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Nutritional yeast culture has specific anti-microbial properties without affecting healthy flora. Preliminary results*

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ABSTRACT

The objective of this study was to perform a preliminary assessment of the potential antimicrobial effects *in vitro* of nutritional yeast culture (Diamond V XP™), containing *Saccharomyces cerevisiae*, media on which it was grown, and metabolites produced during fermentation. It was tested against *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, *Candida tropicalis* ATCC13803, and oral flora from human saliva. Serial dilutions of each microbe were mixed with either water or an aqueous extract of yeast culture, and plated on aerobic, coliform, or yeast/mold Petrifilm™, respectively. Colony counts and appearances showed that yeast culture induced strong inhibition of *C. tropicalis* growth, moderate inhibition of *E. coli*, and only minimal effect on growth of *S. aureus* or oral flora. Metabolic activity of *E. coli* was affected, as seen by reduced beta-glucuronidase activity and lack of gas formation indicative of lactose fermentation. The data suggest that yeast culture consumption may support beneficial composition of intestinal flora while promoting containment of some microbial species.

KEY WORD: nutritional yeast culture, *Saccharomyces cerevisiae*, *Escherichia coli*, *Candida tropicalis*, normal flora, antimicrobial

INTRODUCTION

Enterotoxigenic *Escherichia coli*-associated diarrhoea represents one of the major threats to health and survival among farm animals. Escalated antibiotic re-

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sistance is a major concern, not only with regards to economical loss to farmers world-wide but also for associated human health hazards. Intestinal flora in animals and humans constitute a reservoir of resistance genes, or potentially pathogenic bacteria (van den Bogaard and Stobberingh, 2000). Transfer of antibiotic resistance genes between commensal and pathogenic organisms includes transfer of antimicrobial resistance from potentially pathogenic bacteria in farm animals to commensal organisms in human intestinal flora *via* consumed meat products (Witte, 2000).

Once an animal is compromised by pathogenic enteric bacteria such as some forms of *E. coli*, other opportunistic infections can further contribute to the deterioration of the animal. Examples include infections with *Candida* subspecies in post-weaning multisystemic wasting syndrome in pigs (Zlotowski et al., 2006) and, increasingly, mastitis among dairy cows in areas of Africa (Kivaria and Noordhuizen, 2006). In addition, *Candida* subspecies may also be primary causes of diarrhoea in newborn calves (Elad et al., 1998).

Dietary strategies involve feed supplements such as yeast culture, which is used as a feed additive in all types of farm animals and has been shown to increase milk production (Dann et al., 2002) and feed efficiency in dairy cows (Schingoethe et al., 2004), weaning weight of pigs (Kim et al., 2006), and egg production in chickens (Tangendjaja and Yoon, 2002). In addition, yeast culture increases the ratio of lactobacilli over coliform bacteria in laying hens (Liu et al., 2002). Therefore, the objective of this preliminary study was to evaluate evidence for specific antimicrobial effects of yeast culture *in vitro*.

MATERIAL AND METHODS

Three different types of Petrifilm™ were purchased from 3M Microbiology: aerobic culture (AC), *Escherichia coli*/coliform (EC), and yeast and mold (YM) plates. Each Petrifilm plate is a sample-ready, self-contained, self-sealing system, and manufacturer's protocols for each plate are validated methods by AOAC International. Each plate consists of a counting surface with a grid of 1 cm^2 fields, pre-coated with microbe-specific nutrients, a cold-water soluble gelling agent, and precursor dyes. These dyes are transformed into colour indicators by microbial enzyme processes and help to visualize microbial colonies upon incubation.

An extract containing soluble metabolites from the yeast culture was prepared in the following 3-step protocol: 1. An amount of 0.5 g yeast culture (Diamond V XP™ Yeast Culture, Diamond V Mills) was weighed and added to 5 ml phosphate-buffered saline (PBS, Sigma-Aldrich). This mixture was vortexed for 30 sec and allowed to sit at room temperature for one h. No homogenization was

performed to disrupt yeast cells in the product. 2. Remaining solids were removed by centrifugation at 2400 rpm for 10 min to remove the majority of solids. 3. The supernatant was harvested and any remaining solids were removed by filtration using a 0.22 micron syringe filter. The resulting extract contained soluble metabolites without yeast cell walls and was orange in colour.

