Food Chemistry 38 (1990) 289–296

Effect of Diet on Caries-Inducing *Streptococcus mutans* Growth

S. Tandon, A. R. Prabhakar

Department of Pedodontics and Preventive Dentistry,
College of Dental Surgery, K.M.C., Manipal, India

&

S. Udupa

Department of Biochemistry, K.M.C., Manipal, India

(Received 27 June 1989; revised version received and accepted 3 January 1990)

ABSTRACT

Different food preparations have been tested for support of growth of the bacteria Streptococcus mutans in vitro *both before and after chewing. Fish fry and the sweet dishes*—kesari baath *and* kadla payiasa—*supported the growth of* S. mutans *maximally, whereas bananas and potato chips did not support growth at all. All other foodstuffs supported from mild (*dosa, upma*) to moderate (rice preparations, yogurt) degrees.* Dosa *and* upma *supported growth of* S. mutans *to a greater extent after chewing, whereas in all other cases, chewing did not further enhance the growth.*

INTRODUCTION

It is well established that the initial phase of dental caries involves demineralization of tooth enamel by organic acids produced due to the presence of a number of micro-organisms in the oral cavity. *Streptococcus mutans* species are among the various micro-organisms implicated as the major and most virulent initiators of dental caries. These acidogenic bacteria accumulate in the dental pellicle formed on the tooth surfaces and produce organic acids by their anaerobic metabolism of left-over food particles. The

Food Chemistry 0308-8146/90/$03·50

degree of support rendered by a particular foodstuff for the growth of *Streptococcus mutans* could serve as an index to the cariogenic potential of that foodstuff. In an earlier epidemiological study, the prevalence of dental caries was found to be very high in South Kanara children and it showed a highly significant correlation with the type of food consumed (Shetty & Tandon, 1988). Hence, it was considered worthwhile to determine the extent to which some of the most commonly consumed local foodstuffs were supportive to the growth of *Streptococcus mutans.*

MATERIALS AND METHODS

Selection of reference food items

A preliminary survey was conducted to discover the most common food items that were consumed by the children of South Kanara. The survey comprised 2000 children representing 12 schools in and around Manipal. All the children who took part in the survey were asked to record 24-h diet charts for 7 days and to mark those food items which they commonly used at home in a food check-list supplied to them by the investigator. Based on the above information collected from the children, 13 food items were selected as the most routine weekly diet of a child of average socio-economic status and were categorized in the following groups:

Fruits	Bananas
Fried foods	Potato chips, ground-nuts, fish fry
Breakfast items	*Dosa, idli* (rice and *urad*)
Snacks	*Upma*
Meals	Boiled rice, boiled rice gruel, yogurt
Sweet diets	*Kadla payiasa, kesari baath*

Brief recipes of the food items are given in the appendix.

Selection of children

The children involved in the present study were randomly selected in the age group of 7–13 years. Their teeth were healthy with normal occlusion and were cleaned by the investigator before starting the experiment.

Preparation of nutrient and glucose broths

This was prepared by mixing 1% peptone (w/v), 1% beef extract (w/v) and 0·5% NaCl (w/v) in 100 ml of water. The ingredients were mixed and dissolved by heating briefly in a steamer. The contents were cooled and the

pH adjusted to 7·5. The solution was filtered and the clear solution was autoclaved at 121°C for 15 min. Glucose broth was prepared similarly but with the inclusion of 1% glucose (w/v), and the resulting broth made sterile by free steaming for 1 h.

Determination of the effect of foodstuffs on the growth of *S. mutans*

Each of the freshly prepared solid reference food items was ground separately in a sterile 10 Broeck tissue grinder (Achyuth Rao & Kotian, 1976). Approximately 10 g of each food item was transferred into a 250 ml conical flask containing 100 ml of sterile nutrient broth. Liquid food items (10 ml) were directly transferred into similar conical flasks containing 100 ml of nutrient broth. Next, approximately 10 g or 10 ml of each of the foodstuffs (solid or liquid) was given to each of the children. They were asked to chew/swish the foodstuff for 40 s. The oral contents were collected separately into sterile conical flasks. One gram or 1 ml of the oral contents thus obtained was then placed in 250 ml sterile conical flasks containing 100 ml of nutrient broth. All the conical flasks with their respective food contents and nutrient broth were subjected to free steaming for 1 h. After cooling to room temperature each medium was inoculated with 0·0125 cm^3 of the overnight glucose broth culture of the *S. mutans* (Fig. 1), which was delivered

