S.mutans</i>	25	200
23	P16	<i>S.mutans</i>	≤12.5	200
24	P16	<i>S.mitior</i>	25	100
25	P17	<i>S.mitior</i>	25	200
26	P17	<i>S.salivarius</i>	50	100
27	P18	<i>S.salivarius</i>	25	200
28	P19	<i>S.mutans</i>	25	200
29	P19	<i>S.milleri</i>	50	100
30	P20	<i>S.mutans</i>	≤12.5	200
31	P21	<i>S.mitior</i>	25	100
32	P22	<i>S.mutans</i>	25	200
33	P23	<i>S.mutans</i>	≤12.5	200
34	P23	<i>S.mitior</i>	25	200
35	P24	<i>S.salivarius</i>	25	100
36	P24	<i>S.mutans</i>	25	100
37	P25	<i>S.sanguis</i>	25	200
38	P25	<i>S.mutans</i>	50	100
39	P26	<i>S.mutans</i>	25	100
40	P27	<i>S.mutans</i>	25	200
41	P28	<i>S.salivarius</i>	25	100
42	P29	<i>S.mutans</i>	25	100
43	P30	<i>S.mutans</i>	25	200

Table 2 Continued

44	P31	<i>S.mutans</i>	25	100
45	P32	<i>S.mutans</i>	25	200
46	P33	<i>S.salivarius</i>	25	100
47	P34	<i>S.mutans</i>	25	200
48	P35	<i>S.mutans</i>	≤12.5	100
49	P36	<i>S.mutans</i>	25	100
50	P37	<i>S.mitior</i>	25	100
51	P38	<i>S.mitior</i>	25	100
52	P39	<i>S.mitior</i>	25	100
53	P39	<i>S.mutans</i>	25	100

Table 3 MIC s of the isolates.

MIC	No of isolates	
	Methanol	Water
≤12.5	4	
25	35	
50	14	
100		25
200		28

genes from oral *Streptococci* to *Streptococcus pneumoniae*⁸. This necessitates identification, isolation and characterization of molecules from natural sources for the effective control of cariogenic bacteria.

Mangifera indica is a common indigenous material used for the maintenance of oral hygiene. Mango leaves have been reported to have significant antibacterial properties against certain species of *Staphylococci*, *Pneumococci* and *Lactobacilli*⁹. Mangiferin, a compound present in mango leaves has been reported to be the active principle⁵. Mangiferin is also found to have anti allergic and anti helminthic activities¹⁰. Studies have shown that caries experience in people using mango leaf as an oral hygiene aid is similar to that of the group that used tooth brush⁵. Works comparing the efficacy of various indigenous oral hygiene methods like neem extracts, charcoal, common salt, babul etc prove the superiority of *M.indica* over the others¹¹. Western methods of caries prevention as such will not be successful here and they need to be modified to suit the local needs and habits. Hence we tried to evaluate the effect *M. indica* leaf extracts on Viridans streptococci isolated from dental caries, especially on *S. mutans*, which is the established etiological agent of dental caries.

Materials and Methods

Paraffin stimulated whole saliva samples were collected from 39 patients all of whom were suffering from

dental caries. 100µL of whole saliva was transferred to tubecontaining 1mL of Brain heart infusion broth. This was incubated at 37°C for 24 hours and then sub cultured on to Mitis Salivarius agar containing (g/L), peptone,5, Beef extract,5, Sucrose,10, Dextrose,10, Dipotassium phosphate,4, Trypan blue,.075, Crystal violet,.0008, Agar,20 and 1% filter sterilized Potassium tellurite. The isolates from MSA agar plates were identified on the basis of colony characteristics, Gram staining and sugar fermentation studies. The fermentation tests were performed by sub culturing the isolates to Brain Heart infusion broth containing specific sugars(Glucose, Mannitol, Sorbitol, Sucrose, Starch & Aesculin hydrolysis)¹².

The susceptibility of all these isolates was tested against water and methanol extract of *M. indica* leaves. Extracts were prepared by successively extracting the dried and powdered leaves in a Soxhlet apparatus using water and methanol.

The preliminary susceptibility testing was done by agar well diffusion method. Isolated organisms were swabbed on to Mueller Hinton agar supplemented with blood. Wells were cut on the agar plate and 100 µL of water and methanol extracts were added to separate wells and incubated. Wells containing pure solvents were used as controls. After incubation, the plates were observed for zones of inhibition around the wells.

Determinations of MIC of extracts to the isolates were done by plate dilution method¹³. Various amounts of extracts were added to 10 mL of Mueller Hinton agar supplemented with blood to get final concentrations of 100, 50, 25 &12.5 mg/mL for methanol extract and 400,200,100&50 mg/mL for water extract. Isolates were cultured on these plates using spot inoculation method. A plate without any added extract was incubated as growth control. The plates were incubated at 35°C for 48 hrs in a 5% CO₂ atmosphere and observed for the presence or absence of growth.

Results and Discussion

A total of 53 isolates were identified from 39 samples based on their morphological, cultural and biochemical characteristics. Among these, 31 were (58%) identified as *S. mutans* and 9 each (16%) as *S. salivarius* and *S. mitior*. 2 isolates (4%) each of *S. milleri* and *S. sanguis* were also identified. Thirteen out of 39 samples produced more than one type of bacterial species. This further confirms the previous studies that the chief agent of dental decay is *S. mutans* (Table 1).

In the screening test using agar well diffusion method, a zone of inhibition of more than 10 mm was observed for all isolates for both water and methanol extract.

The agar dilution assay using methanol extract showed an MIC $\leq$ 50mg/mL for all isolates. MIC of 35 isolates (66%) was 25 mg/mL. 21 out of 31 *S. mutans* isolates (67%) were inhibited by 25mg/mL. MIC of 4 isolates were $\leq$ 12.5mg/mL (Table 2, 3).

MIC of water extracts were found to be much higher. MIC of all isolates was $\leq$ 200mg/mL. 25 isolates (47%) required only 100 mg/mL of water extract for inhibition of growth (Table2).

In general, methanol extract was found to be more active than water extract. Further purification and characterization of methanol extract for identification of bioactive molecules against oral *Streptococci* is being carried out.

Acknowledgements The authors are grateful to Dr Prakash Kumar B, Department of Biotechnology, St Thomas College, Pala for constant encouragement and support.

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