MYCOLOGY

Competition for glucose between *Candida albicans* and oral bacteria grown in mixed culture in a chemostat

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The competition for glucose as a growth-limiting substrate between *Candida albicans* and a mixed community of oral bacteria was investigated. A chemostat was operated under glucose-limiting and glucose excess conditions at a dilution rate of 0.05/h. A mixed population of oral bacteria was established and after a steady state had been reached the chemostat was inoculated with *C. albicans*. Seven bacterial species – *Streptococcus sanguis*, *S. sobrinus*, *S. mitis*, *Lactobacillus casei*, *Veillonella dispar*, *Eubacterium saburreum* and *Fusobacterium nucleatum* – were able to establish stable populations under glucose-limiting conditions. The yeast was unable to grow with the bacteria under glucose limitation. Only three bacterial species, *S. sobrinus*, *L. casei* and *E. saburreum*, became established under glucose-excess conditions. *C. albicans* was also able to become established in the glucose-excess chemostat and could grow and maintain a steady state in a mixed culture with these organisms. *L. casei*, *S. mitis* and *S. sobrinus* had faster glucose consumption rates than *C. albicans*. All the bacteria, except for *F. nucleatum*, had maximum specific growth rates higher than *C. albicans*. The results suggest that glucose may act as a growth-limiting substrate for *C. albicans* in the establishment and growth of the yeast in a mixed community of oral bacteria.

Introduction

Oral candidosis is one of the most common fungal infections in man and is usually caused by the yeast *Candida albicans*. This micro-organism is a member of the commensal oral microbiota and occurs in 30–40% of clinically healthy mouths [1]. However, under certain conditions it can act as an opportunist pathogen and may cause several forms of oral candidosis. In the acute form, it may cause severe candidosis in AIDS patients and in patients who receive immunosuppressive drugs, chemotherapy or radiotherapy. In the chronic form, it causes oral discomfort [2].

Many factors can predispose individuals to oral candidosis. Some are mechanical, such as ill-fitting dentures. Some are related to the underlying disease status of the individual, while others are short-term, such as courses of antibiotic therapy [3]. Antibiotic treatment alters the composition of the endogenous microbiota, which then leads to overgrowth of the yeast

[2, 4]. In theory, most antibacterial antibiotics possess the ability to eliminate bacteria that normally compete with the yeast for nutrients. Under these conditions, the yeast is able to multiply more readily and its overgrowth may then lead to infection [2].

Glucose is a highly utilisable substrate for *C. albicans*, both in culture media [5] and in human saliva [6], and it has been suggested that the competition for glucose between the yeast and bacteria plays a major role in preventing overgrowth of the yeast [7]. Candidosis occurs more often when there is high availability of glucose, as in persons with diabetes and in patients receiving total parenteral nutrition [8]. A carbohydrate-rich diet can also promote oral colonisation with *Candida* spp. [9, 10]. High carbohydrate levels in the gut tend to favour multiplication of the yeast and predispose to candidosis [7, 11]. In-vitro studies have also shown that carbon limitation or excess plays an important role in controlling the establishment of organisms in mixed microbial populations [12–14]. Several in-vitro studies on the interactions of *C. albicans* with other micro-organisms have been published [2, 15–18]. However, most of the experiments have been done under simplified environmental conditions in which only one or two organisms were

involved. This is not representative of the huge amount of different bacterial species that normally inhabit the oral cavity [19] and cannot be expected to reflect the interactions of a complex microbiota. One study was done on the interaction of *C. albicans* with a complex microbiota in a mixed culture and under continuous-flow conditions [4]. However, this study was directed towards the ecology of the large intestine and used organisms totally different to those found in the oral cavity. Furthermore, the results of these experiments conflict quite markedly. For example, *Lactobacillus* spp. have been claimed both to enhance [15] and to inhibit [16] the growth of *C. albicans*. Liljemarm and Gibbons [17] were able to suppress growth of *C. albicans* in germ-free mice contaminated with *Streptococcus salivarius* and *S. mitior*. On the other hand, in-vitro experiments could not demonstrate any interaction between *S. salivarius* and *C. albicans* [18].

Although Cormane and Goslings [7] studied the influence of glucose on the growth of *C. albicans* *in vitro*, they were unable to clarify which mechanism was responsible for their observed growth inhibition of *C. albicans*. Nevertheless, they observed that the growth of the yeast in the absence and the presence of bacteria, and its rate of inhibition in mixed cultures, were markedly influenced by the concentration of glucose.

