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Probiotic Activity of *Saccharomyces cerevisiae* var. *boulardii* Against Human Pathogens

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Summary

Infectious diarrhoea is associated with a modification of the intestinal microflora and colonization of pathogenic bacteria. Tests were performed for seven probiotic yeast strains of *Saccharomyces cerevisiae* var. *boulardii*, designated for the prevention and treatment of diarrhoea. To check their possible effectiveness against diarrhoea of different etiologies, the activity against a variety of human pathogenic or opportunistic bacteria was investigated *in vitro*. In mixed cultures with *S. cerevisiae* var. *boulardii*, a statistically significant reduction was observed in the number of cells of *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, by even 55.9 % in the case of *L. monocytogenes* compared with bacterial monocultures. The influence of yeasts was mostly associated with the shortening of the bacterial lag phase duration, more rapid achievement of the maximum growth rates, and a decrease by 4.4–57.1 % (*L. monocytogenes*, *P. aeruginosa*), or an increase by 1.4–70.6 % (*Escherichia coli*, *Enterococcus faecalis*, *Salmonella* Typhimurium) in the exponential growth rates. Another issue included in the research was the ability of *S. cerevisiae* var. *boulardii* to bind pathogenic bacteria to its cell surface. Yeasts have shown binding capacity of *E. coli*, *S.* Typhimurium and additionally of *S. aureus*, *Campylobacter jejuni* and *E. faecalis*. However, no adhesion of *L. monocytogenes* and *P. aeruginosa* to the yeast cell wall was noted. The probiotic activity of *S. cerevisiae* var. *boulardii* against human pathogens is related to a decrease in the number of viable and active cells of bacteria and the binding capacity of yeasts. These processes may limit bacterial invasiveness and prevent bacterial adherence and translocation in the human intestines.

Key words: yeasts, *Saccharomyces cerevisiae* var. *boulardii*, probiotic activity, human pathogens, antibacterial antagonism, adhesion

Introduction

According to the FAO/WHO definition, probiotics are live microorganisms which, when administered in adequate amounts, confer health benefits to the host (*1*). *Saccharomyces cerevisiae* var. *boulardii* is the only known yeast with clinical effects and the only yeast preparation with proven probiotic efficiency in double-blind studies (*2*). Probiotic yeast cultures have been used as both a preventive and a therapeutic agent for the treatment of a variety of diarrhoeal diseases. *S. cerevisiae* var. *boulardii* is reported to be effective in the treatment of antibiotic-associated diarrhoea, traveller's diarrhoea, diarrhoea in human immunodeficiency virus-infected patients and patients with irritable bowel syndrome, as well as of acute and chronic diarrhoea in children and adults (*3*,*4*). The most common opportunistic and pathogenic bacteria related to diarrhoeal conditions are *Clostridium difficile*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Klebsiella oxytoca*, *Shigella* sp. and *Clostridium perfringens* (*4–6*).

Beneficial effects of *S. cerevisiae* var. *boulardii* against enteric pathogens involve different mechanisms, such as prevention of bacterial adherence and translocation in

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the intestinal epithelial cells, production of factors that neutralize bacterial toxins and modulation of the host signalling pathway with proinflammatory response during bacterial infection (*4*,*7*). In the case of many pathogens, adhesion to the epithelial cells is the precondition for the development of their pathogenicity in the intestine. So far, it has only been documented for *Escherichia coli* and *Salmonella* Typhimurium that *S. cerevisiae* var. *boulardii* binds them to the cell surface (*8*). In this context, the question has been raised if the binding capacity of *S. cerevisiae* var. *boulardii* could be a universal way of its probiotic activity against human pathogens.

The study was conducted for *S. cerevisiae* var. *boulardii* isolates originating from commercial specimens developed for the prevention and treatment of diarrhoea to check their action against an extended variety of pathogenic and opportunistic bacteria. Reducing the number of viable and active cells of these bacteria may result in limiting their invasiveness, which may have a preventive or supporting influence in diarrhoea of different etiologies.

Materials and Methods

Strains

In this study, seven strains of *Saccharomyces cerevisiae* var. *boulardii* isolated from medicines and marked as: E – Enterol® 250 mg, Biocodex; H – Hamadin® N, Dr. Willmar Schwabe; O – Omniflora® Akut, Novartis; P1 – Perocur®, Hexal; P2 – Perenterol® forte, UCB GmbH; S – Santax® S, Asche; and Y – Yomogi®, Ardeypharm were examined. Isolates were kept on yeast extract-peptone--dextrose (YPD) agar slants and maintained at –20 °C in YPD broth with 200 g/L of glycerol.

The influence of *S. cerevisiae* var. *boulardii* on the following opportunistic or pathogenic bacteria was determined: *Escherichia coli* ATCC 10536, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 19115, *Pseudomonas aeruginosa* ATCC 15442, *Salmonella* Typhimurium ATCC 14028, *Staphylococcus aureus* ATCC 6538, and *Campylobacter jejuni* ATCC 33291.

