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GLUCAN PRODUCTION BY VIRIDANS GROUP STREPTOCOCCI

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ABSTRACT

A preliminary screening to determine the qualitative and quantitative glucan producing potential of VGS (Viridans group streptococci) was performed. VGS were recovered from oral cavity of individuals from a previous study based on prevalence of dental caries. Overall, out of 525 isolates, 41.5% were glucan producers while 58.5% were glucan non-producers. Species-wise comparison revealed the highest frequency of *S. mutans* (80.3%) exhibiting glucan producing potential followed by *S. oralis* (61.1%), *S. intermedius* (50%), *S. anginosus* (36.7%), *S. mitis* (35%) and *S. salivarius* (14.9%). None of the isolates of *S. uberis* and *S. acidominimus* showed the ability to produce glucan. On the basis of prevalence of dental caries, the frequency of isolates having glucan producing potential was observed higher from carious subjects (46.3%) as compared to non-carious subjects (39.1%). Whereas, species-wise distribution of isolates indicated that the prominent glucan producing species were *S. sanguinis* (100%, 3/3) and *S. mutans* (76%, 22/29) from carious and *S. mutans* (83%, 39/47) from non-carious subjects. The VGS isolates producing large amount of glucan were selected to be estimated quantitatively glucan producing potential of VGS. In this case, *S. sanguinis* produced largest quantity (276.2 mg mean, 206.2-324.6 range) followed by *S. mutans* (143.5 mg mean, 43.5-521.1 range).

Key words: VGS, glucan, dental caries, carious and non-carious subjects

INTRODUCTION

Glucans (extracellular polysaccharides) are D-glucose polymers (polyglucosan) linked by glucosidic bonds. The production of glucans is catalyzed by most of VGS in the presence of glucosyltransferase enzymes (GTFs) using dietary sucrose. Generally, glucans are of two types i.e. soluble and insoluble. Glucans have difference in their physical and chemical characteristics such as molecular weight, solubility in water, intrinsic viscosity, specific rotation, nature of solid products and difference in the proportion of glucosidic bonds (glucopyranosidal units) (Qader *et al*., 2005). According to molecular weight, soluble glucans are less in weight (20-50,000 Daltons) as compared to insoluble glucans (10^6-10^7 Daltons). Mainly, soluble glucans consist of α- 1, 6glucosidic bond linkages whereas insoluble glucans comprise high numbers of α- 1, 3glucosidic bonds linkages (Yu *et al*., 2018). Compared to soluble glucans, the insoluble glucans are recognized as virulence factor for the cariogenicity of various species of VGS and development of dental caries. The insoluble glucans play role as a barrier against the diffusion of salivary buffer which neutralize the acids formed in dental plaque by the activity of cariogenic species of VGS. Glucans can also promote adherence of bacteria to the tooth surfaces. In addition, glucans consequently increase the demineralization of tooth enamel and have a major role in the porosity and structural integrity of biofilm (Palmer *et al*., 2018; Ccahuana-Vasquez and Cury, 2010; Qader *et al*., 2005). Glucans have also been reported as an antigen and haptene. Glucan producing ability of VGS has considerable importance in the cariogenicity as well as in the formation of dental plaque (Ito *et al*., 2012). In view of above, the aims of present study were to determine qualitative and quantitative estimation of glucan producing potential of VGS.

MATERIALS AND METHODS

A total of 525 isolates of 09 different species of VGS viz., *S. anginosus* (281), *S. mutans* (76), *S. mitis* (60), *S. uberis* (34), *S. intermedius* (24), *S. sanguinis* (20), *S. oralis* (18), *S. salivarius* (07) and *S. acidominimus* (05) were used in the present study. These isolates were recovered from carious and non-carious subjects of all age groups, Karachi, Pakistan. All the isolates of VGS were maintained on Sodium azide blood agar slants (Baron *et al*., 1994; Facklam, 2002). Qualitative and quantitative estimation of glucan producing potential of VGS was carried out by method as described by Masumoto *et al*. (1987), Baron *et al*. (1994) and Wiater *et al.* (1999). Brain Heart Infusion Broth (BHIB) (Merck) was used for preparation of inoculum and 5% Sucrose broth containing 0.04% Sodium azide was used for the qualitative and quantitative estimation of glucan production by VGS. Qualitative glucan production was indicated by partial or complete gelling of the broth or by formation of gelatinous or adherent deposits on the

bottom and walls of the tube while absence of glucan was noted when no gelling or deposits were formed (Baron *et al*. 1994). Quantitative glucan production was noted in terms of the dry weight in milligram (mg). VGS isolates producing large amount of glucan were selected for quantitative estimation of glucan production.

