MYCOLOGY

The expression of secreted aspartyl proteinases of *Candida* species in human whole saliva

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The expression of secreted aspartyl proteinases (Saps) by clinical isolates of *Candida albicans*, *C. tropicalis* and *C. parapsilosis* in human saliva supplemented with glucose and in a proteinase-inducing medium (YCB-BSA), was investigated. Also, yeast growth, pH fluctuation and total protein concentration of the saliva cultures during incubation were measured. Sap expression was assessed by evaluating the enzyme activity as well as the antigen concentration. Saps were expressed well in human saliva supplemented with glucose by all three *Candida* species, although greater expressions was found in YCB-BSA medium. *C. albicans* isolates were significantly more proteolytic than the non-*albicans* isolates. In general, for all three species, the rate of yeast growth, pH fluctuation and percentage reduction of total salivary protein concentration concurred with the degree of expression of Saps. These data strongly suggest that Saps of *C. albicans*, *C. tropicalis* and *C. parapsilosis* may play an active role in the progression of oral candidoses, particularly with regard to the abundance of low pH micro-environments in the oral cavity, which are regularly replenished with dietary carbohydrates.

Introduction

Candida spp. – of which *C. albicans* is of foremost medical importance, followed by *C. tropicalis* and *C. parapsilosis* – are notorious opportunist pathogens that cause a variety of oral infections. These three species are distinguished from other *Candida* spp. by their ability to secrete aspartyl proteinase *in vitro* [1, 2]. Ruchel *et al.* [3] and Odds [4] studied the ability of different *Candida* spp. to produce secreted aspartyl proteinases (Saps) and reported variable activity among different *Candida* spp.; e.g., *C. tropicalis* and *C. parapsilosis* produce less proteinase (Sapt and Sapp, respectively) than *C. albicans*. These proteinases are routinely expressed *in vitro* in candidal cultures containing high mol. wt nitrogen sources, such as bovine serum albumin (BSA), at around pH 5.0 [4]. The Saps appear to correlate well with virulence, as

proteolytic wild-type strains of *C. albicans* cause more extensive infection in mice than Sap-deficient mutants [5]. Others have also found correlations between proteinase activity and virulence [6, 7].

Nine iso-enzymes are expressed by different *C. albicans* strains [8], with Sap2 being the most abundant; it has an M_r in the range 43–45 kDa [9]. The optimal pH for SAP2 gene expression is 4.4 and it can be denatured irreversibly above pH 8.0 [10]. Two iso-enzymes of Sapt have been verified so far [11]; they are 43-kDa glycoproteins with pH optima between 3.4 and 3.8, and a pI of 4.5 [12]. Two isoforms of Sapp have been confirmed, Sapp1 and Sapp2 [11]; they are distinguished by a lower M_r of 33 kDa and a higher pI of 5.7 [12, 13]. Immunological studies suggest the existence of common and specific antigenic domains among the aspartyl proteinases of the three species [4].

Although considerable evidence indicates that Saps are potential virulence factors of *C. albicans*, *C. tropicalis* and *C. parapsilosis*, there is little information on their expression and activity in biological fluids such as saliva. Saliva contains antimicrobial proteins including immunoglobulins, lysozyme (muramidase), lactoferrin and the peroxidase system [14]. Lactoferrin and peroxidase are likely to be degraded proteolytically in the

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same manner as the secreted immunoglobulins by proteinases of *Candida* spp., although lysozyme is resistant due to its basic iso-electric point and high charge [5]. The attenuation of *C. albicans* Sap production by sublethal concentrations of lysozyme (unpublished observations) indicates that the Sap activity may be modulated by salivary constituents *in vivo*. As oral yeasts reside in a milieu bathed in this fluid medium, it is important to verify the extent to which candidal Saps are active in saliva and the salivary factors, if any, that may modify this phenomenon.