In the light of the possible role of competition for glucose amongst populations in maintaining candidal homeostasis in the oral cavity, it was decided to investigate the competition for glucose between *C. albicans* and a mixed community of oral bacteria. This would be difficult to perform *in vivo* because of the different parameters in the oral cavity that could influence the growth of the organisms and which are uncontrollable *in vivo*. It has been shown that the chemostat can produce stable communities of oral bacteria that are reproducible in their microbial composition and biochemical activity [12, 20–21]. A chemostat also has the advantage that it can be used as an environmentally-related laboratory model that can be used to investigate cause-and-effect relationships by changes in a single parameter [21]. Therefore, the present study used the chemostat to investigate the effect of glucose limitation and excess on the establishment of *C. albicans* in a mixed community of oral bacteria under conditions related to the oral environment.

Materials and methods

Growth conditions

A BioFlo model C32 chemostat (New Brunswick Scientific, Edison, NJ, USA) was operated at a dilution rate of 0.05/h, a temperature of 37°C and a working volume of 600 ml under a gas phase of CO₂ 5% + N₂ 95% (v/v). The gas was bubbled into the medium through a sparge tube at a gas flow rate of 50 ml/min.

The pH was maintained at 6.8 ± 0.1 by the automatic addition of 2 M NaOH. The growth medium contained (/L of distilled water) proteose peptone (Oxoid) 5 g, tryptone (Oxoid) 5 g, LabLemco powder (Oxoid) 1 g, yeast extract (Oxoid) 2 g, cysteine hydrochloride (Merck, Darmstadt, Germany) 5 g, NaCl (Merck) 5 g, haemin (Sigma) 5 mg, menadione (Sigma) 1 mg, disodium hydrogen phosphate dodecahydrate (Merck) 14.8 g, potassium di-hydrogen phosphate (Merck) 1.2 g. The medium was supplemented with 28 mM glucose (BDH, Poole) to achieve glucose limitation. To obtain glucose-excess conditions, the medium was supplemented with 170 mM glucose (BDH). The medium and the glucose were autoclaved separately and the glucose was added aseptically after sterilisation.

Bacterial strains

The organisms were chosen for their known ability to become established in mixed culture in a chemostat [20]. The bacterial strains were obtained from the National Collection of Type Cultures (NCTC; Colindale Avenue, London) and the yeast was obtained from the National Collection of Pathogenic Fungi (NCPF; Colindale). The strains were: *S. sanguis* NCTC 10904, *S. sobrinus* NCTC 10921, *S. mitis* NCTC 10712, *Lactobacillus casei* NCTC 10302, *Veillonella dispar* (parvula) NCTC 11463 and *Fusobacterium nucleatum* ATCC 25586. The yeast was *C. albicans* strain NCPF 3118. The *Eubacterium saburreum* strain (B618) was isolated from a chemostat culture in this laboratory in a previous study [20].

Chemostat inoculation

Each organism was grown separately in anaerobic batch cultures in the chemostat growth medium for 12 h. Then, 10 ml of each culture (except the yeast) were inoculated into the chemostat. Approximately 300 ml (half the working volume) of culture medium was pumped into the growth vessel to control the pH and temperature. The chemostat was re-inoculated on two subsequent occasions to establish all strains. Before each re-inoculation, a 100-ml volume of culture was removed. After a steady state had been reached, the chemostat was inoculated with 10 ml of a 12-h culture of *C. albicans* grown in the same medium. Re-inoculation with the yeast was performed on two subsequent occasions when it was noticed that the yeast was washed from the chemostat.

Viable counts of the microbial community

At the time of inoculation with the yeast and also at the termination of the experiment, viable counts of the chemostat communities were made. One-ml samples were removed from the growth vessel, dispersed, serially diluted in reduced transport fluid (RTF) [22] and plated on selective media. The media used were

TYC medium [23] for the identification of the streptococci, Rogosa SL Agar (Difco) for lactobacilli, horse blood 5% agar (Blood Agar Base No. 2, Oxoid) supplemented with haemin (Sigma) 5 mg/L and menadione (Sigma) 1 mg/L for the estimation of the total count and for enumeration of *E. saburreum*, and blood agar supplemented with vancomycin (Sigma) 2.5 µg/ml for enumeration of the gram-negative bacteria. All media were incubated anaerobically for 5 days in an atmosphere of CO₂ 10% + H₂ 10% + N₂ 80% (v/v). After inoculation with *C. albicans*, yeast counts were performed daily to monitor growth. A sample was removed from the chemostat and 1 ml of this sample was dispersed, serially diluted in RTF and plated on Sabouraud Agar (Oxoid). The plates were incubated aerobically for 24 h at 37°C and the yeast colonies were counted.