Antibacterial activity of S. cerevisiae var. boulardii

Antibacterial activity of yeasts was investigated on the YPD medium using the agar slab method (*9*). The method was based on the observation of parallel growth of the strains: the indicator and the antagonistic ones. Agar slabs of 14 mm in diameter were aseptically cut out of the YPD agar overgrown with a lawn of *S. cerevisiae* var. *boulardii* incubated for 48 h at 37 °C, and placed on plates with the agar media (Nutrient Agar, Merck, Darmstadt, Germany) inoculated with the indicator strain (10^5–10^6 CFU/mL). After 18 h of incubation, the diameters of growth inhibition zones around the agar slabs were measured. The results are given in mm, minus the agar slab diameter.

Mixed cultures of S. cerevisiae var. boulardii and bacteria

Growth experiments were carried out in the modified medium reproducing the conditions in a human colon (*10*). The modified medium consisted of the following constituents (in g/L of distilled water): starch 5.0, pectin 2.0, guar gum 1.0, porcine gastric mucin 4.0, oatspelt xylan 2.0, arabinogalactan from larch wood 2.0, inulin 1.0, casein 3.0, peptone 3.33, tryptone 5.0, raffinose 10.0, bile salts no. 3 (Oxoid, Basingstoke, UK) 0.4, yeast extract 4.5, $FeSO_4{\cdot}7H_2O$ 0.005, NaCl 6.16, KCl 4.5, KH_2PO_4 0.5, $MgSO_4{\cdot}7H_2O$ 1.25, $CaCl_2{\cdot}6H_2O$ 0.15, $NaHCO_3$ 1.5, cysteine 0.8, hemin 0.05, and Tween 80 1.0; pH was adjusted to 6.2.

The inoculation rates for yeasts and bacteria were 10^5 CFU/mL and the incubation took place at 37 °C under anaerobic conditions (anaerobic jars with AnaeroGen Anaerobic System, Oxoid). The number of microorganisms was estimated by the count plate method after 0, 4, 8, 12, 24 and 32 h of incubation. For yeasts, CFU/mL values were determined by plating appropriate dilutions into the YPD agar medium with the addition of gentamicin (40 mg/100 mL), and for bacteria by plating into nutrient agar (Merck) supplemented with nystatin (210 mg/100 mL).

Sedimentation assay

S. cerevisiae var. *boulardii* cells were cultured by shaking for 24 h at 37 °C in 50 mL of the YPD medium. *Campylobacter jejuni* cells were activated in Preston broth (in g/L: meat extract 10.0, peptone 10.0, NaCl 5.0, and in mL/L: lysed horse blood 50) in microaerobic atmosphere (CampyGen, Oxoid), whereas the other bacteria were cultured in nutrient broth (Merck). Bacterial cultures were incubated at 37 °C for 24 h. The cells were harvested four times by centrifugation at 3000×*g* for 10 min (4 °C) and suspended in PBS buffer (in g/L: NaCl 8.0, KCl 0.2, Na_2HPO_4 1.44, KH_2PO_4 0.24; pH=7.4). Standardized suspensions of yeasts contained approx. 10^8 CFU/mL, and suspensions of bacteria 10^{10} CFU/mL. The sedimentation assay was performed by mixing 1000 μL of the yeast suspension and 50 μL of the bacterial culture in a 1.5-mL conical microcentrifuge tube (*11*). The mixture was incubated at room temperature for up to 3 h and the sediments were subjectively evaluated by the pellet size observed at the bottom of the tube. The negative control was a suspension containing probiotic yeasts alone.

Microscopic analysis

The sample preparation for fluorescence microscopy was carried out as described above for the sedimentation method. Before mixing the suspensions of yeasts and bacteria, the cells of the microorganisms were stained. Bacterial cultures were stained with 0.1 % DAPI solution (Sigma-Aldrich, Munich, Germany) and were incubated at 37 °C for 20 min in the dark. Yeast cells were stained with 0.1 % primuline (Sigma-Aldrich, Dorset, UK) at room temperature for 5 min (*12*). For image acquisition, the fluorescence microscope Olympus BX 41 (Hamburg, Germany) equipped with a digital camera was used.

Statistical analysis

All experiments were performed in triplicate. The data were tested for statistical significance using 3-way ANOVA at the significance level of $p<0.05$ (*13*). The re-

sults concerning the population viability were presented as the arithmetic mean of three assays with the standard error of the mean.

In order to compare the growth curves, the Gompertz equation in conjunction with the DMFit program v. 2.1 were used (*14*). The Gompertz parameter values (A, B, C, M) were applied to calculate the increase of the population biomass: N=C–A, the exponential growth rate: EGR=BC/e, and the lag phase duration: LPD=M–(1/B), as previously described by Zaika *et al.* (*15*).