Germaine and Tellefson [15] were the first to report the growth of *C. albicans* *in vitro* under acidic conditions and its degradation of salivary proteins. They observed marked proteolysis at pH 4.0, less at pH 5.0, and a complete lack of proteolysis at pH 6.0 and 7.0. However, they also found that saliva inhibited candidal Sap synthesis and concluded that the enzymes did not contribute to virulence *in vivo* [15]. These observations contradict those of Samaranayake *et al.* [16, 17], who demonstrated the growth of *C. albicans* in human mixed saliva and concomitant salivary proteolysis. As only *C. albicans* and *C. tropicalis* isolates were used in these studies, there is no information available on the expression of Sapp by *C. parapsilosis* in salivary cultures.

The aim of the present investigation was to study aspartyl protease expression by three *Candida* spp. in human mixed saliva supplemented with glucose. The study entailed examination of growth, and the enzyme activity and antigen concentrations of the proteinases by spectrophotometry, enzyme-linked immunosorbent assay (ELISA) and immunoblotting (Western blotting). pH fluctuations and total protein concentrations during incubation of the saliva-yeast cultures were also monitored.

Materials and methods

Organisms

Nine oral *Candida* isolates were investigated; these were proteinase positive and belonged to three species – *C. albicans* (three isolates), *C. tropicalis* (three) and *C. parapsilosis* (three). The *C. albicans* isolates BM20617, BS742 and BU1010) were from HIV-infected individuals in Hong Kong attending the Oral AIDS Research Clinic, Faculty of Dentistry, Hong Kong. Two isolates each of *C. tropicalis* (T155700 and T155646) and *C. parapsilosis* (P154538(1) and P134023K(2)) were from healthy individuals in Hong Kong. *C. tropicalis* 003T and *C. parapsilosis* N6 were from healthy individuals from Tanzania and Norway, respectively. All isolates were derived from oral rinse or swab samples of individuals without signs or symptoms of oral candidosis. The yeasts were identified with API 20C test kits (API Laboratory Products, Basingstoke) and by the germ-tube test [18].

Saliva preparation

Unstimulated whole saliva was collected from a healthy young individual by expectoration into a universal container held over ice. Samples were always collected between 10.00 and 12.00 am, then pooled and centrifuged at 10 000 rpm for 20 min at 4°C. The supernate was then filtered through a 2.0-µm pore membrane filter (Corning Glass Works, Corning, NY, USA). To ascertain the sterility of the supernate, 0.05 ml of streptomycin-penicillin solution (10 000 µg/ml and 10 000 U/ml, respectively; Gibco Laboratories, Paisley) was added to 10-ml samples of supernate, and they were immediately stored at –20°C. The frozen samples were used within 24 h.

Before each experiment, saliva was supplemented by adding sterile 1 M glucose solution to yield a final concentration of 200 mM glucose. The sterility of the saliva samples was checked routinely by culture on blood agar and Sabouraud's Dextrose Agar (Gibco Laboratories), before each assay.

Growth and proteinase production

The isolates were grown aerobically on Sabouraud's dextrose agar at 37°C for 24 h. One loopful of the resultant culture was pre-cultured in YCB-BSA medium – yeast carbon base 23.4 g, yeast extract 2.0 g (Difco, Detroit, MI, USA) and bovine serum albumin 4.0 g/L (Sigma) adjusted to pH 5 and filter-sterilised – which induces proteinase expression [19], in an orbital incubator at 37°C for 24 h. The cells were harvested (5000 rpm for 10 min) and washed twice with sterile saline, transferred into the prepared saliva and the initial inoculum was adjusted to 1×10^6 cells/ml with a haemocytometer. Then the saliva candida suspension was incubated with agitation (150 rpm) at 37°C, for 72 h in an orbital shaker. Samples were removed at 12-h intervals and assayed for cell growth, pH value, protein concentration and Sap level. Growth was determined by the assessment of cfu/ml of the sample on Sabouraud's dextrose agar. Growth assays were conducted concurrently in YCB-BSA medium for comparison with the saliva cultures. In this experiment, one isolate from each *Candida* sp. was used, i.e., *C. albicans* BM20617, *C. tropicalis* 003T and *C. parapsilosis* P154538(1).