Fig. 1. *Streptococcus mutans* colonies grown on blood agar (coloured mesh).

with a Pasteur pipette whose bevel tip was cut at 56 Moorse gauge. The inoculated samples were incubated at 37°C for 24 h. The *S. mutans* inoculated in nutrient broth alone and nutrient broth containing 1% glucose were also incubated similarly. These served as controls.

Measurement of *S. mutans* growth

The growth of *S. mutans* was measured by comparing the turbidity with Brown's opacity tubes (Brown, 1919). The amount of growth noted with respect to each food item was obtained by comparing the turbidity of the nutrient broth inoculated with *S. mutans* with food before and after chewing with standard Brown's tubes. The standard Brown's tubes range from No. 1 to No. 7, depending upon the different degrees of opacity which indicate the various degrees of bacterial growth. In this study, No. 1 to No. 3 Brown's tubes were used indicating dense (+ + +), moderate (+ +) and mild growth (+), respectively (Fig. 2). To ascertain that the turbidity observed in the inoculated nutrient broth was due to the presence of *S. mutans* alone, a subculture was performed and confirmed.

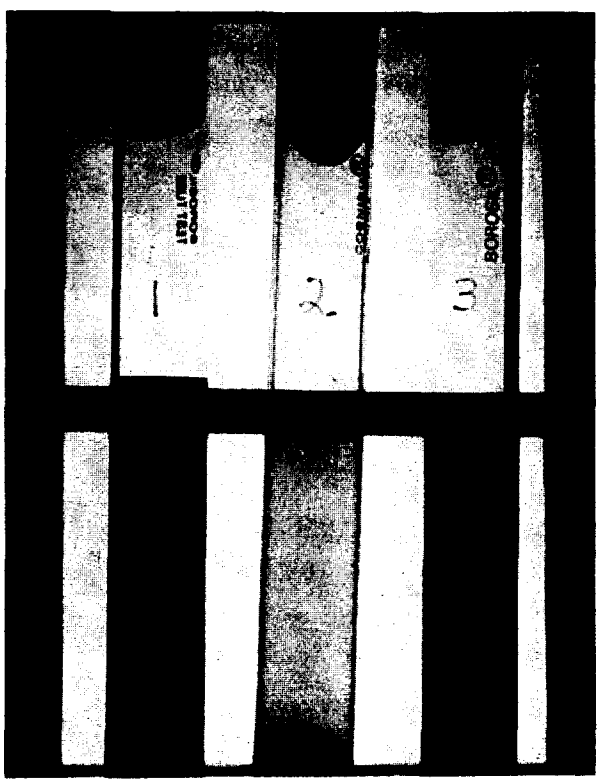

Fig. 2. Gradation of growth on liquid media: 1, + + +; 2, sterile; 3, + +.

RESULTS AND DISCUSSION

The degree of support rendered by the various food items to the growth of *Streptococcus mutans* is depicted in Table 1. The sweet dishes, *kadla payiasa* and *kesari baath*, showed the highest degree of support (+ + +) towards the growth of *S. mutans* both before and after chewing. This observation was expected, since these foodstuffs are rich in sucrose. Krasse (1975) reported that the most important substrate for the involvement of *S. mutans* in the caries process is the disaccharide sucrose. Guggenheim (1968) has reported the ability of bacteria to stick to the tooth surface as an absolute requirement for the formation of plaque. The sticky surface has been found to be imparted by the intermediary products of sucrose and other dietary carbohydrates (glucans and fructans) (Berman & Gibbons, 1966).

All the rice preparations (rice *idli*, *urad idli*, boiled rice and boiled rice gruel) as well as yogurt showed only a moderate (+ +) degree of support to the growth of *S. mutans*. This could be related to the presence of moderate amounts of carbohydrates and proteins as their main ingredients. The inherent pH of these foodstuffs is higher than that of other foodstuffs (Prabhakar *et al.*, 1989). In addition, the major carbohydrate in these foodstuffs is starch, which is less cariogenic than mono- or disaccharides (Wendy *et al.*, 1985; Udupa *et al.*, 1989).

