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Evaluation of probiotic potential of yeasts isolated from traditional cheeses manufactured in Serbia and Croatia

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ABSTRACT

Aim: The aim of this study was to investigate the *in vitro* probiotic potential of dairy yeast isolates from artisanal cheeses manufactured in Serbia and Croatia. **Materials and Methods:** Twelve yeast strains isolated from artisanal fresh soft and white brined cheeses manufactured in Serbia and Croatia were used in the study. Survival in chemically-simulated gastrointestinal conditions, adherence to epithelial intestinal cells and proliferation of gut-associated lymphoid tissue (GALT) cells were evaluated. **Results:** The results revealed that two strains of *Kluyvereomyces lactis* ZIM 2408 and ZIM 2453 grew above one log unit (Δ log CFU/ml) in the complex colonic medium during 24 h of cultivation, while *Torulaspora delbrueckii* ZIM 2460 was the most resistant isolate in chemically-simulated conditions of gastric juice and upper intestinal tract. It was demonstrated that the strains *K. lactis* ZIM 2408 and ZIM2441 and *Saccharomyces cerevisiae* ZIM 2415 were highly adhesive to Caco-2 cells, while strains *K. lactis* ZIM 2408 and *Debaryomyces hansenii* ZIM 2415 exhibit the highest adhesion percentage to HT29-MTX cells. All strains significantly ($P < 0.0001$) decreased the proliferation of GALT cells, suggesting the possible strain-specific immunomodulatory potential of the isolates. **Conclusion:** The dairy yeast isolates exhibit strain-specific probiotic properties, particularly the strain *K. lactis* ZIM 2408, which appears to be the best probiotic candidate in terms of all three criteria. Taking into account their immunomodulatory potential, the yeast isolates could be further tested for specific probiotic applications and eventually included in functional food formulated for patients suffering from diseases associated with an increased inflammatory status.

KEY WORDS: Caco-2, HT29-MTX, GALT, host-microbe interactions, immunomodulation, *Kluyvereomyces lactis*

INTRODUCTION

The Western Balkan Countries (WBC) harbour a variety of traditional fermented foods produced by spontaneous or controlled fermentation from cow's, ewe's and goat's milk. These products feature a rich diversity of lactic acid bacteria (LAB) as part of their natural microflora, with relevant genetic, metabolic and technological features, making these bacteria

potential candidates as probiotic microorganisms [1-6]. However, the diversity and probiotic potential of yeasts in WBC artisanal dairy products is still insufficiently explored.

Probiotics, defined as 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host' [7], have been demonstrated to exert health-promoting effects through various proposed mechanisms: (a) Competition with pathogenic bacteria for nutrients and binding sites in the gut epithelium, (b) inactivation of the toxins and metabolites produced by pathogens, (c) the production of antimicrobial substances which inhibit the growth of pathogenic microorganisms, (d) stimulation/modulation of the immune response, or (e) anti-carcinogenic action [8]. The beneficial effects of probiotics are shown to be strain specific, pointing to the need to use various screening systems to identify specific probiotics to treat specific disorders and symptoms [9]. Human colon tumorigenic cell lines such as Caco-2 and mucin-producing HT29-MTX are recognized as good models for elucidation of the mechanisms involved in host-microbe interactions, although they lack the complexity of the human immune system [8,10-14]. In addition, probiotics can interact with gut-associated lymphoid tissue (GALT) and bind to epithelial surface receptors, inducing humoral and cellular immune responses [15]. Furthermore, recent studies have shown that probiotics exhibit beneficial health effects by directly modulating or down-regulating the immune system through modification of the immune response in GALT, thereby preventing the symptoms of inflammatory bowel disease, allergies and asthma [16]. Hence, GALT primary cells have been suggested as an improved *in vitro* model for studying the interactions of microorganisms, because they are non-transformed, non-tumorigenic and produce mucin [17].