Measurement of pH value

The pH value of the saliva cultures during incubation for 72 h was measured with a pH meter (Sentron instruments, Model 501, The Netherlands). The pH meter was calibrated before use at two pH levels (pH 7.0 and pH 4.0), with standard buffers.

Assessment of protein concentration of the saliva culture

The protein concentration of the saliva cultures was determined by the Coomassie Blue assay [20]. Samples

(80 μ l) of saliva culture were applied to each well of a micro-test plate (Dynatech Microelisa, CA, USA) with dye reagent (BioRad Laboratories, USA) 20 μ l and mixed thoroughly. The final reading was obtained by measuring the OD₅₉₅ in an automated microreader (BioRad). The protein concentration of the samples was calculated from a standard curve obtained by plotting the OD₅₉₅ of standard BSA solutions (Sigma) at a range of 200–14 000 μ g/ml (each 0.1 μ l of BSA solution was mixed with 50 μ l of the dye reagent to conduct the standard assay).

Assessment of proteinase activity

Two methods, spectrophotometric assay [21] and ELISA [22], were used to evaluate the proteinase expression of three *Candida* spp. in whole saliva and YCB-BSA medium. For spectrophotometry, each assay included 0.1 ml of culture supernate and 0.4 ml of 0.1 M citrate buffer (pH 3.2) containing BSA 1%. The control consisted of identical ingredients and pepstatin A (Sigma) 50.0 μ g/ml which acted as the proteinase inhibitor. After incubation at 37°C for 15 min, the reaction was stopped with trichloroacetic acid (TCA) 5% on ice, the mixture was centrifuged, the absorbance of the supernate was read at 280 nm against a blank containing distilled water. One unit of enzyme activity was expressed as the amount, in micromoles, of tyrosine equivalents released/min/ml of culture supernate [8].

Proteinase antigen concentration was also assessed by ELISA. For this, the antisera against *C. albicans* [22] and *C. tropicalis* [12] Saps were raised in rabbits; the antiserum against Sapp was derived from guinea-pig [13]. The antisera against Sap2 and the purified Sap2 were kind gifts from Professor P. A. Sullivan (New Zealand). The antisera against Sapt and Sapp and the purified Sapt and Sapp were from Professor R. Ruchel (Germany). Dilution of the proteinase antiserum used was 1 in 5000 for *C. tropicalis* and *C. parapsilosis* and 1 in 500 for *C. albicans*. The secondary antibodies for *C. albicans* and *C. tropicalis* were phosphatase-conjugated goat anti-rabbit IgG (Sigma) and for *C. parapsilosis* phosphatase-conjugated goat anti-guinea-pig IgG (Sigma); these were used at a dilution of 1 in 1000.

First, 100- μ l samples of the culture supernate were mixed with 0.5 ml of TCA 50% (w/v), incubated for 30 min on ice and centrifuged at 10 000 rpm for 10 min; the pellets were washed twice with ethanol 95%, dissolved in SDS 1%, boiled for 3 min, diluted in 0.2 M sodium carbonate buffer, pH 9.5, and applied (70 μ l) to the micro-test plates (Dynatech Microelisa). The plates were incubated overnight at 4°C, rinsed three times with phosphate-buffered saline (PBS, 0.1 M, pH 7.4) containing Tween 20 0.05% v/v and blocked with BSA 1% PBS buffer for 1 h at 37°C. Anti-proteinase serum (100 μ l) was added at various dilutions in PBS and incubated for 2 h at 37°C. After

washing, 100 μ l diluted secondary antibody were added and incubated for 2 h at 37°C. Finally, the reaction was detected with *p*-nitrophenyl phosphate (Sigma) and terminated by adding 20 μ l of 3 M NaOH after 20 min. The plates were read with an automated microreader (BioRad) at 405 nm, blanked against air. The amounts of proteinase were calculated from a standard curve (1–110 ng) determined with purified Sap as coating antigen under the conditions described above. Reaction controls included negative tests without coating antigen or antibodies.

