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In vitro growth and acid production by mutans streptococci on traditional African foods

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Abstract

The growth rate and production of acids by mutans streptococci (MS) are influenced by their ability to ferment different dietary carbohydrates. This suggests that the nutrient environment in the oral cavity affects bacterial virulence. The aim of this study was to investigate the effect of maize, samp and brown bread on the growth and acidogenicity of this species. Six laboratory references and five clinical strains isolated from the dental plaque of South African black and 'colored' (historical race classification) children were studied in batch culture on maize, samp (coarsely ground maize), brown bread and compared against a 3% sucrose control. The doubling time of bacterial strains was prolonged in maize (1.9–17.5 h) and samp (2.4–18.4 h), and the number of cell divisions was low. Staple foods accounted for 25% ($F = 5.98$; $P = 0.0007$) and MS strains 30.78% ($F = 2.84$; $P = 0.009$) of the total variance. The fermentation of samp and maize showed the least drop in pH of the culture medium, ranging between 0.54 and 1.06 and 0.69 and 2.28 pH units respectively, with variation between strains most significant in maize ($F = 33.62$; $P < 0.0001$). The total mean concentration of acids produced was highest in bread (25.13 mM/mL) and samp (17.00 mM/mL) which was comparable to Brain Heart Infusion broth (16.49 mM/mL) and a basal synthetic medium (17.96 mM/mL) containing 3% sucrose, but the yield of lactate, acetate and formate was low during the fermentation of samp (0.50 mM/mL), BHI + 3% sucrose (4.12 mM/mL) and brown bread (0.06 mM/mL) respectively. Results indicated that maize and samp do not optimally support the growth or acid production by MS, and the varying response of test strains demonstrates the strain variability of this species to different carbohydrate sources in the diet.

Keywords: Mutans streptococci; pH; Doubling time; Lactate; Acetate; Formate; Maize; Samp; Bread

1. Introduction

In Africa [1,2] and South Africa [3] investigations have shown a downward trend in the prevalence of dental caries, while the numbers of mutans streptococci (MS) have remained similar in children with and without caries. In addition, studies on urban and rural black preschool children in South Africa, have found that diet was not clinically relevant to caries prevalence and experience [4,5]. This implied that good dental health is possible with the presence of caries-promoting factors in the diet as well as a high prevalence of MS, but raises speculation that the decrease in dental caries in South African populations is perhaps due to differences in virulence among the species of MS isolated from these populations. Other host factors besides the human diet [6] that can modify the caries process include the endogenous oral microorganisms found in dental plaque [7], the composition of teeth and saliva, salivary buffering, the acquired pellicle and the host immune system [8].

The MS belong to the indigenous micro-flora of the mouth, but possess virulent caries-promoting properties expressed only under specific environmental conditions. Two species of the MS, *Streptococcus mutans* and *S. sobrinus* are specifically implicated in dental caries

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[9–11]. These bacteria produce large quantities of acid, particularly lactic acid and are highly acid tolerant which allows them to colonize and persist, causing demineralization of enamel and eventual cavitation [12,13]. The production of acids, a by-product of fermentation is dependant on the fermentable carbohydrates available in the host diet. Although diet can have a limited influence on caries [14,15], some fermentable carbohydrates promote virulence factors such as acid and extracellular polymer production more than others and this can influence streptococcal strain selection and survival in the mouth.

A review by Seow [12] reported several studies that have shown that sugars such as sucrose, glucose and fructose are mainly associated in early childhood caries whereas foods such as milk and cheese had a cariostatic effect. However, people of distinct ethnic groups have specific food preferences [16] and in South Africa maize is one traditional African food staple eaten in the form of a soft porridge or 'stiff porridge'. Studies on maize have shown little potential to produce caries [17] and an in vivo study by Pollard et al. [18], showed that the pH drop in plaque was minimal after exposure to a maize challenge in South African black children. No studies to-date has been done on the effect of traditional African staple foods on the growth of MS strains and the production of acids in vitro. Hence, the purpose of this study was to investigate the in vitro acidogenic and growth response of MS reference and clinical strains to three traditional African staple foods; maize, samp and brown bread.

2. Material and methods

2.1. Bacterial Strains

The following MS strains were purchased from the National Collection of Type Cultures (Colindale, London); *S. mutans* type strain NCTC 10449 (Sims) and 10923 (LM7), *S. sobrinus* type strain 12277 (SL-1), 11061 (OMZ 65), 10922 (OMZ 176) and 10919 (Z/AHT). A total of five clinical strains were investigated of which two isolates were collected from the plaque of a 5-year-old child with caries (c) and the remaining isolates were from three, 12-year-old caries-free (cf) children of black and colored (European-Malay–black) descent whose diets comprise principally of maize, samp and brown bread. The codes assigned to these isolates for this study were; 942007(cf), 942036(cf), 942037(cf), 953012A(c) and 953012B(c). The identities of fresh isolates were confirmed by colony morphology on a selective medium, trypticase yeast cystine bacitracin (TYCSB) agar containing 15% sucrose, gram staining and verified by the biochemical identification scheme by Shklair and Keene [19] supplemented by the aesculin hydrolysis test [20] and production of hydrogen peroxide [21]. Further differentiation of these strains was done by amplification of the *gtfBC*, *gtfI* and *gtfA* gene fragments by the polymerase chain reaction, which helped to confirmed the identity of these clinical isolates (Table 1). The bacterial cultures were kept frozen at −20°C in a freeze-down fluid containing glycerol and fortified with foetal calf serum, until used.

Table 1
Biochemical characteristics of mutans streptococci clinical isolates and PCR verification by amplification of the *gtfBC*, *gtfI* and *gtfA* gene fragments

Biochemical test fermentation of	No caries			Caries	
	942036	942037	942007	953012A	953012B
Mannitol	+[a]	+	+	+	+
Sorbitol	+	+	+	+	+
Raffinose	−[b]	+	−	+	−
Melibiose	−	+	−	+	−
NH3 from L-arginine	−	−	−	−	−
Mannitol + 2 U/mL bacitracin	−	+	+	+	+
Aesculin hydrolysis	−	−	+	+	−
PCR amplification of					
gtfBC	−[c]	+	+	+	−
gtfI	+[d]	−	−	−	+
gtfA	−	+	−	+	−

[a] + = positive fermentation reaction.
[b] = negative fermentation reaction.
[c] = gene absent.
[d] + = gene present.

2.2. PCR verification of test bacteria

Bacterial DNA was prepared by growing the test strains in brain heart infusion (BHI) broth (Biolab Diagnostics, Merck, Midrand, SA) supplemented with 3% sucrose. At mid-log phase, the cells were pelleted by centrifugation and extracted using a technique by Popovic et al. [22] using the phenol–chloroform method with modification. The modification was extension of lysis time so that the rigid streptococcal cell walls were ruptured while preserving DNA integrity. Briefly, the cells were placed on a rotary shaker at 37°C for 45 min in 1 mL of 10 × TE buffer (pH 8) containing lysozyme (5 mg/mL) (Boehringer Mannheim, GmbH, Germany) and Rnase A (0.1 mg/mL) (Boehringer Mannheim, GmbH, Germany). Sodium dodecyl sulphate was added to a final concentration of one percent and samples were incubated in a water bath at 65°C for 15 min. Proteinase K (Roche Diagnostics, Indianapolis, USA) was added