Measurement of the maximum specific growth rates of the microbial species

The maximum specific growth rate of each organism was measured by growing each in pure batch culture in the chemostat under the same growth conditions used in the continuous culture experiments. The chemostat was loaded with 600 ml of medium supplemented with 28 mM glucose and brought to 37°C. The apparatus was inoculated with 10 ml of a 12-h culture and growth was monitored for optical density while being stirred as a batch culture. Cell dry weights were determined by taking duplicate 5-ml samples of killed culture (formalin 10% by volume), centrifuging, washing the cells once in distilled water and drying the washed deposit of cells at 110°C for 2 days. The maximum specific growth rate (μ_m) of each organism was obtained by plotting the natural logarithm of the cell mass during the exponential growth phase against time, the best straight line being fitted by the method of least squares. The value of μ_m was obtained by determining the slope of the straight line.

Measurement of glucose consumption rates of the microbial species

The glucose consumption rate of each organism was measured by growing the organisms in pure culture in the chemostat under the same growth conditions used in the mixed culture experiments. The chemostat was loaded with 600 ml of medium supplemented with 28 mM glucose and brought to 37°C. The apparatus was inoculated with 10 ml of a 12-h culture and growth was monitored for optical density while being stirred at a dilution rate (*D*) of 0.05/h. Cell dry weights were determined by taking duplicate 5-ml samples of killed culture (formalin 10% by volume within 15 s), centrifuging, washing the cells once in distilled water and drying the washed deposit of cells at 110°C for 2 days. The residual glucose concentration of the supernate was determined by the method described by Kingsley and Getchell [24]. The rate of glucose

consumption was calculated from the equation of Herbert and Kornberg [25]:

$$q_s = D(S_0 - S)x$$

where q_s = the amount of glucose utilised (mg glucose/mg dry mass of cells/h), D = the dilution rate (/h), S_0 = the glucose concentration in the inflowing medium (mg/ml), S = the residual glucose concentration in the culture (mg/ml) and x = dry mass of cells (mg/ml).

Calculation of the theoretical washout rate

The theoretical washout rate and the rate of loss of *C. albicans* from the chemostat were calculated from the equation

$$\log_e x_t = \log_e x_0 - Dt$$

where x_t is the number of particles after time t , x_0 is the initial number of particles and D is the dilution rate [26].

All the experiments in this study were repeated three times and all values in each experiment were obtained in duplicate.

Results

Growth of bacterial species

The microbial composition of the cultures after 10 volume changes (10 generations) is shown in Table 1. The seven bacterial species which were inoculated into the glucose-limited chemostat all became established. A total population level of log₁₀ 10.4 (SD 2.1) cfu/ml (total anaerobic count) was reached. *S. sanguis* and *S. sobrinus* dominated the culture. The asaccharolytic species *V. dispar* occurred at comparatively high counts while *L. casei* had the lowest count.

The glucose concentration of the growth medium (excess glucose) affected the microbial composition of the steady state communities. In contrast to the glucose-limited chemostat, only three of the seven bacterial species inoculated into the glucose-excess chemostat became established and reached a steady state – *L. casei*, *S. sobrinus* and *E. saburreum* (Table 1). A total population level of log₁₀ 10.5 (SD 0.5) cfu/ml was reached. Both *L. casei* and *S. sobrinus* cell counts were higher than in the glucose-limited cultures and *S. sanguis* was replaced by *S. sobrinus* as the dominant species.

Growth of *C. albicans*

C. albicans was unable to establish and compete for a niche in the glucose-limited chemostat. Directly after inoculation the yeast counts were log₁₀ 4.2 (SD 0.2) cfu/ml. However, immediately after inoculation their numbers started to decline (Fig. 1). After 4 days the yeast cell counts were below the detection level of

Table 1. The composition of the steady-state bacterial communities under conditions of glucose limitation and excess, the maximum specific growth rates (μ_m) and the glucose consumption rate (q_s) of the microbial species at $D = 0.05/\text{h}$

Species	Mean $\log_{10}$ cfu/ml ($n = 6$) under		μ_m (/h)	q_s (mg glucose/ mg dry cells/h)
	glucose limitation	glucose excess		
<i>S. sanguis</i>	9.2	ND	0.514	4.9
<i>S. sobrinus</i>	8.8	10	0.610	2.7
<i>S. mitis</i>	6.1	ND	0.232	3.8
<i>L. casei</i>	5.0	9.7	0.303	3.2
<i>V. dispar</i> *	8.1	ND	0.355	0.0
<i>E. saburreum</i> *	6.1	4.4	0.143	0.0
<i>F. nucleatum</i> *	5.2	ND	0.153	0.16
<i>C. albicans</i>	ND	2.9	0.197	1.5
Total population	10.4 (SD 2.1)	10.5 (SD 0.5)	...	...