Western blotting

Western blotting was performed as follows. Supernates from fresh YCB-BSA and saliva culture were collected after incubation for 36 h. SDS-PAGE of proteins was performed in vertical slab acrylamide 10% w/v gels (BioRad). Proteins were transferred electrophoretically from polyacrylamide gels to nitrocellulose membranes with the semi-dry electrophoretic transfer cell (BioRad) for 1 h at 15 V and 180 mA. After complete transfer, as verified by a silver staining, the membrane was incubated with BSA 3% in Tris-buffered saline (TBS, pH 7.6) for 1 h at 37°C, and then in proteinase (Sap, Sapt and Sapp) antiserum (diluted 1 in 200) in BSA 1% TBS for 2 h at 37°C, with gentle agitation. The membrane was rinsed twice for 10 min with Tween 20 0.05% in Tris-buffered saline (TABS), then once briefly with distilled water, and incubated with the second antibody. The second antibodies for *C. albicans* and *C. tropicalis* were goat anti-rabbit IgG-peroxidase conjugate (Sigma); for *C. parapsilosis* the second antibody was goat anti-guinea-pig IgG-alkaline phosphatase conjugate (Sigma). The dilution of the three antibodies was 1 in 500 in BSA 1% TBS. After rinsing in TABS twice for 10 min, then once in distilled water, the membrane was exposed to the substrates. Chromogenic diaminobenzidine (Sigma) was used as the substrate for *C. albicans* and *C. tropicalis*; 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) (Sigma) was used as the substrate for *C. parapsilosis*.

In the yeast growth experiments, a selected isolate from each species (three isolates in total) was grown in saliva on a single occasion, whereas in pH studies, a total of three isolates from each of the three *Candida* species (nine isolates) was used.

Results

Yeast growth in whole saliva and YCB-BSA medium

Data on the growth of *C. albicans* BM20617, *C. tropicalis* 003T and *C. parapsilosis* 154538(1) in whole saliva and YCB-BSA medium are presented in Fig. 1. All the isolates grew well in whole saliva, but *C. albicans* demonstrated more rapid and abundant growth than the other two species. *C. albicans*

Fig. 1. Growth curves of *C. albicans* BM20617 (—, ■), *C. parapsilosis* 154538(1) (□, ■) and *C. tropicalis* 003T (▲, ▼) isolates in human whole saliva (—, ■, ▼) and YCB-BSA medium (—, □, ▲).

BM20617 took 36 h to reach the stationary phase of growth (when the cell concentration was 7.1×10^6 cfu/ml), whereas *C. tropicalis* 003T and *C. parapsilosis* 154538(1) reached a stationary phase after c. 24 h (when the cell concentration was 2.8×10^6 cfu/ml and 3.1×10^6 cfu/ml, respectively).

The three *Candida* isolates grew better during incubation for 72 h in YCB-BSA medium than in saliva (Fig. 1). No significant difference was observed between the growth of *C. parapsilosis* 154838(1) and *C. tropicalis* 003T in saliva or YCB-BSA medium.

pH profiles of saliva cultures

The pH curves of the saliva cultures of the three *Candida* spp. during the 72-h incubation period are shown in Fig. 2. The pH fell slightly immediately after the addition of the *Candida* isolates in all saliva cultures. In the cultures of *C. albicans* isolates BM20617, BU1010 and BS742, the pH fell (from 7.9) to 2.7, 2.9 and 3.0 respectively, over a period of 36 h. The pH fall induced by both *C. tropicalis* and *C. parapsilosis* was less than that of *C. albicans*; the lowest pH induced by *C. albicans* was 2.7, while the equivalent value was 5.4 for *C. parapsilosis* and 4.9 for

C. tropicalis. Also, the pH decrease induced by *C. tropicalis* during incubation for 72 h was not significantly different from that induced by *C. parapsilosis*.