A number of studies have suggested that the administration of probiotics plays a role in the promotion of human health. Numerous products intended for human consumption containing live microorganisms have been declared to have probiotic activity. Although, the design of foods containing probiotics has focused primarily on *Lactobacillus* and *Bifidobacterium* [18-22], the use of yeast probiotics is limited. *Saccharomyces boulardii* is considered a probiotic and has been widely used in lyophilized form for the prevention and treatment of human gastrointestinal tract (GIT) diseases [10,23-28]. Recently, several other yeast strains belonging to the genera *Saccharomyces*, *Debaryomyces*, *Torulaspora*, *Kluyveromyces*, *Pichia*, and *Candida* have also been shown to have probiotic potential in terms of their ability to survive simulated conditions of the GIT, and to adhere to different mammalian intestinal epithelial cells [29-32].

In our previous study, we isolated and characterized 69 yeast strains from artisanal white pickled and fresh soft cheeses manufactured in Serbia and Croatia, respectively [33]. Due to the interest of the food industry in novel candidate probiotic strains, the current study was designed to select yeast isolates with probiotic potential. The aim of this study was to challenge natural yeast isolates to a chemically simulated GIT transit and to test their ability to adhere to epithelial intestinal cell (EIC) lines, as well as to induce and modulate the proliferation of GALT cells in the presence of UV-irradiated strains. Here we present the probiotic potential of 12 dairy yeast isolates.

MATERIALS AND METHODS

Yeast Strains

The twelve autochthonous yeast strains used in this study were previously isolated from traditional cheeses manufactured in Serbia and Croatia: *K. lactis* ZIM 2408 (ENA ID HE660059); ZIM 2441 (ENA ID HE799667); ZIM 2453 (ENA ID HE660074); ZIM 2456 (ENA ID HE660077); *Torulaspora delbrueckii* ZIM 2436 (ENA ID HE660081); ZIM 2458 (ENA ID HE660079); ZIM 2460 (ENA ID HE799671); *Torulaspora quercuum* ZIM 2412 (ENA ID HE660063); *Debaryomyces hansenii* ZIM 2415 (ENA ID HE799657); ZIM 2440 (ENA ID HE799666); *Galactomyces geotrichum* ZIM 2422 (ENA ID HE799659); *Saccharomyces cerevisiae* ZIM 2447 (ENA ID HF545670) [33]. The strains were either the predominant yeast species in the cheese samples [33] and/or strains isolated from the cheeses which are allowed to be added intentionally to food (qualified presumption as safe) [34]. The strains were cultivated on YPD agar (Sigma Chemical Co., St. Louis, MO, USA) at 28°C.

Survival of Yeasts in Simulated Chemical Conditions Encountered in the GIT

The ability of the yeast strains to survive in chemical conditions that simulate those encountered in the GIT was assessed. One colony of each yeast strain was resuspended in YPD medium and grown overnight at 28°C and 220 rpm (with shaking, in aerobic conditions). The cells were harvested by centrifugation (2880 g force, 5 min) and washed twice in phosphate-buffered saline (PBS) (PBS: NaCl 8 g/l, KCl 0.2 g/l, Na_2HPO_4 1.44 g/l, KH_2PO_4 0.24 g/l; pH 7.2). The final cell concentration was adjusted to 7.0 log cells/ml. The cells were further suspended in PBS solution of pH 2 with added pepsin (3 mg/ml) at 37°C for 3 h and in PBS solution of pH 7.2 with 0.3% (w/v) Oxgall at 37°C for 4 h simulating stomach and small intestine conditions, respectively. Resistance to the chemical conditions encountered in the human colon were tested in a complex colonic model growth medium (CMGM) [35] under anaerobic conditions for 24 h. After incubation, viable colony counts were determined and survival rate was expressed as means of Δlog units of duplicates.

Adhesion of Yeast Strains to Intestinal Cell Lines

The colonocyte-like cell lines Caco-2 and HT29-MTX were used to determine the adhesion ability of the yeast isolates. Caco-2 cell lines were purchased from the European Collection of Cell Cultures (ECACC No. 86010202) and HT29-MTX was kindly supplied by Dr. T. Lesuffleur (INSERM UMR S 938, Paris, France) [36]. The culture and maintenance of the cell lines were carried out following standard procedures [5] using Advanced DMEM medium (Gibco Invitrogen, Paisley, UK) for Caco-2 and HT29-MTX supplemented with heat inactivated