The following microbial strains were obtained from the American Type Culture Collection (ATCC): *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Candida tropicalis* ATCC 13803. Healthy oral flora was obtained by unstimulated saliva collection from human subjects. Stock solutions of microbes were prepared by rehydration of lyophilized organisms from the ATCC vials into 5 ml sterile water, which was allowed to sit at 4°C overnight. Oral flora was obtained by mixing 1 ml unstimulated saliva with 9 ml sterile water. Plating of serial dilutions allowed assessment of numbers of viable organisms per ml. The stock suspension was adjusted to approximately 10^4 viable microbes per ml. Serial, 10-fold dilutions were made from this stock.

Serial dilutions of all 4 microbial suspensions were made in sterile PBS. Each dilution was then mixed in equal proportions with either PBS (control) or yeast culture extract immediately before plating *E. coli*, *S. aureus*, oral flora, and *C. tropicalis* onto the appropriate type of Petrifilm (EC, AC, AC, and YM plates, respectively). *Escherichia coli*/coliform and AC plates were incubated for 24 and 48 h at 35°C. Yeast and mold plates were incubated at 25°C for up to 6 days. Colonies were counted, and Petrifilm plates were photographed.

RESULTS

Growth of *C. tropicalis* in the presence of yeast culture was almost completely undetectable over a 1,000-fold dilution range. Even at the highest concentration of plated *C. tropicalis*, only minor colony formation was observed. These colonies were smaller than colonies on untreated control plates, and only occurred along the edges of the plate (Figure 1, Table 1).

Growth of *E. coli* was reduced in the presence of yeast culture. Even when high concentrations of *E. coli* were plated, colonies that were observed were extremely small compared to colonies on untreated control plates (Figure 2, Table 1). There was also evidence that *E. coli* were less metabolically active in the presence of yeast culture. This was indicated by reduction in the indicator dye for beta-glucuronidase activity and reduced metabolic gas formation from lactose fermentation associated with each colony. Gas formation can be seen on Figure 2 (indicated by arrows) at the 1:100 dilution of *E. coli* stock, in the top panel of control cultures.

Table 1. Colony counts for microbial growth in the absence versus presence of yeast culturea[1]

Dilutions	*C. tropicalis*		*E. coli*		*S. aureus*		Oral flora	
	control	XP	control	XP	control	XP	control	XP
1:1	500	0	++++[2]	++[3]	268	175	++	++
1:10	216	0	+++	+	114	44	+	+
1:100	74	0	14	7[4]	31	15	158	129
1:1000	16	0	0	0	7	9	21	19

[1] the data shown are representative of three independent tests; [2] plusses indicate that the colony density was too numerous to count, and that relative growth was estimated; [3] the *E. coli* colonies growing in the presence of XP were more diffuse, smaller, and less distinct, in part due to a lower amount of beta-glucuronidase activity, as reflected by the lower level of indicator dye associated with the colonies; [4] these colonies were barely visible due to a low amount of beta-glucuronidase activity. These colonies were not associated with gas bubbles, indicating low or no lactose fermentation activity

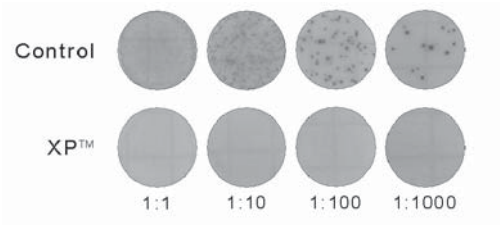

Figure 1. Growth inhibitory effect of yeast culture on *Candida tropicalis*. Serial dilutions of microbial culture were mixed with an equal amount of yeast culture extract or PBS and plated onto Petrifilm. Colonies appeared within 3 days on untreated film (top row), whereas no colonies were seen on films treated with yeast culture (bottom row). Data shown are representative of three separate experiments