Proteinase activity of *Candida* spp. in saliva cultures

Proteinase activity was expressed by all three *Candida* spp. during the 72-h incubation period in YCB-BSA medium and whole saliva. In general, with all three species, the proteinase activity peaked at c. 36 h of fungal growth and declined thereafter (Fig. 3). Furthermore, proteinase activity was consistently lower in saliva cultures than in YCB-BSA medium for all species. However, the proteinase activity of *C. albicans* in saliva culture was still higher than that of *C. parapsilosis* and *C. tropicalis* (Fig. 3); a similar pattern was observed in YCB-BSA medium. There was no significant difference between the proteinase activity of *C. parapsilosis* and *C. tropicalis*.

Proteinase antigen concentration of *Candida* spp. in saliva cultures

In addition to spectrophotometric assessment of the proteinase activity reported above, the proteinase

Fig. 2. pH curves of the saliva culture medium during the 72-h incubation period with three isolates each of *C. albicans* (■), *C. parapsilosis* (▲) and *C. tropicalis* (○) and an uninoculated control saliva sample (□).

antigen concentration of the *Candida* isolates was evaluated by ELISA. In general, proteinase concentration of all nine *Candida* isolates, belonging to three different species, peaked at *c.* 48 h in both whole saliva and YCB-BSA medium and declined thereafter (Fig. 4). The proteinase concentration of the *Candida* isolates was consistently lower in whole saliva than in YCB-BSA medium. Enzyme production by *C. albicans* was significantly higher than that of *C. parapsilosis* and *C. tropicalis* isolates; there was no significant difference between *C. parapsilosis* and *C. tropicalis* isolates.

Western blotting

Western blotting was performed to confirm the identity of the antigens detected by ELISA (Fig. 5). Probing whole saliva culture supernates (36 h) of *C. albicans* BM20617, *C. tropicalis* 003T and *C. parapsilosis* 154538(1) with the antiserum of each species revealed positive bands of 43 kDa in the case of *C. albicans* and *C. tropicalis*, and 33 kDa in the case of *C. parapsilosis*. Protein bands with identical molecular sizes were also obtained from YCB-BSA culture supernates of all three *Candida* spp. (Fig. 5). These bands derived from

Candida saliva/YCB-BSA cultures were of the same molecular size as the purified proteinase of each species obtained from external sources.

Percentage reduction in salivary total protein concentration

When the percentage reduction in salivary proteins was compared, *C. albicans*, in general, produced the largest reduction in salivary proteins compared with the other two species (Fig. 6). On the other hand, the proteolytic activity of *C. parapsilosis* and *C. tropicalis* isolates was similar (Fig. 6). In quantitative terms, after incubation for 72 h, the mean percentage reduction of salivary proteins by the three *C. albicans* isolates was 62.7%; *C. parapsilosis* and *C. tropicalis* isolates produced 42.2% and 49.3% reduction, respectively.

Discussion

These results demonstrate clearly that isolates of *C. albicans*, *C. parapsilosis* and *C. tropicalis* have the ability to grow well in whole saliva supplemented with glucose, although less profusely than in the YCB-BSA

Fig. 3. Secreted aspartyl proteinase activity measured by spectrophotometry of (a) *C. albicans* BM20617 (■, ●), BU1010 (▲) and BS742 (◆); (b) *C. parapsilosis* P154538(1) (■, ●), N6 (▲) and P134023K(2) (◆); (c) *C. tropicalis* 003T (■, ●), T155700 (▲) and T155646 (◆) during incubation for 72 h in human whole saliva (●, ▲, ◆) and YCB-BSA medium (■).