ND, not detected.

*Asaccharolytic species.

Fig. 1. The washout rate of an inert particle (◆) and the loss of *C. albicans* cells under glucose-limiting conditions (experiments 1, ■; 2, △; 3, ×).

10 cfu/ml. The yeast cells were lost at a mean rate of 0.038/h. This was faster than the theoretical washout rate of 0.02/h. There was no change in the bacterial composition of the chemostat culture after inoculation with the yeast.

On the other hand, *C. albicans* was able to become established and grow to a steady state in the glucose-excess chemostat. During the first 2 days after inoculation, the yeast cell counts declined at a mean rate of 0.02/h, equal to the theoretical washout rate. However, after 2 days the yeast growth rate stabilised at the dilution rate and maintained a steady state with a mean yeast count of $\log_{10}$ 2.9 (SD 0.6) cfu/ml (Fig. 2).

As with the glucose-limited culture, no change in the bacterial composition occurred after inoculation with the yeast.

Growth rates and substrate utilisation of the species

The maximum specific growth rates of the organisms that were able to utilise glucose as carbon substrate ranged from 0.197/h to 0.610/h (Table 1). Of these organisms, *C. albicans* had the lowest maximum specific growth rate and *S. sobrinus* had the highest maximum specific growth rate.

Fig. 2. The washout rate of an inert particle (◆) and the growth of *C. albicans* under glucose-excess conditions (experiments 1, ■; 2, △; 3, ×).

Glucose did not act as a growth-limiting substrate for *E. saburreum*, *F. nucleatum* or *V. dispar*.

Discussion

In the glucose-limited chemostat, the experimental conditions were as follows: the dilution rate and hence the growth rate was constant, the culture was in a steady state, growth was limited by the deficiency of a single nutrient and the culture was perfectly mixed. Chemostat theory predicts that under these conditions, unless microbial interactions or mixed substrate utilisation occurs, a single bacterial population will competitively displace all others if a culture is inoculated with a mixture of organisms [27–30]. In the present study, a mixed microbial population comprising seven bacterial species was able to become established and maintain a steady state in the glucose-limited chemostat culture. This indicates that the populations that established themselves were not all dependent on glucose as carbon substrate, but made use of mixed substrate utilisation to develop into a complex community. This is in accordance with the results of McKee *et al.* [21]. The mixed substrate utilisation could be due to the interaction between primary feeders (those that use substrates present in the original growth medium) and secondary feeders (those that use metabolic by-products) [27]. It could also be related to the ability of some of the populations to avoid direct competition for the overall growth-limiting nutrient (glucose) of the community by using amino acids for their growth and energy requirements. For example, *Veillonella* spp. use lactate produced by other organ-

isms [31]. *Veillonella* spp. have also been found to be associated with *E. saburreum* [32]. The co-existence of *S. sanguis* with other oral bacteria in glucose-limited chemostat cultures can be explained by the utilisation of arginine by this organism as a source of energy when glucose is unavailable [33, 34]. *F. nucleatum* also has the ability to use amino acids as carbon and energy supplies [35].

The bacterial composition of the community was markedly influenced by excess glucose concentration. The glucose-excess chemostat had a lower species diversity. Four of the seven bacterial species in the inoculum were unable to colonise the chemostat, even after repeated inoculations. A lower species diversity in the presence of excess glucose was also observed by Marsh *et al.* [12] and McKee *et al.* [21]. This could be ascribed to a shortage of nitrogen supply in the form of a limitation of certain amino acids under the glucose-excess conditions. McKee *et al.* [21] found that at least three amino acids were depleted from the culture medium under conditions of glucose excess, suggesting that competition for amino acids is an important factor in the establishment of some oral populations. The four bacteria that were unable to grow in the glucose-excess chemostat probably had to compete for a nitrogen source as a result of the depletion of amino acids. For example, as *V. parvula* needs amino acids for growth [36], it probably had to compete with *L. casei*, *S. sobrinus* and *E. saburreum* for essential amino acids. Similarly, the absence of *S. sanguis*, *S. mitis* and *F. nucleatum* in the glucose-excess culture could be due to a depletion of essential amino acids. On the other hand, the ability of *S. sanguis* and *F. nucleatum* to use

amino acids as carbon and energy supplies [33, 35] could have enabled them to grow in the glucose-limited chemostat. *L. casei*, *S. sobrinus* and *E. saburreum* became established in both the glucose-limited and glucose-excess cultures, suggesting that they did not compete either for glucose or for the same amino acids.