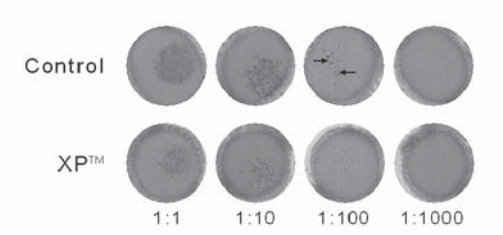

Figure 2. Anti-coliform effect of yeast culture on cultures of *Escherichia coli*. Serial dilutions of microbial culture were mixed with an equal amount of yeast culture extract or PBS and plated onto Petrifilm. Colonies appeared within 24 h on both untreated films (top row) and films treated with yeast culture (bottom row). The colonies grown in the presence of yeast extract were more difficult to count, due to their diffuse appearance, lack of metabolic activity as reflected by low amount of indicator dye for beta-glucuronidase activity, lack of gas formation associated with lactose fermentation (see arrows on control plate), and smaller size. Data shown are representative of three separate experiments

Growth of *S. aureus* was only mildly affected by inclusion of yeast culture. Growth inhibition of *S. aureus* was only evident at the highest concentrations of plated microbes, where slightly smaller colonies were seen (Figure 3, Table 1). This indicated that a changed profile of essential nutrients as a result of addition of yeast culture, rather than a direct antimicrobial effect, was providing less-than-optimal culture conditions at high population densities with higher competition for nutrients.

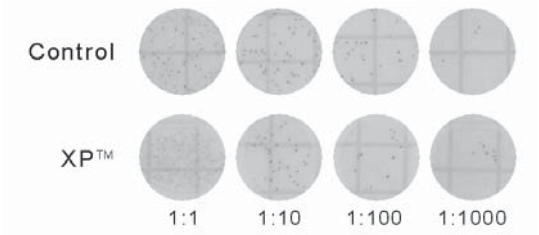

Figure 3. Anti-microbial effect of yeast culture on cultures of *Staphylococcus aureus*. Serial dilutions of microbial culture were mixed with an equal amount of yeast culture extract or PBS and plated onto Petrifilm. Colonies appeared within 24 h. Almost no difference was seen between untreated (top row) and yeast culture-treated (bottom row) cultures. Data shown are representative of three separate experiments

As a control, healthy oral flora was used as a mixed source of predominantly commensal organisms. Growth of oral bacteria from healthy saliva samples were almost unaffected by the addition of yeast culture (Table 1).

DISCUSSION

Yeast culture is a *Saccharomyces cerevisiae* based product that is used as a nutritional additive to animal feed (Schingoethe et al., 2004; Barnes et al., 2006). Data presented here show that XP yeast culture specifically inhibited growth of *E. coli* and *C. tropicalis*, with only a minor inhibition of growth of *S. aureus* or healthy oral flora. The effect was dose-dependent. It was estimated that XP yeast culture inhibited the growth of *C. tropicalis* 1,000-fold, while also inhibiting the growth as well as the metabolic activity of *E. coli*. This selective anti-microbial effect is promising, as it supports the argument that consumption of XP yeast culture may help reduce harmful coliform bacteria and fungal organisms such as *Candida* subspecies, while not causing an imbalance of healthy intestinal flora.

Specific anti-microbial effects may be contributing factors to the previously observed effects of yeast culture *in vivo*, as the support of commensal organisms while containing potentially pathogenic organisms is particularly important around the time of weaning, when protective effects of colostrum cease and the animal is only beginning to develop immunity. There may be very little time and chance for vaccination to provide adequate protection, and dietary compounds such as yeast culture may offer protective effects during this period.

CONCLUSIONS

Based on these studies *in vitro*, XP yeast culture has a modulating effect on microbial growth, favouring some aerobic bacteria while inhibiting the growth and metabolism of *E. coli* and *C. tropicalis*. To what extent this extends to other coliform and fungal microorganisms remains to be tested. This effect is separate from, and possibly synergistic with, the previously reported beneficial effects on intestinal flora reported for mannan oligosaccharides (Rozeboom et al., 2005) as the test was performed only with soluble metabolites extracted from fully fermented yeast culture without cell wall components of yeast.

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