TABLE 1
Effect of Various Food Items on the Growth of *Streptococcus mutans*[a]

Food item	*Growth in food + nutrient broth*	*Growth in chewed food + nutrient broth*	*Growth in nutrient broth*	*Growth in glucose broth*
Bananas	−	−	−	+ +
Potato chips	−	−	−	+ +
Dosa	+	+ +	−	+ +
Upma	+	+ +	−	+ +
Urad idli	+ +	+ +	−	+ +
Rice *idli*	+ +	+ +	−	+ +
Fried ground-nut	+ +	+ +	−	+ +
Boiled rice	+ +	+ +	−	+ +
Boiled rice gruel	+ +	+ +	−	+ +
Yogurt	+ +	+ +	−	+ +
Fish fry	+ + +	+ + +	−	+ +
Kesari baath	+ + +	+ + +	−	+ +
Kadla payiasa	+ + +	+ + +	−	+ +

[a] Growth compared to Brown's opacity tube No. 1 (+ + +), No. 2 (+ +), No. 3 (+).

Dosa and *upma* showed only a mild (+) degree of support to *S. mutans* growth. However, the chewed food seemed to support the growth to a greater extent. It is possible that some component(s) of the foodstuff is able to inhibit the bacterial enzymes. Kashket *et al.* (1985), in an in-vitro study, found that glucosyl transferase from dental plaque *S. mutans* was strongly inhibited by some beverages and fruit juices. Possibly in the cases of *dosa* and *upma* this inhibition may be reversed by salivary components, thereby enhancing *S. mutans* growth upon chewing.

The fried foods—fish fry, fried ground-nuts and potato chips—showed varying degrees of support, from dense to no support, respectively. The variations may be due to the difference in types of ingredients they contained. Two animal studies (Rosen *et al.*, 1984; Wendy *et al.*, 1985) have shown a heavy oral population of *S. mutans* with the cheese, rich in protein, and no significant growth of *S. mutans* with the maize starch. This could explain why fish and ground-nuts have shown severe to moderate growth of *S. mutans* in the present study, whereas potato chips showed no growth at all.

Banana, too, showed no support to *S. mutans* growth. This interesting finding of the present study is not in agreement with an earlier study (Gupta *et al.*, 1985) where banana was found to be highly cariogenic. This could be due to difference of inherent pH of the banana used. The type of banana which was used for the present study had a high inherent pH of 6·73 (Prabhakar *et al.*, 1989) compared to an inherent pH of 5·4 for the type of banana used in the previous study.

Many studies have established the existence of an intimate relationship between *S. mutans* and dental caries (Clarke, 1924; Drucker *et al.*, 1972; Kirk *et al.*, 1972; Fitzgerald, 1976). Dental plaque is a concentrated aggregation of micro-organisms, mucopolysaccharides and other components. The survival of micro-organisms on dental plaque is by the anaerobic metabolism of carbohydrates and proteins present in foodstuffs retained in the oral cavity (Berman & Gibbons, 1966; Skinner & Naylor, 1972; Wendy *et al.*, 1985). The acids produced by the fermentation process are believed to cause demineralization of tooth enamel. Hence, foodstuffs which promote the growth of *S. mutans* could be considered potentially cariogenic.

In the present study the variation in the degree of support by *dosa* and *upma* to *S. mutans* growth, observed in different experimental conditions, could not be explained convincingly. With the exceptions of banana and potato chips, all the foodstuffs supported the growth of *S. mutans* in varying degrees. Hence, contrary to the popular belief that sweet foods alone are cariogenic, many of the non-sweet foodstuffs can also be considered potentially cariogenic. Although the present study is not enough to produce unequivocal evidence that each reference food which supported the growth of *S. mutans* has definite cariogenic potential, it definitely provides

information to recommend intelligent use of these commonly consumed foodstuffs by South Kanara children. However, an in-depth study is required with reference to those food items which showed non-supportive trends or variation towards the growth of *S. mutans*.