Results and Discussion

The research aimed at determining the influence of seven probiotic yeasts *S. cerevisiae* var. *boulardii* on human pathogenic or opportunistic bacteria: *Escherichia coli*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium and *Staphylococcus aureus*. In the case of three bacterial strains, *L. monocytogenes*, *P. aeruginosa* and *S. aureus*, a decrease in the number of bacterial cells was observed in the mixed cultures with probiotic yeasts (Table 1). The reduction in the number of *L. monocytogenes* and *P. aeruginosa* cells in the mixed cultures with probiotic yeasts, compared to the proper bacterial monocultures, equaled respectively 19.1–55.9 and 2.1–22.5 %, and these reductions were statistically significant. On the other hand, probiotic yeasts influenced the growth of *S. aureus.* During the culturing process, a reduction in the number of *S. aureus* cells was noted by 2 log units after 8 h of coincubation. It was then followed by bacterial growth, although the number of bacteria was still lower by 0.5–1 log unit than in the monoculture. The growth curve of *S. aureus* grown with yeasts is presented for *S. cerevisiae* var. *boulardii* E (Fig. 1). The growth curve of the remaining yeast strains had a similar course. The effect of probiotic yeasts was also related to the change in the exponential growth rates, the times at which the cultures achieved their maximum growth rates and the lag phase durations. The vast majority of bacterial strains showed a significant shortening of the lag phase duration by 0.3–6.15 h. Only in the case of *E. faecalis* in the mixed cultures with yeasts H and O, and of *S.* Typhimurium in the culture with strain E, the logarithmic phase was longer than in the monocultures, respectively by 0.42, 0.62 and 0.21 h (Table 1). The shortening of the logarithmic phase was also related to a reduction in the time at which the cultures achieved maximum growth rate (M values). The bacteria reached their maximum growth rates within a time shorter by 0.23–3.98 h than in the case of monocultures. Additionally, *E. coli*, *E. faecalis* and *S.* Typhimurium in mixed cultures with *S. cerevisiae* var. *boulardii* demonstrated most frequently an increase in the growth rate, equalling, compared to bacterial monocultures, from 1.4 % for *S.* Typhimurium cultured with strain P2 to 70.6 % for *E. faecalis* in the presence of yeasts O (Table 1). On the other hand, in the case of *P. aeruginosa* and *L. monocytogenes*, the presence of probiotic yeasts resulted in a decrease of the bacterial growth rate, by 4.4–31.1 and 31.8–57.1 %, respectively.

The lack of clear antagonistic effect of probiotic yeasts on human pathogens was also confirmed by marking the inhibition zones of bacterial growth with the use of the agar slab method. A clear antagonism of yeasts against bacteria was only confirmed for *S. aureus* (Fig. 2 presents an exemplary picture for *S. cerevisiae* var. *boulardii* E). Consistent results were obtained for all the tested strains of *S. cerevisiae* var. *boulardii*, and the precipitation areas equaled from (7.8±0.06) to (10.0±0.17) mm depending on the yeast. However, the growth of *S. aureus* in the mixed cultures with yeasts indicates a reduction in the number of cells, but not the total inhibition of the bacterial growth (Fig. 1). The remaining tested bacterial strains did not manifest growth inhibition zones around the agar slabs with *S. cerevisiae* var. *boulardii* cultures.

One of the hypotheses proposed to explain the probiotic activity of *S. cerevisiae* var. *boulardii* against enteropathogenic microorganisms is antagonism by production of inhibitory compounds (*3*,*16*–*19*). However, there are only a few investigations which have shown that this yeast inhibits the growth of various bacteria. This influence has been demonstrated for *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella typhi*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella atypical* and *Escherichia coli* (*16*,*17*). Also, Bornet and Bergogne-Berezin (*18*) report a decrease in bacterial counts of *P. aeruginosa* and *S. aureus* in the presence of *S. cerevisiae* var. *boulardii* in dietetic mixtures designated for enteral feeding in intensive care patients. Although the reduction in the number of cells of both bacterial strains was statistically significant, it equaled only 2 log units. Similarly, Zbinden *et al.* (*19*) showed a drop in the number of *S.* Typhimurium cells after 5 h in the presence of *S. cerevisiae* var. *boulardii*. At the initial level of $1.4 \cdot 10^7$ CFU/mL, *S.* Typhimurium grew to $6.6 \cdot 10^8$ CFU/mL in the absence and to $1.9 \cdot 10^8$ CFU/mL in the presence of *S. cerevisiae* var. *boulardii*. However, the *in vivo* research indicates that the protection against *Salmonella* Typhimurium, *Shigella flexneri* and *Clostridium difficile* obtained in mice previously treated with yeasts is not due to the reduction of the bacterial populations in the intestines (*20*,*21*). There are some other properties that could explain the protective effect against the enteropathogenic bacteria such as immunomodulation, modulation of toxin production or action, and competition for adhesion sites or nutrients in the presence of yeasts (*3*,*7*,*8*,*21*).

Prevention of bacterial adherence and translocation in the intestinal epithelial cells result from the fact that the cell wall of *S. cerevisiae* var. *boulardii* has the ability to bind enteropathogens. In our study, we used the sedimentation and microscopic methods to evaluate the adhesion of bacterial cells to the probiotic yeast cell wall. In the sedimentation method, based on a subjective assessment of the pellet size observed at the bottom of the tube compared to the control sample (tube 1), clearly positive results were obtained for *E. coli*, *C. jejuni* and *E. faecalis* (Fig. 3).