RESULTS AND DISCUSSION

To our knowledge, there is limited research studies demonstrating about the glucan producing potential of VGS isolated from oral cavity. In the present study, the preliminary qualitative screening was conducted to determine the glucan producing potential of 525 isolates of 09 different species of VGS viz., *S. anginosus* (281), *S. mutans* (76), *S. mitis* (60), *S. uberis* (34), *S. intermedius* (24), *S. sanguinis* (20), *S. oralis* (18), *S. salivarius* (07) and *S. acidominimus* (05). The results are mentioned in Table 1. Of 525 isolates, 218 (41.5%) isolates of VGS were found glucan producers and 307 (58.5%) were glucan non-producers.

Among different species of VGS, out of 218 glucan producing isolates, the highest frequency was noted for *S. mutans* (80.3%, 61/76) followed by *S. oralis* (61.1%, 11/18), *S. intermedius* (50%, 12/24), *S. mitis* (38.3%, 23/60), *S. anginosus* (36.7%, 103/281), *S. sanguinis* (35%, 07/20) and *S. salivarius* (14.9%, 01/07) (Table 1). In case of glucan non-producers, the highest frequencies were found for *S. uberis* (100%, 34/34) and *S. acidominimus* (100%, 05/05) followed by *S. salivarius* (85.7%, 06/07), *S. sanguinis* (65%, 13/20), *S. anginosus* (63.3%, 178/281), *S. mitis* (61.7%, 37/60), *S. intermedius* (50%, 12/24), *S. oralis* (38.9%, 07/18) and *S. mutans* (19.7%, 15/76) (Table I). The present study is correlated with research of Barrientos and Rodriguez (2011) who reported isolation of *S. mutans* producing glucan and glucosyltransferase from non-carious subjects. This indicates that there are conditions in the oral cavity different from these factors that keep the subjects free from dental caries, which should be investigated in the further research for strategies to control the dental disease. There could be possibilities of the apparently less cariogenic strains of *S. mutans* appear to compete more successfully against other oral commensals than do the more cariogenic strains of *S. mutans* isolated from non-carious subjects. These less cariogenic strains then occupy the niche for *S. mutans*-like organism within the dental plaque biofilm and thus help maintain its low cariogenicity (Peter Holbrook and Magnusdottir, 2011).

In the present study, the incidence of isolates having glucan producing potential was also compared with respect to prevalence of dental caries (Table I). Out of 175 isolates obtained from carious subjects, 81 (46.3%) isolates were found glucan producers and 94 (53.7%) isolates were glucan non-producers. While, in case of 350 VGS isolates obtained from non-carious subjects, 137 (39.1%) and 213 (60.9%) isolates were found as glucan producers and glucan non-producers respectively (Table 1).

Species-wise distribution of glucan producers was also noted with respect to prevalence of dental caries (Table I). In case of 81 glucan producing isolates obtained from carious subjects, *S. mutans* was the most prominent species as 76% (22/29) isolates exhibited glucan producing potential. It was followed by *S. oralis* (75%, 03/04), *S. mitis* (69.2%, 09/13), *S. intermedius* (55.6%, 05/09), *S. anginosus* (40%, 38/95) and *S. salivarius* (16.7%, 01/06). While, all the three isolates of *S. sanguinis* also produced glucan. In case of non-carious subjects, distribution of glucan producers with respect to different species also revealed the highest frequency for *S. mutans* (83%, 39/47) followed by *S. oralis* (57.1%, 08/14), *S. intermedius* (46.7%, 07/15), *S. anginosus* (35%, 65/186), *S. mitis* (29.8%, 14/47) and *S. sanguinis* (23.5%, 04/17) (Table 1).