The dilution rate of the chemostat was less than the maximum specific growth rate of all the organisms involved (Table 1). Therefore, no washout of any of the organisms as a result of a faster dilution rate could have occurred. Nevertheless, *C. albicans* was unable to colonise and compete for a niche in the mixed community of oral bacteria in the glucose-limited chemostat. In contrast, it could establish itself and grow in a steady state under glucose-excess conditions.

Except for the availability of glucose, all other growth conditions were favourable for the growth of the yeast in the glucose-limited chemostat. Although *Candida* spp. grow best under aerobic conditions, the yeast will grow under anaerobic conditions, but at reduced growth rates. Webster and Odds [37] found that the growth rate of the yeast will be reduced by *c.* 16% when grown anaerobically in an atmosphere containing CO₂ 5%. With an aerobic maximum growth rate of 0.237/h in the same chemostat growth medium [20], a reduction in growth rate of 16% would give a growth rate of *c.* 0.198/h. This was the observed growth rate of the yeast in this study, which was much faster than the dilution rate of the chemostat.

A number of interactions [27, 29] could be responsible for the inability of the yeast to establish in the glucose-limited chemostat. Excretion of metabolic by-products can easily give rise to population interactions additional to competition for nutrients. However, no antifungal substance was produced by the bacterial species when tested individually against the yeast (no mention of any antifungal substance, produced by the oral bacteria, could be found in previous reports). Furthermore, when the glucose-limited chemostat effluent was filter-sterilised and supplemented with glucose, the yeast could grow in this medium. The latter finding, and the fact that the yeast could become established in the glucose excess chemostat, further indicate that no auto-inhibitory substance was produced. In the absence of protozoa, the effects of predation can be excluded. Parasitism such as a viral element similar to a bacteriophage is possible but unlikely, especially in view of the fact that the yeast was able to grow in the filter-sterilised effluent after the addition of glucose. This suggests that the yeast could not establish in the glucose-limited chemostat because it could not compete with the bacteria for the limited available glucose. Starvation of the yeast cells as a result of the shortage of a carbon source could possibly explain why a death rate of 0.018/h (0.038–0.020/h) was observed.

If no other interactions between populations occur, the

outcome of competition under single substrate limitation is dependent on the actual specific growth rates and substrate affinity of the organisms involved [29]. The dilution rate of the chemostat was fixed at $D = 0.05/\text{h}$, which was less than the maximum specific growth rate of all the organisms (Table 1). Therefore, the substrate affinity of the different organisms could be a determining factor in the final outcome of the competition. The yeast would not be able to compete against the bacteria – *L. casei*, *S. mitis* and *S. sobrinus* – with faster glucose consumption rates. However, under conditions of glucose excess, the bacterial competition as a result of the faster glucose consumption rates would be eliminated and enough glucose would be available for the normal metabolism of the yeast, while the dilution rate of 0.05/h would be slow enough to prevent the yeast from being washed out.

Previous studies have shown that the chemostat can produce stable and reproducible communities of oral bacteria under conditions relevant to those found in the oral cavity [12, 20, 21]. The dilution rate of 0.05/h, the temperature of 37°C and the gas phase of CO₂ 5% + N₂ 95% were chosen to simulate oral conditions. The particular species were chosen because of their known ability to become established and maintain a steady state under these conditions [20]. However, it must be emphasised that the chemostat cannot be used to simulate the oral cavity. It can only be used as an environmentally related model with which to control the large number of environmental parameters which would make an in-vivo study of this nature very difficult. Therefore, the results cannot be directly extrapolated to the oral cavity but the present study has demonstrated that the availability of glucose could act as a growth-limiting substrate for *C. albicans* when the yeast competes for a niche in a mixed microbial community under these particular environmental conditions. It also shows that *L. casei*, *S. mitis* and *S. sobrinus* might play a role in this respect.

In 1963, Cormane and Goslings [7] speculated on the importance of glucose for the growth of *C. albicans* in the oral cavity and suggested that the competition for glucose between bacteria and the yeast plays a major role in the survival and growth of the yeast. The importance of glucose was also shown by the fact that the yeast can grow only in glucose-supplemented saliva and not in saliva alone [6]. The results of the present study emphasise the possible role of glucose in the homeostasis of the yeast in a mixed microbial habitat. Clearly, more research is needed to clarify the role of glucose and other parameters involved. Future studies are under way in which this system is being used to study the effect of other parameters of oral significance on the homeostasis of *C. albicans* in the oral cavity.

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