In addition to this, based on the microscopic observations, adhesion was found in the case of *E. coli*, *S.* Typhimurium, *S. aureus*, *C. jejuni* and *E. faecalis* (Fig. 4). No binding was observed for bacteria *L. monocytogenes* and *P. aeruginosa* to the cell wall of *S. cerevisiae* var. *boulardii*.

Table 1. Growth kinetics values for bacterial strains cultured with probiotic yeasts

Bacteria	Mixed culture with	A	N	M	EGR	LPD
		log CFU/mL	log CFU/mL	h	h^{-1}	h
E. coli	–	5.01±0.15	4.28±0.16	9.35±0.46	0.65±0.020	4.09±0.35
	E	4.94±0.38	4.25±0.42	5.76±0.26	0.69±0.020	0.87±0.19
	H	4.99±0.14	4.16±0.16	6.32±0.19	0.75±0.014	1.83±0.06
	O	4.97±0.08	4.23±0.10	5.57±0.09	0.77±0.024	1.18±0.11
	P1	5.00±0.03	4.08±0.17	5.57±0.14	0.90±0.026	1.85±0.11
	P2	5.04±0.18	4.07±0.18	6.57±0.18	0.74±0.006	2.04±0.09
	S	4.95±0.23	4.14±0.21	5.45±0.26	0.90±0.018	1.74±0.12
	Y	5.08±0.16	4.05±0.19	6.66±0.36	0.80±0.024	2.44±0.14
E. faecalis	–	5.03±0.18	3.69±0.31	6.12±0.13	0.68±0.014	1.40±0.10
	E	5.17±0.20	3.51±0.20	6.93±0.10	0.52±0.010	0.77±0.12
	H	4.99±0.07	3.69±0.08	5.05±0.24	0.99±0.010	1.82±0.11
	O	5.00±0.04	3.57±0.12	4.73±0.13	1.16±0.046	2.02±0.06
	P1	4.92±0.12	3.85±0.16	5.25±0.10	0.61±0.007	0.039±0.01
	P2	4.92±0.11	3.80±0.13	5.89±0.47	0.67±0.010	1.11±0.14
	S	4.99±0.14	3.59±0.16	5.21±0.06	0.76±0.012	1.05±0.14
	Y	4.98±0.13	3.82±0.14	5.11±0.32	0.81±0.016	1.10±0.06
L. monocytogenes	–	5.13±0.10	3.45±0.17	11.13±0.46	0.63±0.017	6.15±0.60
	E	5.24±0.10	2.25±0.13	7.97±0.66	0.38±0.021	0.72±0.10
	H	5.11±0.20	2.42±0.11	8.26±0.21	0.43±0.017	1.97±0.12
	O	5.09±0.07	1.88±0.17	9.51±0.10	0.27±0.026	0.027±0.012
	P1	5.09±0.10	2.94±0.23	9.16±0.09	0.28±0.019	0.027±0.02
	P2	5.11±0.10	2.51±0.13	9.67±0.21	0.29±0.006	0.026±0.017
	S	5.09±0.18	1.52±0.18	7.15±0.28	0.34±0.009	0.015±0.02
	Y	5.06±0.14	2.79±0.08	9.92±0.51	0.33±0.009	0.034±0.033
P. aeruginosa	–	5.11±0.12	4.48±0.16	10.10±0.42	0.45±0.012	2.31±0.19
	E	4.89±0.10	4.39±0.14	7.98±0.44	0.43±0.013	0.10±0.029
	H	5.19±0.14	3.71±0.11	8.46±0.38	0.39±0.011	0.019±0.01
	O	5.18±0.22	3.47±0.16	8.43±0.13	0.38±0.026	0.025±0.015
	P1	5.05±0.09	4.20±0.06	10.57±0.21	0.32±0.022	0.018±0.011
	P2	5.14±0.14	3.99±0.15	9.22±0.28	0.36±0.006	0.090±0.046
	S	5.17±0.09	4.01±0.20	10.90±0.39	0.31±0.008	0.024±0.012
	Y	5.04±0.18	3.71±0.13	6.87±0.29	0.59±0.045	1.44±0.13
S. Typhimurium	–	5.03±0.16	4.30±0.17	6.34±0.18	0.73±0.053	1.65±0.11
	E	4.89±0.23	4.23±0.20	5.16±0.35	1.02±0.087	1.86±0.08
	H	4.95±0.14	4.37±0.10	4.64±0.13	0.74±0.008	0.034±0.023
	O	4.91±0.14	4.38±0.19	4.78±0.15	0.89±0.043	0.96±0.14
	P1	4.98±0.10	4.27±0.14	4.22±0.09	0.81±0.037	0.01±0.017
	P2	4.95±0.38	4.21±0.10	5.41±0.28	0.62±0.027	0.068±0.046
	S	4.99±0.13	4.38±0.18	4.17±0.23	0.83±0.034	0.015±0.02
	Y	4.94±0.11	4.31±0.32	4.05±0.36	0.84±0.010	0.015±0.02

The values are presented as mean±standard error of the mean (SEM); A=initial level of bacteria, N=increase between the initial and final cell numbers of bacteria, EGR=exponential growth rate, LPD=lag phase duration, M=time at which the culture achieves its maximum growth rate, by application of the Gompertz model (*14*)

At the same time, identical results were obtained for all the tested strains of probiotic yeasts.

