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MINI-REVIEW

Probiotic *Saccharomyces cerevisiae* strains as biotherapeutic tools: is there room for improvement?

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Abstract The probiotic yeast *Saccharomyces cerevisiae* var *boulardii* is widely used as a low cost and efficient adjuvant against gastrointestinal tract disorders such as inflammatory bowel disease and treatment of several types of diarrhea, both in humans and animals. *S. boulardii* exerts its protective mechanisms by binding and neutralizing enteric pathogens or their toxins, by reducing inflammation and by inducing the secretion of sIgA. Although several *S. cerevisiae* strains have proven probiotic potential in both humans and animals, only *S. boulardii* is currently licensed for use in humans. Recently, some researchers started using *S. boulardii* as heterologous protein expression systems. Combined with their probiotic activity, the use of these strains as prophylactic and therapeutic proteins carriers might result in a positive combined effort to fight specific diseases. Here, we provide an overview of the current use of *S. cerevisiae* strains as probiotics and their mechanisms of action. We also discuss their potential to produce molecules with biotherapeutic application and the advantages and hurdles of this approach. Finally, we suggest future directions and alternatives for which the combined effort of specific immunomodulatory effects of probiotic *S. cerevisiae* strains and ability to express desired foreign genes would find a practical application.

Keywords Probiotics · *Saccharomyces cerevisiae* · *Saccharomyces cerevisiae* var *boulardii* · Mechanisms of action · Expression of heterologous proteins

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Introduction

Probiotics are defined by the World Health Organization (WHO) as "live microorganisms that can provide benefits to human [and animal] health when administered in adequate amounts, which confer a beneficial health effect on the host" (Vieira et al. 2013). *Saccharomyces cerevisiae* var *boulardii* (henceforth mentioned as *S. boulardii*), the only yeast approved as human probiotic, was first isolated in Indochina during a cholera epidemic, in 1923. Henri Boulard, a French scientist, whilst looking for novel *Saccharomyces* strains with laboratorial or industrial applications, noticed that some locals who drank a tea made of the tropical fruits mangosteen and lychee were refractory to the symptoms of the disease. Analyzing the skin of those fruits, he succeeded in isolating *S. boulardii*, which later proved to have probiotic properties (McFarland 2010). Although *S. boulardii* belongs to the same species of *S. cerevisiae*, it was found to possess a different

genetic profile, including chromosome IX trisomy and higher copy numbers of particular sets of genes, including some related with stress responses (Edwards-Ingram et al. 2004; Edwards-Ingram et al. 2007; Khatri et al. 2013). We also assessed recently that *S. boulardii* has approximately 150× less δ transposon sequences than *S. cerevisiae* s288c (Douradinha et al. 2014), probably due to the lack of haploid mitotic growth and sporulation processes in the former (Edwards-Ingram et al. 2004). The referred genetic differences probably account for the dissimilar metabolic and physiological properties shown by *S. boulardii* (Fietto et al. 2004; Khatri et al. 2013), as optimal growth temperature at 37 °C, the mammal host internal temperature, whilst most strains of *S. cerevisiae* prefer temperatures no higher than 30 °C (Fietto et al. 2004). Moreover, *S. boulardii* is able to survive the harsh environmental conditions found in the gastrointestinal tract (GIT), tolerating low pH in the stomach and the presence of bile salts and pancreatic proteases, both in vitro (Fietto et al. 2004) and in vivo (Rodrigues et al. 2000). Thus, both its survival fitness and probiotic potential are most probably related to the above-mentioned genetic differences (Edwards-Ingram et al. 2007).

Strains of *S. boulardii*, *S. cerevisiae*, and *Saccharomyces unisporus* have also been isolated from alcoholic beverages such as Brazilian cachaça (Martins et al. 2005), Ghanaian sorghum beer (Van der Aa Kühle et al. 2005) and Northern Caucasus kefir (Diosma et al. 2014), from Greek feta cheese (Kourelis et al. 2010), Italian sourdough bread (Perricone et al. 2014), and from children's feces (Kourelis et al. 2010). All these strains have demonstrated probiotic potential, as indicated by optimal growth temperature at 37 °C, resistance to GIT stresses, and other properties. Also, besides *S. boulardii*, other *S. cerevisiae* strains have been approved as animal feed additives and veterinary probiotics (Pérez-Sotelo et al. 2005; Ferraretto et al. 2012; Zanello et al. 2013).