Species-wise distribution of glucan non-producers revealed that among 94 glucan non-producers obtained from carious subjects, *S. uberis* was the most prominent species as 100% (15/15) isolates were found glucan non-producers. It was followed by *S. salivarius* (83.3%, 05/06), *S. anginosus* (60%, 57/95), *S. intermedius* (44.4%, 04/09), *S. mitis* (30.8%, 04/13), *S. oralis* (25%, 01/04) and *S. mutans* (24.1%, 07/29). The single isolate of *S. acidominimus* also did not produce glucan. While, among 213 glucan non-producers isolated from non-carious subjects, the highest number of isolates was also noted for *S. uberis* (100%, 19/19) followed by *S. acidominimus* (100%, 04/04), *S. sanguinis* (76.5%, 13/17), *S. mitis* (70.2%, 33/47), *S. anginosus* (65%, 121/186), *S. intermedius* (53.3%, 08/15), *S. oralis* (42.9%, 06/14) and *S. mutans* (17%, 08/47). The single isolate of *S. salivarius* did not produce glucan (Table 1).

In the present study, fifty three isolates belonging to 6 different species of VGS viz., *S. mutans* (26), *S. anginosus* (12), *S. sanguinis* (05), *S. intermedius* (05), *S. oralis* (04) and a single isolate of *S. salivarius* were used to check quantitative estimation of glucan production. The results are shown in Table 2. Different codes were assigned for respective isolates used in the study i.e. SMUC and SMUN for *S. mutans* isolated from carious and non-carious subjects respectively followed by SAC and SAN for *S. anginosus*, SSNC and SSNN for *S. sanguinis*, SIC and SIN for *S. intermedius*, SOC and SON for *S. oralis* (04) and SSLC for *S. salivarius*.

Table 1. Screening of glucan producing potential of viridans group streptococci isolated from carious & non-carious subjects.

Organisms	Total No. of isolates	Subjects	Number of isolates	Number of isolates having glucan producing potential							
				Positive		Total		Negative		Total	
				No.	%	No.	%	No.	%	No.	%
S. anginosus	281	Carious	95	38	40	103	36.7	57	60	178	63.3
		Non-carious	186	65	35			121	65		
S. mutans	76	Carious	29	22	76	61	80.3	07	24.1	15	19.7
		Non-carious	47	39	83			08	17		
S. mitis	60	Carious	13	09	69.2	23	38.3	04	30.8	37	61.7
		Non-carious	47	14	29.8			33	70.2		
S. uberis	34	Carious	15	0	0	0	0	15	100	34	100
		Non-carious	19	0	0			19	100		
S. intermedius	24	Carious	09	05	55.6	12	50	04	44.4	12	50
		Non-carious	15	07	46.7			08	53.3		
S. sanguinis	20	Carious	03	03	100	07	35	0	0	13	65
		Non-carious	17	04	23.5			13	76.5		
S. oralis	18	Carious	04	03	75	11	61.1	01	25	07	38.9
		Non-carious	14	08	57.1			06	42.9		
S. salivarius	07	Carious	06	01	16.7	01	14.9	05	83.3	06	85.7
		Non-carious	01	0	0			01	100		
S. acidominimus	05	Carious	01	0	0	0	0	01	100	05	100
		Non-carious	04	0	0			04	100		
Total	525	Carious	175	81	46.3	218	41.5	94	53.7	307	58.5
		Non-carious	350	137	39.1			213	60.9		

Table 2.Quantitative estimation of glucan producing potential of viridans group streptococci.