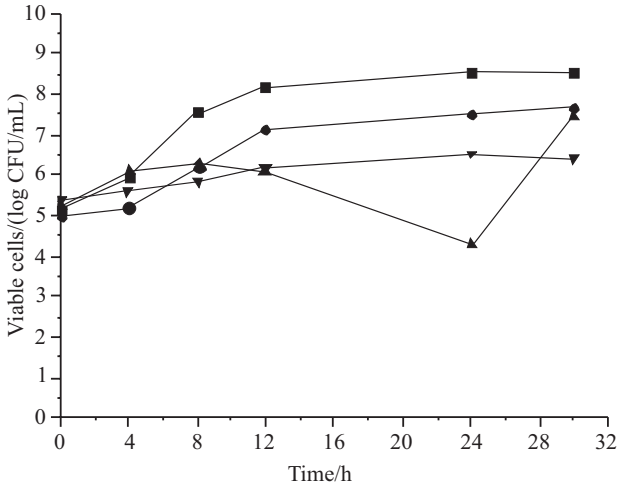

Fig. 1. Growth of *S. aureus* and probiotic yeast E in the monocultures and in the mixed cultures: –■– *S. aureus*, –●– *S. cerevisiae* var. *boulardii* E, –▲– *S. aureus* in the mixed culture, –▼– *S. cerevisiae* var. *boulardii* E in the mixed culture

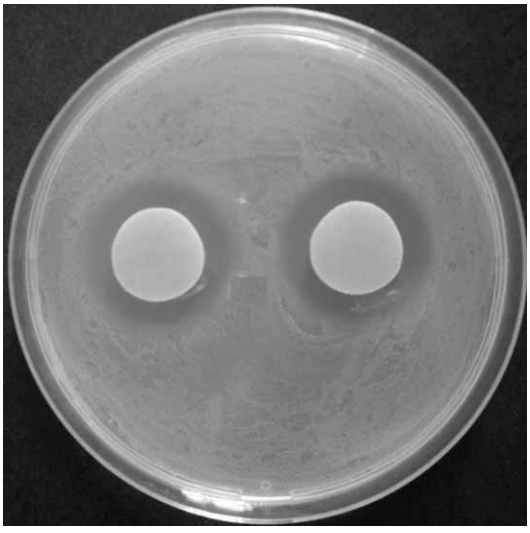

Fig. 2. Influence of *S. cerevisiae* var. *boulardii* E on the growth of *S. aureus* after 18 h of incubation

Our results are consistent with the reports stating that the cell wall of *S. cerevisiae* var. *boulardii* has shown binding capacity to enterohaemorrhagic *Escherichia coli* and *Salmonella* Typhimurium (*8*,*22*). The probiotic activity of *S. cerevisiae* var. *boulardii* based on the binding of *E. coli* cells has also been confirmed in *in vivo* research, which demonstrated that administration of *S. cerevisiae* var. *boulardii* reduces the adherence of enterotoxigenic *E. coli* to mesenteric lymph node in pig intestine (*23*). Probiotic yeast treatment on *Salmonella*-induced infection in mice also reduced the weight loss and mortality, and modulated the signalling pathways involved in the inflammatory activation in a murine model of typhoid fever. This preventing effect was due to a preferential binding of the *S.* Typhimurium to the yeast than to gut epithelial cells (*24*). According to our knowledge, our paper is the first one presenting the results demonstrating the ability of *S. cerevisiae* var. *boulardii* to bind bacterial strains other than *E. coli* and *S.* Typhimurium, *i.e. Staphylococcus aureus*, *Enterococcus faecalis* and *Campylobacter jejuni*, even though the mechanism of these effects may be different. It has already been shown that adherence of enteric bacteria is mostly due to common type 1 pili and may be inhibited by D-mannose or α-mannosidase, although bacterial flagella have also been connected with bacterial adhesion to human buccal cells, mouse spleen lymphocytes and yeast cells (*25*–*27*). Furthermore, yeast mannoproteins may serve as receptors for protein-protein interactions between yeasts and bacteria (*28*).

The ability of yeasts to bind bacterial cells results from the presence of mannoproteins in the cell wall structure, and therefore, it is not a unique feature assigned to *S. cerevisiae* var. *boulardii*, but rather a universal feature of all strains of the genus *Saccharomyces* (*11*,*25*,*26*,*28*,*29*). However, the scanning electron microscopy shows that the bacteria of type 1 fimbriated *E. coli* were more strongly bound to the surface of *S. cerevisiae* var. *boulardii* than to the surfaces of probiotic *Saccharomyces cerevisiae* strains (*8*,*25*). The differences in the binding capacity may also be due to the culturing conditions such as temperature, humidity and pH (*11*,*30*).