foetal bovine serum (5% for Caco-2, 10% for HT29-MTX), L-glutamine (2 mM) and with a mixture of antibiotics (10 U/ml penicillin, 10 μg/ml streptomycin, 50 μg/ml gentamicin). Media and reagents were purchased from PAA (Pasching, Austria). Intestinal cells were seeded in 24-well plates and cultivated until a confluent differentiated state was reached (monolayers). Yeasts were cultured for 24 h as described above and after washing twice with Dulbecco´s PBS solution (Sigma) were resuspended in the corresponding cell-line media without antibiotics at a concentration of about 10^8 CFU/ml. Cellular monolayers were also carefully washed with Dulbecco´s PBS solution (Sigma), and yeast suspensions were added at an MOI (multiplicity of infection) ratio of about 10:1 (yeast: eukaryotic cell). Adhesion experiments were carried out for 1 h at 37°C, 5% CO_2 and, afterwards, wells were gently washed to release unattached yeasts before proceeding with the lysis of cellular monolayers using 0.25% Trypsin–ethylenediaminetetraacetic acid (EDTA) solution (PAA, Pasching, Austria). Dilutions of samples, before and after adhesion, were made in PBS solution and yeast counts were performed on YPD agar plates.

The adhesion was calculated as: % CFU adhered yeasts/CFU added yeasts. Experiments were carried out in two replicated plates and in each plate two wells were used per sample.

Proliferation of GALT in the Presence of Non-viable Yeast Strains

The yeast strains were grown in the same manner as described above. Overnight yeast cultures were harvested by centrifugation, washed two times with PBS buffer and resuspended in 5 ml of the same buffer with a final cell suspension of 10^7 CFU/ml determined by plate counting. Cells were inactivated by UV light (in the UV chamber, 15 W) for 3 cycles of 30 min each. Plate counting was carried out after UV treatment to corroborate the absence of live yeasts that could be able to recover in the proper medium. UV-inactivated yeasts were then divided into single-use aliquots, frozen in liquid N_2 and stored at −80°C until use [37]. All experimental procedures and protocols conformed to institutional guidelines for the care and use of animals in research no. 2/09 (Ethical Committee of the Faculty of Pharmacy, University of Belgrade). Animal manipulations were approved by the Ethical Committee for Experimentation on Laboratory Animals of the Faculty of Pharmacy, University of Belgrade. A total number of 3 Wistar rats (healthy female adults between 6 and 8 weeks old) were purchased from the Farm of the Military Medical Academy, Belgrade. For the experiments each animal was anaesthetized with CO_2 and, once assured of the loss of corneal reflex, its intestine was excised from the jejunum to the ileocaecal junction. The whole small intestine was placed in cold Hank´s balanced salt solution (HBSS without calcium and magnesium ions, prepared according to the formula of Gibco, Invitrogen) and kept at 4°C until processing. Finally, the animals were sacrificed using the increase of CO_2 concentration. The isolation of lymphocytes from GALT (Peyer´s Patches lymphocytes and IEL) was carried out as previously described [38]. Briefly, small pieces of the cleaned small intestine were incubated with HBSS without Ca and Mg ions, with antibiotics (gentamicin 500 mg/ml [AppliChem GmbH, Darmstadt, Germany], penicillin 20 IU/ml, streptomycin 2 μg/ml [PAA], and 10 mM Hepes). Treatment with HBSS-DTT (HBSS with 2 mM DTT and 10 mM Hepes, pH 7.2) and HBSS-EDTA (HBSS with 1 mM EDTA and 1 mM Hepes, pH 7.2) was used to release the IEL lymphocyte subset. Incubation in complete RPMI medium with antibiotics (RPMI-1640 with 2 mM L-glutamine and 25 mM HEPES (PAA, Austria), 10% heat-inactivated FSB, 100 mg/mL streptomycin (Sigma) and ampicillin (Sigma) with collagenase 100 IU/ml (Gibco Invitrogen) was used to isolate the PP subset of lymphocytes. The PPL and IEL present in the supernatants were purified by Percoll (Sigma) gradient (66%-47%-25%) and then resuspended in complete RPMI medium with antibiotics.