medium. Although only results from a single representative isolate from each species are shown (Fig. 1), all isolates demonstrated the same trend. Whilst the growth of *C. albicans* and *C. tropicalis* in human saliva culture has been reported by Samaranayake *et al.* [17], this is the first study demonstrating *C. parapsilosis* growth in human saliva *in vitro*. These results together with previous work [17, 23] convincingly demonstrate the ability of yeasts to grow in saliva while expressing their extracellular proteolytic activity. The latter phenomenon, in particular, has been questioned by

Germaine and Tellefson [15], who studied only a single *C. albicans* isolate, and the current data refute their contention.

A marked fall in pH was detected in human mixed saliva candida cultures, especially for *C. albicans* and it is likely that this is due to the acidogenic nature of *C. albicans* compared with the other two species. Although the acidogenic and aciduric nature of *C. albicans* and *C. tropicalis* cultured in glucose-supplemented media has been reported by others [16, 17,

Fig. 4. Secreted aspartyl proteinase antigen concentration assessed by ELISA in (a) *C. albicans* BM20617 (■, ●), BU1010 (▲) and BS742 (◆); (b) *C. parapsilosis* P154538(1) (■, ●), N6 (▲) and P134023K(2) (◆); (c) *C. tropicalis* 003T (■, ●), T155700 (▲) and T155646 (◆) during incubation for 72 h in human whole saliva (●, ▲, ◆) and YCB-BSA medium (■).

24, 25], the current results highlight these properties for *C. parapsilosis*. In general, pH minima were reached with all nine isolates at *c.* 36 h, after which an increased pH was detected – particularly in *C. albicans* cultures. One reason for this may be the salivary buffering action which neutralises the yeast-derived carboxylic acids that have rapidly accumulated during the log phase of yeast growth. It is known that acid production in yeast-salivary cultures peaks at the log phase of growth and declines or abates thereafter

[17, 23]. An acidic pH is crucial for proteinase expression in *Candida* spp. The fact that the species with the most robust acidogenic potential (*C. albicans*) was able to induce a higher degree of proteinase expression and proteolytic activity than *C. tropicalis* and *C. parapsilosis* which are known to be less acidogenic, implies that the latter characteristic is a contributory factor in virulence.

The study of *C. albicans* Saps is complex, as their

Fig. 5. Western blot of supernates from three *Candida* spp. cultured in human whole saliva and YCB-BSA medium. Lane 1, purified Sap; 2, saliva culture with *C. albicans* BM20617; 3, purified Sapt; 4, YCB-BSA medium with *C. tropicalis* 003T; 5, saliva culture with *C. tropicalis* 003T; 6, saliva culture with *C. parapsilosis* 154538(1); 7, YCB-BSA medium with *C. parapsilosis* 154538(1); 8, purified Sapp. The molecular size of both purified Sap and Sapt used in this study was 43 kDa, while that of Sapp was 33 kDa.

production is encoded by a multigene family with at least nine members responsible for the production of Sap1 – Sap9. Recent investigations with Sap-deficient mutants have indicated clearly that Sap2 is the most important member of the group [26]. Furthermore, Sap2 appears to be a prerequisite for candidal growth in media with protein as the sole nitrogen source (as in the current study), although Sap4, Sap5 and Sap6 play a subsidiary role in the induction of Sap2 [26]. The current results have, for the first time, confirmed the hypothesis of differential expression of Sap, Sapt and Sapp in whole saliva, by means of ELISA and Western blotting techniques. Although previous workers have calibrated Sap expression in whole saliva in semi-quantitative terms [17], the antigenic and electrophoretic properties of the expressed Saps of *Candida* spp. have not been described hitherto.