An important initial event in bacterial pathogenesis is the adherence of bacteria *via* their surface lectins to the host intestinal cells (*31*). The use of *S. cerevisiae* var. *boulardii* as a probiotic in humans may counteract the adhesion of pathogens to the host tissues by providing

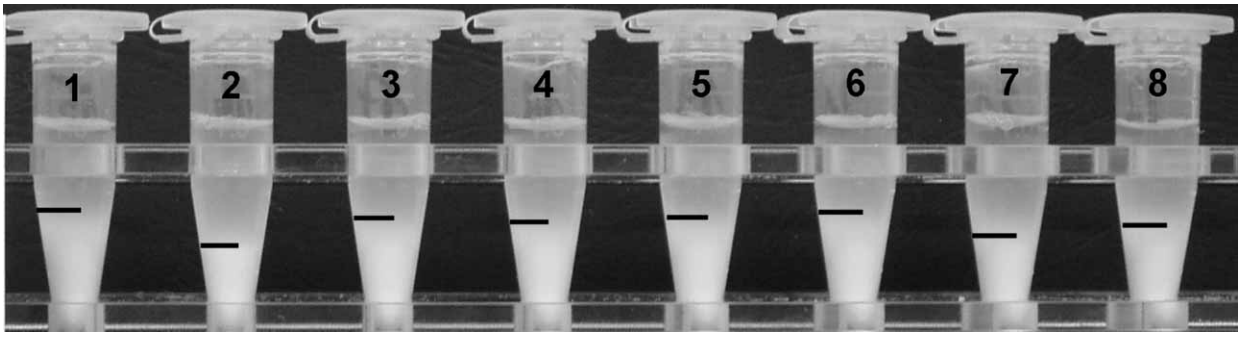

Fig. 3. Sedimentation assays for (1) *S. cerevisiae* var. *boulardii* E and (2–8) its mixtures with the tested bacteria: (2) *E. coli*, (3) *S.* Typhimurium, (4) *S. aureus*, (5) *L. monocytogenes*, (6) *P. aeruginosa*, (7) *C. jejuni* and (8) *E. faecalis*; lines indicate sediment levels