To quantify the response of GALT to the different factors tested, 2×10^5 lymphocyte cells were incubated with UV-inactivated yeasts (at a ratio 1:5) for 4 days at 37°C in complete RPMI medium with antibiotics at 37°C with 5% CO_2. All cultures were performed in triplicate (GALT) in 96-well round-bottom microtiter plates. After 4 days of incubation, the proliferation of GALT-lymphocytes was determined with a Cell Proliferation Assay Kit (Millipore Corporation, Billerica, MA, USA) following the manufacturer's instructions. Results were compared with a negative control (lymphocytes growing in complete RPMI medium with antibiotics) to test the capability of each factor to induce GALT-proliferation.

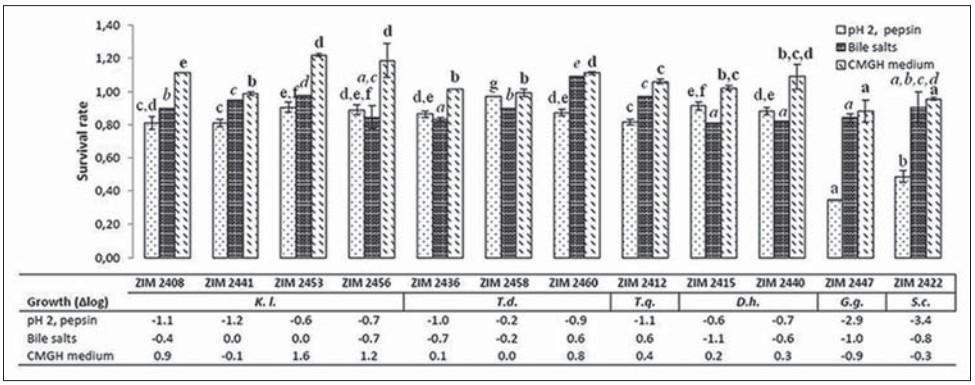
Statistical Analysis

After checking the normal distribution of the proliferation data (NORMDINST), one-way ANOVA tests were used to determine differences between each factor and the negative control. Finally, one-way ANOVA tests, together with the mean comparison test less significant difference, were used to compare the differences between the three strains. Results were represented by mean ± standard deviation or standard error. The SPSS 15.0 Statistical Software Package (SPSS Inc) was used for all determinations and the value $P < 0.05$ was considered as significant.

RESULTS

In Vitro GIT Survival of Yeast Strains

The ability of the 12 yeast strains to survive chemical conditions similar to the conditions found in the GIT was tested. The results showed that most of the strains survived moderately under simulated gastric conditions (from 81% to 97%; Δlog CFU/ml from -0.2 to 1.2) with the exception of the strains belonging to *G. geotrichum* and *S. cerevisiae* which showed a poor survival rate (35% and 49%; Δlog CFU/ml -2.9 and 3.4, respectively) [Figure 1]. Moreover, the rate of survival was either maintained or slightly decreased in the presence of bile salts for all tested strains (from 81% to 109%; Δlog from −1.1 to 0.6). Two strains of *K. lactis* ZIM 2453 and ZIM 2456 grew above one log unit (Δ log CFU/ml) in CMGM during 24-h of cultivation. Nevertheless, *T. delbrueckii* strains ZIM 2458 and



	ZIM 2408	ZIM 2441	ZIM 2453	ZIM 2456	ZIM 2436	ZIM 2458	ZIM 2460	ZIM 2412	ZIM 2415	ZIM 2440	ZIM 2447	ZIM 2422
Growth (Δlog)	*K. l.*				*T.d.*			*T.q.*	*D.h.*		*G.g.*	*S.c.*
pH 2, pepsin	-1.1	-1.2	-0.6	-0.7	-1.0	-0.2	-0.9	-1.1	-0.6	-0.7	-2.9	-3.4
Bile salts	-0.4	0.0	0.0	-0.7	-0.7	-0.2	0.6	0.6	-1.1	-0.6	-1.0	-0.8
CMGH medium	0.9	-0.1	1.6	1.2	0.1	0.0	0.8	0.4	0.2	0.3	-0.9	-0.3

Figure 1: Survival rate and Δlog CFU/ml of yeast strains under simulated stomach conditions (pH 2), in an environment simulating upper gastrointestinal tract (bile salt) and under simulated colonal conditions in a CMGM medium, *K.l.* - *Kluyveromyces lactis*; *T.d.* - *Torulaspora delbrueckii; T.q.* - *T. quercuum; D.h.* - *Debaryomyces hansenii; G.g.* - *Galactomyces geotrichum; S.c.* - *Saccharomyces cerevisiae*. Within each GIT challenge, columns that do not share the same letter are statistically different (p<0.05).