REFERENCES

Achyuth Rao, K. M. & Kotian, M. (1976). A typical myocobacteria and sterility. *Lancet*, **i**, 1301–2.

Berman, K. S. & Gibbons, R. J. (1966). Iodophilic polysaccharide synthesis by human and rodent oral bacteria. *Arch. Oral Biol.*, **11**, 533–5.

Brown, H. C. (1919). Brown's opacity tubes. *Ind. J. Med. Res.*, **7**, 238–40.

Clarke, J. K. (1924). On the bacterial factor in the aetiology of dental caries. *Br. J. Exp. Path.*, **5**, 141–5.

Drucker, D. B., Green, R. M. & Blackmore, D. K. (1972). Caries induced in rats in three weeks by a *Streptococcus* isolated from man. *J. Dent. Res.*, **51**, 1510–11.

Fitzgerald, R. J. (1976). The microbiology of plaque in relation to dental caries. *Abstr. Micro.*, **3**, 849–52.

Guggenheim, B. (1968). Streptococcus of dental plaque. *Caries Res.*, **2**, 147–52.

Gupta, R. P., Tiwari, A. & Chawla, H. S. (1985). Consumption of fresh fruit on the hydrogen ion modulation of human dental plaque. *J. Ind. Soc. Pedo. Prev. Dent.*, **1**, 28–30.

Kashket, S., Paolino, V. J., Lewis, D. A. & Van Houte, J. (1985). *In vitro* inhibition of glucosyl transferase from the dental plaque bacterium *Streptococcus mutans* by common beverages and food extracts. *Arch. Oral Biol.*, **30**, 821–6.

Kirk, C., Hoerman, H., Harris, J., Keene, I. & Shklair, L. (1972). The association of *Streptococcus mutans* with early carious lesions in human teeth. *J. Am. Dent. Assoc.*, **95**, 1349–51.

Krasse, B. (1975). The effect of caries inducing *Streptococci* in hamsters fed diets with glucose or sucrose. *Arch. Oral Biol.*, **10**, 1223–5.

Prabhakar, A. R., Tandon, S. & Udupa, S. (1989). Cariogenic potential of commonly consumed food-stuffs by South Kanara children. *J. Ind. Soc. Pedo. Prev. Dent.*, **7**(1), 1–6.

Rosen, S., Min, D. B., Harper, D. S., Harper, W. J., Beck, E. X. & Beck, F. M. (1984). Effect of cheese, with and without added sucrose on dental caries and recovery of *Streptococcus mutans* in rats. *J. Dent. Res.*, **63**, 894–7.

Shetty, N. & Tandon, S. (1988). Prevalence of dental caries as related to risk factors in school children of South Kanara. *J. Ind. Soc. Pedo. Prev. Dent.*, **6**, 30–7.

Skinner, A. & Naylor, M. N. (1972). Influence of sugar type on the pattern of acid production by *Streptococcus mutans*. *J. Dent. Res.*, **51**, 1022–4.

Udupa, S. L., Prabhakar, A. R. & Tandon, S. (1989). Alpha amylase inhibitors foodstuffs. *Food Chemistry*, **34**, 95–101.

Wendy, A., Horton, E. & Jacob, A. (1985). Cariogenicity of sucrose, glucose and maize starch in gnotobiotic rats mono infested with strains of the bacteria *Streptococcus mutans*, *Streptococcus salivarius* and *Streptococcus milleri*. *Arch. Oral Biol.*, **30**, 777–80.

APPENDIX: BRIEF RECIPES OF THE FOOD PREPARATION

Dosa: Made up of rice and black gram (3:1 w/w), soaked for a few hours, ground to a fine paste, and allowed to ferment overnight. This is then spread out on a pan like a flat pancake.

Idli: Can be made up of rice alone or rice and black gram (3:2 w/w), soaked for a few hours, ground to a thick, coarse paste, allowed to ferment and steamed as small cakes.

Upma: Coarse wheat powder, fried with a little oil, cooked with water, with salt, seasoning.

Ganji: Boiled rice gruel.

Kesari baath: Like *upma*, but instead of salt and seasoning, sugar along with raisins and cashew nuts is added.

Kadla payiasa: Sweet dish prepared by boiling bengal gram with jaggery to which fresh coconut milk is added.