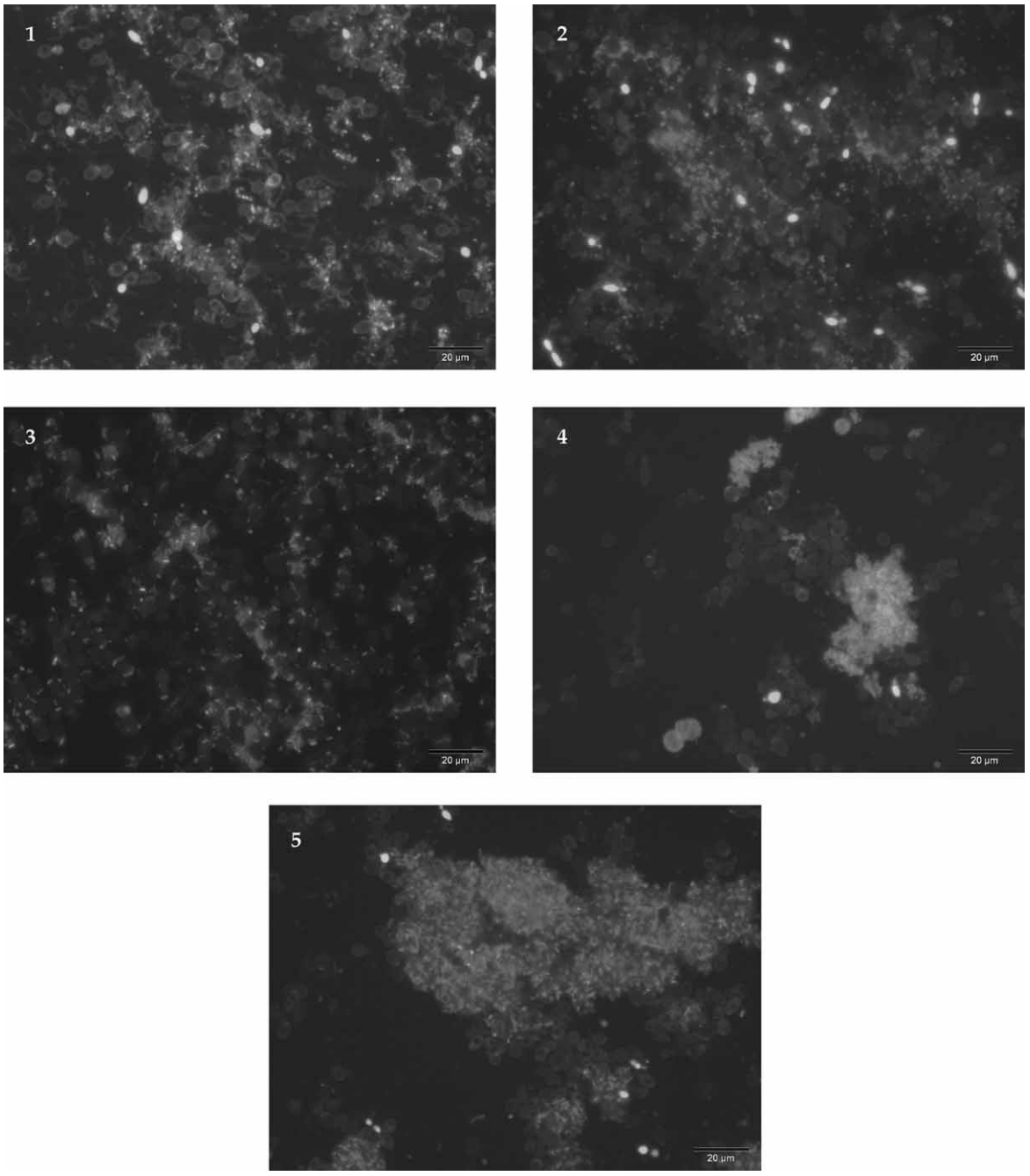

Fig. 4. Exemplary adhesion tests by fluorescence microscopy for *S. cerevisiae* var. *boulardii* E in mixtures with the tested bacteria: positive results for (1) *E. faecalis*, (2) *S. aureus*, (3) *S.* Typhimurium, (4) *C. jejuni* and (5) *E. coli*

alternative adhesion sites to enterobacteria and in that way prevent infections (*23*,*24*). It may also eliminate pathogens from the gastrointestinal tract of infected patients.

Conclusions

It seems that probiotic activity of *S. cerevisiae* var. *boulardii* against human pathogens results from the reduction of bacterial growth or from the adhesion of bacteria to yeast cell walls. Probiotic strains of *S. cerevisiae* var. *boulardii* caused a statistically significant reduction in the number of cells of *L. monocytogenes*, *P. aeruginosa* and *S. aureus*, and a decrease in the growth rate of *L. monocytogenes* and *P. aeruginosa*. The ability of *S. cerevisiae* var. *boulardii* strains to bind enteropathogens was confirmed for *E. coli* and *S.* Typhimurium. Additionally, the yeasts showed binding capacity to *S. aureus*, *C. jejuni* and *E. faecalis*. However, the mechanisms of binding cocci to the yeast cell surface remain to be investigated.

The use of *S. cerevisiae* var. *boulardii* may limit bacterial invasiveness and infections caused by human pathogens through the reduction in the number of viable and active cells of bacteria and the elimination of pathogens bound to yeast surface from the gastrointestinal tract.

