Proteolytic activity of oral streptococci

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1. SUMMARY

*Streptococcus mutans* and *Streptococcus sobrinus* were the least proteolytic of 8 species of oral streptococci while *Streptococcus oralis* and *Streptococcus sanguis* were the most proteolytic. Degradation of FITC-BSA was significantly correlated with the hydrolysis of synthetic endopeptidase substrates. As *S. oralis* strains proliferate in dental plaque in the absence of dietary food their success, in vivo, might be due partially to their greater proteolytic activity compared to other oral streptococci.

2. INTRODUCTION

Streptococci represent a considerable proportion of the bacterial flora on every surface in the oral cavity, including the dentition, tongue and cheeks [1]. Bacteria obtain nutrients from their immediate environment, specifically from host-derived salivary components (proteins and glycoproteins) and to a lesser extent from dietary food [2–7]. Oral streptococci produce a number of glycosidic enzymes and they produce arylamidase and dianinopeptidase activities [8] but they are generally regarded as having little, or no, proteolytic activity towards natural substrates [9–11]. Despite these observations the rates of growth of oral streptococci, in vivo, is generally unaffected by the availability of the host's diet [12–13]. We have however shown that the numbers of 'Streptococcus mitior' (= *Streptococcus oralis* [14–16]) increased in the plaque of fasted monkeys [13]. Clearly, oral streptococci have the ability to acquire substrates for growth from host-derived nutrients. In this study we have determined the production of endopeptidase activities of oral streptococci and assessed their ability to degrade casein and bovine serum albumin in fluorescence-linked assays.

3. MATERIALS AND METHODS

3.1. Bacterial strains

The following strains [16–18] were used: *S. mutans*; NCTC 10449, H212, B14, B89, SE11, T282 and LM7, *S. sobrinus*; TH21, OMZ65, SL1 and K1, *S. mitis*; NCTC 7865, F90A and NCTC 3165, *S. sanguis*; NCTC 7863, SK96, KPE2 and M408, *S. vestibularis*; RW3, AS2, Pv91 and MM1, *S. oralis* I; OPA1, NCTC 7864, LVG1, PC 1467 and ATCC 9811, *S. oralis* II; K208, NS51, HV51,
OP51 and NCTC 10712, S. salivarius; A385, H53, M36 and NCTC 8618, S. anginosus; MILL17, NCTC 10713, NCD0 2227, B448, NCTC 8037, Is57, MILL132, MILL21, MILL136, FW102, G5:3, 415–87 and MILL64.

They were grown for 2 days in candle jars at 37°C on Fastidious Anaerobe Agar (FAA, LabM) and the growth was removed using sterile swabs into 50 mM TES (Sigma, Poole, Dorset, U.K.) buffer, pH 7.5 (TES buffer) and adjusted to an absorbance of 0.1 at 620 nm.

3.2. Degradation of synthetic substrates

The endopeptidase substrates used were N-tert-butoxycarbonyl-val-pro-arg-7-amido-4-methylcoumarin(AMC), N-carbobenzoxy-phe-arg-AMC, N-tert-butoxycarbonyl-leu-ser-thr-arg-AMC, tosyl-gly-pro-arg-AMC, N-tert-butoxycarbonyl-leu-glut-gly-arg-AMC, N-tert-butoxycarbonyl-val-leu-lys-AMC. N-carcobenzoxy-lys-AMC, acetyl-ala-ala-pro-ala-AMC, N-Succinyl-leu-leu-val-tyr-AMC, Glutaryl-phe-AMC N-succinyl-ala-ala-phe-AMC. Assays were set up using 50 μl of bacterial suspension, 20 μL of a 100 μg/ml substrate solution and 130 μl of TES buffer in opaque microtitre trays. The assays were incubated at 37°C and the degradation of substrate was monitored after 1, 6 and 20 h, using excitation and emission wavelengths of 380 and 460 nm, respectively and the mean number of nmol of substrate hydrolysed per h calculated for each strain.

Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>ENDO-1 (μM)</th>
<th>ENDO-2 (μM)</th>
<th>ENDO-3 (μM)</th>
<th>CASEIN (nmol)</th>
<th>BSA (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mutans</td>
<td>0.00</td>
<td>0.00</td>
<td>0.12 (0.06)</td>
<td>86 (18)</td>
<td>81 (40)</td>
</tr>
<tr>
<td>S. sobrinus</td>
<td>0.00</td>
<td>0.00</td>
<td>0.47 (0.16)</td>
<td>54 (14)</td>
<td>67 (9)</td>
</tr>
<tr>
<td>S. mitis</td>
<td>0.49 (0.57)</td>
<td>6.20 (1.78)</td>
<td>0.69 (0.20)</td>
<td>81 (17)</td>
<td>114 (25)</td>
</tr>
<tr>
<td>S. sanguis</td>
<td>0.14 (0.17)</td>
<td>7.73 (1.96)</td>
<td>0.86 (0.16)</td>
<td>69 (51)</td>
<td>190 (46)</td>
</tr>
<tr>
<td>S. vestibularis</td>
<td>0.16 (0.21)</td>
<td>3.49 (2.79)</td>
<td>0.60 (0.51)</td>
<td>79 (32)</td>
<td>154 (19)</td>
</tr>
<tr>
<td>S. oralis I</td>
<td>0.65 (0.39)</td>
<td>6.10 (2.21)</td>
<td>1.64 (0.70)</td>
<td>132 (73)</td>
<td>258 (71)</td>
</tr>
<tr>
<td>S. oralis II</td>
<td>0.62 (0.33)</td>
<td>3.45 (1.96)</td>
<td>1.86 (1.20)</td>
<td>115 (9)</td>
<td>251 (89)</td>
</tr>
<tr>
<td>S. salivarius</td>
<td>0.00</td>
<td>0.79 (1.68)</td>
<td>0.30 (0.05)</td>
<td>93 (14)</td>
<td>77 (52)</td>
</tr>
<tr>
<td>S. anginosus</td>
<td>0.08 (0.13)</td>
<td>4.26 (1.70)</td>
<td>0.69 (0.34)</td>
<td>86 (24)</td>
<td>101 (78)</td>
</tr>
</tbody>
</table>


4.3. Degradation of casein and BSA

Fluorescein isothiocyanate-labelled casein (FITC-casein) (Sigma, 14.4 μg FITC per mg casein) and fluorescein isothiocyanate-labelled BSA (FITC-BSA) (10.2 mol of FITC per mol of BSA) were diluted in TES buffer to 10 μg/ml. Assays were set up as described above but with 2 μl of fluorescein-labelled substrate. This assay is a modification of that described by Twinings [20]. Protein degradation is accompanied by an increase in fluorescence measured using an excitation and emission wavelengths of 495 nm and 525 nm, respectively.

4. RESULTS

4.1. Degradation of endopeptidase substrates

The substrates with arginine adjacent to AMC were not hydrolysed by the mutants streptococci or S. salivarius. The greatest activities were apparent with S. mitis, S. sanguis S. oralis I and S. oralis II; typical results are shown in Table 1. The other species hydrolysed these substrates at slower rates or not at all.

None of the mutants streptococci hydrolysed substrates with lysine adjacent to AMC and the other species hydrolysed N-carbobenzoxy-lys-AMC at similar rates. Elastase-like activity was only detected in strains of S. oralis II and no strain hydrolysed the elastase substrate with valine adjacent to AMC. Chymotrypsin-like activity was...
detected in all species with the S. oralis groups having the highest activities.

4.2. Degradation of FITC-casein and FITC-BSA

S. oralis strains had the greatest activity towards both proteins (Table 1). Proteolysis measured with FITC-casein was not significantly correlated with the extent of degradation of FITC-BSA \( (r = 0.323, P > 0.05) \). The degradation of FITC-casein was not usually correlated with the ability of the bacteria to hydrolyse the synthetic substrates. However the degradation of FITC-BSA was significantly correlated with the rate of hydrolysis of most of the endopeptidase substrates (Fig. 1).

5. DISCUSSION

Previous studies have reported the production of arylamidase activity by oral streptococci but little or no endopeptidase or proteolytic activities have previously been detected when measured using chromogenic protease substrates or by plate assays using native molecules [8–11]. In this report we have used 7-amido-4-methylcoumarin-labelled substrates which are considerably more sensitive than chromogenic substrates for detecting hydrolytic activity. The oral streptococci exhibited a wide range of proteolytic potentials with the mutans streptococci having, overall, the least proteolytic activity towards synthetic protease substrates and FITC-labelled proteins. The most proteolytic were the two S. oralis groups and S. sanguis which hydrolysed the widest range of synthetic substrates and degraded the two proteins to the greatest extent. In the same assays B. gingivalis degraded the endopeptidase substrates, FITC-BSA and FITC-casein at rates up to 1000 times faster than these streptococcal species.

Within the collection of strains named S. anginosus are representatives of the groups Streptococcus intermedius and Streptococcus constellatus (the validity of these as separate species needs further study [18,21]) but there was no significant difference between the proteolytic activities of these three species. The two homology groups of S. oralis have been recognized previously [16,22]. One of these groups (S. oralis I) includes the type strain of S. oralis (LVG1 = NCTC 11427), whereas the other DNA homology group, referred to as S. oralis II, has not been assigned an approved species epithet. S. oralis, the oral streptococcal species with the greatest proteolytic activity, are isolated early in the formation of dental plaque [23,24] and are isolated in significantly increased numbers from the plaque of fasted animals [13]. It is therefore possible that the increased isolation rate of S. oralis, under conditions in which salivary components are the major source of nutrient, is due to their ability to obtain nutrients from salivary components at a rate greater than that of other streptococci.

In conclusion, we have shown that all oral streptococcal species produce proteolytic activity and it is likely that the production of these activities may facilitate the growth of these bacteria in dental plaque and on other intra-oral surfaces.

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REFERENCES