Organisms	Subjects	Total number of isolates	Code No.	Glucan production (mg)	Mean	Range
S. mutans(SMU)	Carious (C)	19	SMUC1	12.2	133.1	12.2 - 541
			SMUC2	39.3		
			SMUC3	39.9		
			SMUC4	46.5		
			SMUC5	52.1		
			SMUC6	54.3		
			SMUC7	54.4		
			SMUC8	54.8		
			SMUC9	59.4		
			SMUC10	67.8		
			SMUC11	88.1		
			SMUC12	99.3		
			SMUC13	111.3		
			SMUC14	122.5		
			SMUC15	177.7		
			SMUC16	221		
			SMUC17	333.2		
			SMUC18	353.6		
			SMUC19	541		
	Non-carious (N)	07	SMUN1	43.5	143.5	43.5 - 521.1
			SMUN2	73.1		
			SMUN3	78.8		
			SMUN4	88		
			SMUN5	98.3		
			SMUN6	101.9		
			SMUN7	521.1		
S. anginosus(SA)	Carious (C)	07	SAC1	73.4	196.9	73.4 - 388.5
			SAC2	81.9		
			SAC3	131.1		
			SAC4	186.2		
			SAC5	244.6		
			SAC6	272.5		
			SAC7	388.5		
	Non-carious (N)	05	SAN1	12.3	65.8	12.3 – 160.7
			SAN2	16.6		
			SAN3	41.8		
			SAN4	97.8		
			SAN5	160.7		
S. sanguinis(SSN)	Carious (C)	03	SSNC1	206.2	276.2	206.2 - 324.6
			SSNC2	297.7		
			SSNC3	324.6		
	Non-carious (N)	02	SSNN1	89.4	105.9	89.4-122.4
			SSNN2	122.4		
S. intermedius(SI)	Carious (C)	03	SIC1	62.5	87.3	62.5 - 101.9
			SIC2	97.4		
			SIC3	101.9		
	Non-carious (N)	02	SIN1	18.7	33.6	18.7 - 48.4
			SIN2	48.4		
S. oralis(SO)	Carious (C)	03	SOC1	34.3	169.1	34.3 - 340.7
			SOC2	132.2		
			SOC3	340.7		
	Non-carious (N)	01	SON1	18.3	18.3	18.3
S. salivarius(SSL)	Carious (C)	01	SSLC1	77.3	77.3	77.3
	Non-carious (N)	None	-	-	-	-

In the present study, overall, in case of carious subjects, the highest mean of glucan production was noted for SSNC i.e. 276.2 followed by SAC (196.9), SOC (169.1), SMUC (133.1), SIC (87.3) and SSLC (77.3) (Table 2). As shown in Table 2, among VGS isolated from non-carious subjects, the high amount of mean of glucan production was noted from SMUN (143.5) followed by SSNN (105.9), SAN (65.8), SIN (33.6) and SON (18.3). Present study showed glucan producing species recovered from non-carious subjects, but their role is unclear. Oral VGS have both harmful and harmless bacteria. However, under special conditions commensal VGS can become opportunistic pathogens, initiating disease and damaging the host. Also researchers have documented that dental caries is multifactorial disease categorized in three different factors i.e. risk factor, risk indicator and risk inhibitor. These factors are bacteria, time, susceptible tooth surface and fermentable carbohydrates. Along with these factors, there are certain behavioral and sociodemographic factors that are likely to increase the risk of dental caries. It is hard to determine one major factor associated with dental caries because they are all interlinked with each other and have importance for development of dental caries. There is possibility that non-carious subjects may not use sugar containing diets and other products and could be maintain their oral hygiene practices which promote to reduce the plaque production and dental caries. As sugar containing diets play an important role in the formation of glucan. Among sugars, sucrose is a key factor for dental caries. Sucrose increases the growth of cariogenic bacteria. Beside sucrose, starch is also a major component present in the diet in different forms. Starch is not easily available for bacteria because bacteria cannot easily break high molecular weight sugars into smaller units as it is an essential step before entering the plaque (Zahara *et al*., 2010). Beside consumption of sugar, frequency of total amount of sugar is also important for promoting dental caries. If sugar is consumed only at meal time, the pH of saliva critically drops for 3 hours whereas pH becomes below than critical level for longer period when sugar is consumed between meals (Harties and Leach, 1975). The process of dental caries becomes negligible if sugars are consumed frequently in low or balance amount and present in a form that are not retained in the oral cavity for longer period.

CONCLUSION

In the present study, different isolates of VGS recovered from oral cavity of carious and non-carious subjects produce virulence factors associated with dental caries, such as glucans. It is one of the major virulence factors in the formation of dental plaque and dental caries. However, its role is unclear in non-carious subjects who carry production of glucan by different species belonging to VGS which should be interrogated in future for finding strategies to control the dental caries.

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