Acknowledgements

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References

1. Evaluation of Health and Nutritional Properties of Powder Milk and Live Lactic Acid Bacteria, FAO/WHO Report, Geneva, Switzerland (2001).
2. H. Kumura, Y. Tanoue, M. Tsukahara, T. Tanaka, K. Shimazaki, Screening of dairy yeast strains for probiotic applications, *J. Dairy Sci. 87* (2004) 4050–4056.
3. L.V. McFarland, Systematic review and meta-analysis of *Saccharomyces boulardii* in adult patients, *World J. Gastroenterol. 16* (2010) 2202–2222.
4. C. Pothoulakis, Review article: Anti-inflammatory mechanisms of action of *Saccharomyces boulardii*, *Aliment. Pharm. Therap. 30* (2009) 826–833.
5. L.V. MacFarland, Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease, *Am. J. Gastroenterol. 101* (2006) 812–822.
6. L. Beaugerie, J.C. Petit, Antibiotic-associated diarrhoea, *Best Pract. Res. Clin. Gastroenterol. 18* (2004) 337–352.
7. D. Czerucka, T. Piche, P. Rampal, Review article: Yeasts as probiotics – *Saccharomyces boulardii*, *Aliment. Pharm. Therap. 26* (2007) 767–778.
8. B.R. Gedek, Adherence of *Escherichia coli* serogroup 0 157 and the *Salmonella typhimurium* mutant DT 104 to the surface of *Saccharomyces boulardii*, *Mycoses*, *42* (1999) 261–264.
9. M. Strus, A new method for testing antagonistic activity of lactic acid bacteria (LAB) on selected pathogenic indicator bacteria, *Med. Dośw. Mikrobiol. 50* (1998) 123–130.
10. G.T. Macfarlane, S. Macfarlane, G.R. Gibson, Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon, *Microb. Ecol. 35* (1997) 180–187.
11. L.S. Pérez-Sotelo, M. Talavera-Rojas, H.G. Monroy-Salazar, S. Lagunas-Bernabé, J.A. Cuarón-Ibargüengoytia, R. Montes de Oca Jimenez, J.C. Vazquez-Chagoyan, *In vitro* evaluation of binding capacity of *Saccharomyces cerevisiae* Sc47 to adhere to the wall of *Salmonella* spp., *Rev. Latinoam. Microbiol. 47* (2005) 70–75.
12. D. Kręgiel, J. Berłowska, Evaluation of yeast cell vitality using different fluorescent dyes, *Sci. Bull. Tech. Univer. Lodz Food Chem. Biotechnol. 73* (2009) 5–14.
13. STATISTICA (Data Analysis Software System), v. 6.0, StatSoft, Inc, Tulsa, OK, USA (2001) (*www.statsoft.com*).
14. J. Baranyi, T.A. Roberts, A dynamic approach to predicting bacterial growth in food, *Int. J. Food Microbiol. 23* (1994) 277–294.
15. L.L. Zaika, J.G. Philips, J.S. Fanelli, O.J. Scullen, Revised model for aerobic growth of *Schigella flexneri* to extend the validity of predictions at temperatures between 10 and 19 °C, *Int. J. Food Microbiol. 41* (1998) 9–19.
16. M. Bizot, Antagonism phenomena between various microorganisms: Yeasts and bacteria, *La Presse Med. 63* (1955) 1251–1252 (in French).
17. S. Brugier, F. Patte, Antagonism *in vitro* between ultra levure and different species of bacteria, *Med. Paris*, *45* (1975) 3–8 (in French).
18. M. Bornet, E. Bergogne-Berezin, Bacterial growth in enteral eliminations value of the addition of *Saccharomyces boulardii*, *Sci. Aliments*, *6* (1986) 63–73.
19. R. Zbinden, E.E. Gonczi, M. Altwegg, Inhibition of *Saccharomyces boulardii* (nom. inval.) on cell invasion of *Salmonella typhimurium* and *Yersinia enterocolitica*, *Microb. Ecol. Health Dis. 11* (1999) 158–162.
20. A.C. Rodrigues, D.C. Cara, S.H. Fretez, F.Q. Cunha, E.C. Vieira, J.R. Nicoli, L.Q. Vieira, *Saccharomyces boulardii* stimulates sIgA production and the phagocytic system of gnotobiotic mice, *J. Appl. Microbiol. 89* (2000) 404–414.
21. F.S. Martins, R.M.D. Nardi, R.M.E. Arantes, C.A. Rosa, M.J. Neves, J.R. Nicoli, Screening of yeasts as probiotic based on capacities to colonize the gastrointestinal tract and to protect against enteropathogen challenge in mice, *J. Gen. Appl. Microbiol. 51* (2005) 83–92.
22. F.S. Martins, G. Dalmasso, R.M.E. Arantes, A. Doye, E. Lemichez, P. Lagadec *et al.*, Interaction of *Saccharomyces boulardii* with *Salmonella enterica* serovar Typhimurium protects mice and modifies T84 cell response to the infection, *PLoS ONE, 5* (2010) e8925.
23. M. Lessard, M. Dupuis, N. Gagnon, E. Nadeau, J.J. Matte, J. Goulet, J.M. Fairbrother, Administration of *Pediococcus acidilactici* or *Saccharomyces cerevisiae boulardii* modulates development of porcine mucosal immunity and reduces intestinal bacterial translocation after *Escherichia coli* challenge, *J. Anim. Sci. 87* (2009) 922–934.
24. F.S. Martins, S.D.A. Elian, A.T. Vieira, F.C.P. Tiago, A.K.S. Martins, F.C.P. Silva *et al.*, Oral treatment with *Saccharomyces cerevisiae* strain UFMG 905 modulates immune responses and interferes with signal pathways involved in the activation of inflammation in a murine model of typhoid fever, *Int. J. Med. Microbiol. 301* (2011) 359–364.
25. T.K. Korhonen, H. Leffler, C.S. Edén, Binding specificity of piliated strains of *Escherichia coli* and *Salmonella typhimurium* to epithelial cells, *Saccharomyces cerevisiae* cells and erythrocytes, *Infect. Immun. 32* (1981) 796–804.
26. K. Jann, G. Schmidt, E. Blumenstock, K. Vosbeck, *Escherichia coli* adhesion to *Saccharomyces cerevisiae* and mammalian cells: Role of piliation and surface hydrophobicity, *Infect. Immun. 32* (1981) 484–489.
27. Y. Eshdat, V. Speth, K. Jann, Participation of pili and cell wall adhesion in the yeast agglutination activity of *Escherichia coli*, *Infect. Immun. 34* (1981) 980–986.
28. K.W. Millsap, H.C. van der Mei, R. Bos, H.J. Busscher, Adhesive interactions between medically important yeasts and bacteria, *FEMS Microbiol. Rev. 21* (1998) 321–326.
29. F.M. Klis, A. Boorsma, P.W.J. De Groot, Cell wall construction in *Saccharomyces cerevisiae*, *Yeast*, *23* (2006) 185–202.
30. A.S. McDermid, A.S. McKee, A.B. Dowset, P.D. Marsch, The effect of environmental pH on the physiology and surface structures of *Salmonella* serotype Enteritidis phage type 4, *J. Med. Microbiol. 6* (1996) 452–458.
31. E.L. Hartland, G. Frankel, S. Knutton: Interactions Between Enteropathogenic *Escherichia coli* and Epithelial Cells. In: *Bacterial Adhesion to Host Tissues*, M. Wilson (Ed.), Cambridge University Press, Cambridge, UK (2002) pp. 277–299.