ZIM 2460 were the most resistant in simulated conditions of gastric juice and upper intestinal tract, respectively.

The Adhesion Ability of Yeast Strains to Intestinal Cell Lines

The adhesion ability to a monolayer EIC was investigated for the 12 yeast dairy strains using human cell lines (Caco-2 and HT29-MTX) carried out as per the procedure documented in Nikolic et al., 2014 [5]. The results are presented in Figure 2. As seen in Figure 2, the results demonstrate that three natural dairy yeast isolates, *K. lactis* ZIM 2408, *K. lactis* ZIM 2441 and *D. hansenii* ZIM 2415, were highly adhesive (more than 60% of added yeasts). The adhesion properties of the strains *K. lactis* ZIM 2408 and *S. cerevisiae* ZIM 2415 followed the same tendency in both intestinal cell lines, although they exhibited lower percentages of adhesion to the mucus-producing cell line HT29-MTX. An exception was strain *K. lactis* ZIM 2441 which exhibited good adhesion to the Caco-2 cell line but significantly lower adhesion to the HT29-MTX cell line ($P < 0.05$).

Proliferation of GALT in the Presence of UV Inactivated Yeasts Isolates

The proliferation indexes of GALT measured in the presence of the stimuli are presented in Figure 3. In general, the results showed that the proliferation of GALT cells was reduced in the presence of the yeast strains compared to the control (RMPI in the absence of the stimulus). Specifically, the strain *K. lactis* ZIM 2408 and three *Torulaspora* isolates (*T. delbrueckii* ZIM 2436, *T. quercuum* ZIM 2412 and *Torulaspora* sp. ZIM 2460) significantly reduced the number of GALT cells in comparison to non-treated GALT cells ($P < 0.0001$ in all cases, except for *S. cerevisiae* ZIM 2415 and *S. cerevisiae* ZIM 2440, $P < 0.001$). None of the strains increased the proliferation of GALT cells.

DISCUSSION

In recent years, an increasing number of studies have suggested that the administration of probiotics plays a role in the promotion of human health. Numerous products intended for human consumption containing live microorganisms have been declared to have probiotic activity. Various studies have confirmed that bacterial species, mostly *Lactobacillus* and *Bifidobacterium* strains, can modulate GALT responses [18,20-22]. The scientific and clinical interest in finding microorganisms with the ability to regulate intestinal immune response has increased due to the accumulating evidence that the GIT microbiota play a critical role in the initiation and prevention of inflammatory bowel diseases, allergies, eczema and various atopic diseases [9,16,39,40]. Although recent reviews indicate the health-promoting properties of yeasts, studies describing the probiotic potential of yeast strains are still limited [41,42].