Inter-species variations in Sap expression have also been demonstrated by the current data. In both the YCB-BSA medium and the saliva cultures, *C. albicans* tended to be more proteolytic than either *C. tropicalis* and *C. parapsilosis*. On the other hand, the proteolytic activity and the proteinase expression in the case of the latter two species were very similar. One reason for this could be the profuse growth of *C. albicans* in the test media as compared with non-*albicans* species, observed here as well as by others [17]. Despite this caveat, the results of the present study tend to confirm the recognised hierarchy of virulence in *Candida* spp. – as *C. albicans* is considered to be the most virulent compared with *C. tropicalis* and *C. parapsilosis* [27]. However, the latter two, together with *C. albicans*, are distinguishable from other *Candida* spp. in possessing aspartyl proteinases that are secreted extracellularly. Indeed, it is interesting to note that *C. parapsilosis*, first considered a harmless yeast (isolated from fruits and other inanimate material) is now regarded as an emerging pathogen capable of causing considerable mortality – especially in hospitalised and other immunocompromised patient groups [28].

When the maxima of proteinase activity and enzyme concentration were compared, (the latter in terms of the antigen level), there was a 12-h time lag between the peaks of these parameters. This phenomenon has also been reported by others [21] and may be due to the fact that ELISA measures the total amount of antigen present compared with spectrophotometric assessment, where only the active enzyme is detected.

It was noteworthy that, over the 72-h period of incubation, total salivary protein concentration decreased at different rates. Similar findings have been reported previously by Samaranayake *et al.* [17] for both *C. albicans* and *C. tropicalis* in human whole saliva. The most likely explanation for this phenomenon is the differential rates of degradation of salivary proteins by the expressed candidal proteinases.

It is debatable whether the in-vitro conditions employed in this study prevail intra-orally and whether the Saps have a role in the pathogenesis of oral infection. Intra-oral habitats with low pH values are found in sites protected from the flushing action of saliva, such as the upper denture fitting surface [29]. Furthermore, regular and prolonged intake of carbohydrate foods lowers the pH in denture plaque to *c.* pH 4–5 [16, 30], conducive to Sap activity. Furthermore, Darlington [31] has demonstrated that salivary glucose and sucrose concentrations may reach up to 500 mM, depending on the frequency and duration of intake of dietary carbohydrates. Also, a high carbohydrate intake is known to initiate or aggravate, or both, *Candida*-associated denture stomatitis [32, 33]. Not only would such an environment stimulate Sap expression, but it may enhance the proliferation [34] and adhesion of *C. albicans* to palatal epithelial cells [35–37] and acrylic denture surfaces [38, 39]. Recent work has shown that in addition to a primary enzymic role, various Saps may act singly or synergically to enhance the adhesiveness of *C. albicans* to human tissues [40], as well as cleaving mucosal immunoglobulins [6], thus facil-

Fig. 6. Percentage reduction of salivary proteins induced by (a) *C. albicans* BM20617 (●), BU1010 (▲) and BS742 (◆); (b) *C. parapsilosis* P154538(1) (●), N6 (▲) and P13402K(2) (◆); (c) *C. tropicalis* 003T (●), T155700 (▲) and T155646 (◆) during incubation for 72 h in human whole saliva and a *Candida*-free saliva control sample (□).

itating yeast colonisation. Taken together, these findings tend to support the theory that the Saps of *Candida* spp. could indeed contribute to the pathogenesis of *Candida*-associated denture stomatitis [5, 41], the most prevalent oral candidal infection in the West.

In conclusion, these results demonstrate that *Candida* spp. secrete aspartyl proteinases of broad substrate specificity with the ability to induce salivary proteo-

lysis and possibly tissue damage. Furthermore, there are significant inter-species differences in Sap expression in whole saliva. However, further studies are needed to differentiate the proteinase iso-enzymes expressed by the yeasts, in biological fluids such as saliva and serum, and to elicit qualitative data on the specific, constituent proteins which are degraded. In addition, in-vivo experimentation, possibly in an animal model, is essential to confirm these findings.

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