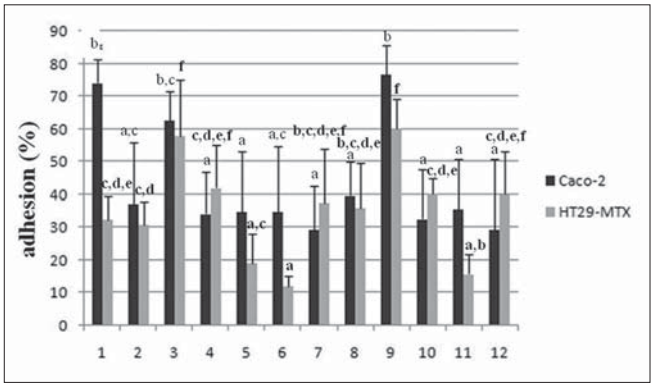


Figure 2: Adhesion of the yeasts isolates to Caco-2 and HT29-MTX cell lines. The asterisk is showing statistically different (p<0.05) adhesion in between cell lines. 1. *Kluyveromyces lactis* ZIM 2441; 2. *K. lactis* ZIM 2453; 3. *K. lactis* ZIM 2408; 4. *K. lactis* ZIM 2456; 5. *Torulaspora delbrueckii* ZIM 2458; 6. *T. delbrueckii* ZIM 2436; 7. *T. quercuum* ZIM 2412; 8. *Torulaspora* sp. ZIM 2460; 9. *Saccharomyces cerevisiae* ZIM 2415; 10. *S. cerevisiae* ZIM 2440; 11. *S. cerevisiae* ZIM 2447; 12. *Debaryomyces hansenii* ZIM 2422. The adhesion was calculated as: % CFU adhered yeasts/CFU added yeasts. Experiments were carried out in two replicated plates and in each plate two wells were used per sample. The Student's *t*-test was used for each strain to determine if the data in between the adhesion to two IEC lines are significantly different from each other, $P < 0.05$ value was considered significant. Within each IEC line, columns that do not share the same letter are statistically different ($P < 0.05$).

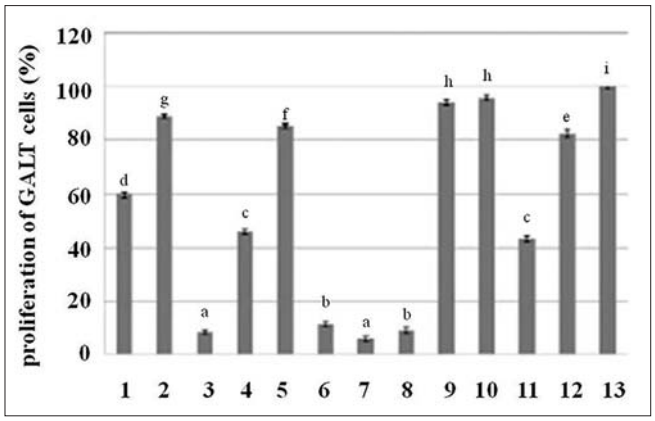


Figure 3: Proliferation of GALT cell isolated from rats, co-cultured for four days in the presence of the yeast isolates at ratio (yeast : cell line) 5:1. 1. *Kluyveromyces lactis* ZIM 2441; 2. *K. lactis* ZIM 2453; 3. *K. lactis* ZIM 2408; 4. *K. lactis* ZIM 2456; 5. *Torulaspora delbrueckii* ZIM 2458; 6. *T. delbrueckii* ZIM 2436; 7. *T. quercuum* ZIM 2412; 8. *Torulaspora* sp. ZIM 2460; 9. *Saccharomyces cerevisiae* ZIM 2415; 10. *S. cerevisiae* ZIM 2440; 11. *S. cerevisiae* ZIM 2447; 12. *Debaryomyces hansenii* ZIM 2422; 13. Control (lymphocytes without the stimuli). The Student's *t*-test was used for each strain to determine if the data in between the proliferation in the presence of yeasts and for the control to two IEC lines are significantly different from each other, $P < 0.001$ value was considered significant. Columns that do not share the same letter are statistically different ($P < 0.05$).

In this study, the probiotic potential of the natural yeast isolates originating from artisanal cheeses was evaluated in terms of their survival under simulated GIT conditions, adhesion to the intestinal epithelial cell lines Caco-2 and HT29-MTX and modulation of GALT cell proliferation. According to the FAO/WHO guidelines for the evaluation of probiotics for human food applications [7], the survival of probiotic strains in gastric and intestinal digestion is one of the desirable properties that strains with probiotic potential should present. The results obtained in this study showed high resistance of the yeast isolates to low pH conditions that could be related to an adaptation to acidic conditions in the natural environment from which the strains were isolated, similar to that reported for lactobacilli of food origin [43,44]. Interestingly, in contrast to LAB of food origin the majority of the yeast isolates proliferated in the colonic model under anaerobic conditions [5]. In general, our results confirmed that the yeasts associated with food have a rather good ability to survive under simulated GIT conditions [30, 45,46]. Nevertheless, as was demonstrated for LAB, the acid and bile resistance were more strain than species dependant properties [47]. The colonization of intestinal mucosa is another important criterion for selection of strains with probiotic potential [7], since their health-promoting effects might be partly dependent on their persistence in the intestine and adhesion to mucosal surfaces [48]. However, adhesion was shown not to be a prerequisite for probiotic yeasts in order to have inhibitory action against pathogenic bacteria [49]. Human colon tumorigenic cell lines such as Caco-2 and mucin-producing HT29-MTX are recognized as good models for elucidation of the mechanisms involved in host-microbe interactions, although they lack the complexity of the human immune system [50,51]. In general, our results showed better adhesion of the yeast strains to the Caco-2 cell lines than to HT29-MTX. The presence of the glycoprotein (mucin) layer in the HT29-MTX cell line might have hindered the availability of the cells as receptors for yeasts. The use of HT29-MTX cells could be advantageous in studying the adhesion ability of microorganisms to EIC, since they represent a mucin-secreting cell culture that expresses similar protein patterns to human intestinal epithelium [51]. On the other hand, the adhesion ability of the different bacterial strains to independent cultures of HT29-MTX cells was shown to be lower in comparison to the Caco-2 cell line [52], suggesting that various models should be used in order to study the adhesion abilities of particular strains.

The next important characteristic of potential probiotic candidates is the capacity to modulate the immune response of the host. Probiotics can interact with GALT and bind to epithelial surface receptors, inducing humoral and cellular immune responses. Furthermore, recent studies have shown that probiotics exhibit beneficial effects by directly modulating or down-regulating the immune system through modification of the immune response in GALT, preventing in that way the symptoms of inflammatory bowel disease, allergies and asthma [16]. Hence, GALT primary cells have been suggested as an improved *in vitro* model for studying the interactions of microorganisms within the host, due to the fact that they are non-transformed, non-tumorigenic and produce mucin [17]. Two main effects of probiotics on a host's immunity, demonstrated in several *in vitro* and *in vivo* studies, are strengthening the immunological barrier through the development of the innate and adaptive immune system and decreasing the immune responsiveness to unbalanced inflammatory conditions [9]. Different yeast species, many of them usually found in fermented food, such as *D. hansenii*, *T. delbrueckii*, *K. lactis*, and *S. cerevisiae*, have also shown tolerance to passage through the GIT, adhesion to intestinal Caco-2 cell lines, and immunostimulatory activity [50]. Interestingly, our results revealed the reduced proliferation of GALT cells in the presence of the yeast isolates, indicating that yeast strains isolated from artisanal cheeses have the potential to modulate the host response and to have possibly immunosuppressive activity, perhaps by up- and down-regulation of various cytokines. Similarly, the results of Romanin et al. [53] indicate that the inhibition of innate epithelial response could be a rather general property of different yeast species.

In general, the use of probiotics yeasts, such as *S. boulardii*, is shown to be safe in healthy populations and, to the best of our knowledge, no adverse effects have been reported in immunocompetent patients. However, a recent systematic review documented that probiotic products based on *S. boulardii* increase the risk of complications, such as fungemia or a rare gastrointestinal allergic reaction, in immunocompromised subjects [54]. Although rare, serious complications from probiotics (i.e., fungemia) in immunocompromised patients, or in those who had central venous catheters, highlight the need to establish the safety profile of these agents when they are used in anyone other than healthy populations.

Taking this safety issue together with the immunomodulatory potential of the yeast isolates tested in this study, they could eventually be included in functional food formulated for patients suffering diseases associated with an increased inflammatory status. The main advantage of using the probiotic yeast isolates instead of bacterial ones could be related to the prevention and treatment of antibiotic-associated diarrhea, since they are not affected by antibiotics.

CONCLUSION

The results obtained in this study demonstrate that dairy yeast isolates exhibit strain-specific probiotic potential, since they are able to survive simulated conditions of the intestinal tract, to colonize the intestine and there is a suggestion of their immunomodulatory activity. In particular, the strain *K. lactis* ZIM 2408 appears to be the best probiotic candidate studied due to its ability to survive under chemically simulated GIT conditions, its adherence to EIC and its immunosuppressive activity, and could be further investigated for specific probiotic applications. Hence, following the FAO/WHO criteria and EFSA recommendations, it is necessary to underline that the safety and health-promoting efficacy of particular yeast probiotic strains need to be further tested in pre-clinical trials.

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