Importantly, as a probiotic, *S. boulardii* is preferred over probiotic bacteria, such as *Lactococcus lactis*, due to its natural resistance to many antibiotics and to the fact that bacterial probiotics and intestinal microbiota can acquire genes which confer resistance to antibiotics and pass them on to bacterial pathogens (Temmerman et al. 2003; Salyers et al. 2004; Mathur and Singh 2005; Czerucka et al. 2007). Thus, this probiotic yeast is commonly used in both adults and children patients who suffer from diarrhea derived from continuous use of antibiotics (reviewed in Czerucka et al. 2007), especially those caused by *Clostridium difficile*, a bacterial pathogen which causes pseudomembranous colitis (reviewed in O'Horo et al. 2014), and to alleviate the secondary effects of the treatment against *Helicobacter pylori*, a gastritis-causing bacteria (reviewed in Girardin and Seidman 2011). *S. boulardii* is used to treat several types of diarrhea, such as infectious diarrhea (caused by pathogens such as *C. difficile* or rotavirus, which can result both in acute diarrhea in children and in traveler's (tourists) diarrhea), diarrhea derived from Acquired Immunodeficiency Syndrome (AIDS) complications, and diarrhea resulting from enteral tube feeding (Sazawal et al. 2006; Whelan 2007; reviewed in Czerucka et al. 2007; Girardin and Seidman 2011; O'Horo et al. 2014; Feizizadeh et al. 2014). *S. boulardii* is also used as an adjuvant in the treatment of irritable bowel syndrome (IBS), colitis and related malaises, and inflammatory bowel disease (IBD), as Crohn's disease (reviewed in Czerucka et al. 2007; Girardin and Seidman 2011; Soares 2014).

In this review, we give an overview of the available information concerning the mechanisms of action and genetic transformation of *S. cerevisiae* probiotic strains. We also provide suggestions of how those features could be combined to develop yeast strains with a therapeutic action against pathogens which affect mainly the gastrointestinal tract.

Mechanisms of action of probiotic *S. cerevisiae* strains

The mechanisms of action of probiotic *S. cerevisiae* yeasts have been elegantly described elsewhere (Czerucka et al. 2007; Pothoulakis 2009; McFarland 2010; Martins et al. 2013; Plaza-Diaz et al. 2014). Not surprisingly, and since S. *boulardii* has been the most widely probiotic yeast studied, its mechanisms of action against enteropathogens and disorders of the GIT are the most well described among all *S. cerevisiae* probiotic strains (Czerucka et al. 2007; Tiago et al. 2012). It has been demonstrated that *S. boulardii* binds and neutralizes pathogenic enteric bacteria (*C. difficile*, *Escherichia coli*, *Vibrio cholera*, *Salmonella*, and *Shigella* spp.) and bacterial toxins (*C. difficile*, *V. cholera*, and *E. coli*) (Brandão et al. 1998; Gedek 1999; Czerucka et al. 2007; Mumy et al. 2008; Martins et al. 2010; Tiago et al. 2012; Martins et al. 2013). *S. boulardii* also affect immune factors, inducing the secretion of sIgA, tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-5, IL-6, IL-10, and IL-12, as demonstrated both in vitro and in the colon of murine models (Rodrigues et al. 2000; Czerucka et al. 2007; Foligné et al. 2010; Kourelis et al. 2010; Smith et al. 2014), while downregulating IL-8 expression by acting in the NF-κB pathway in uninfected enterocytes (Sougioultzis et al. 2006; Martins et al. 2010) and also in MAPK and AP-1 pathways in *Salmonella enterica* serovar Typhimurium-infected enterocytes (Martins et al. 2010; Martins et al. 2013). *S. boulardii* also reduces the levels of pro-inflammatory cytokines IL-6 and TNF-α in models of colitis and infection with pathogenic *E. coli* (Dalmasso et al. 2006; Foligné et al. 2010; Plaza-Diaz et al. 2014). In addition, *S. boulardii* has trophic effects on enterocytes (reviewed in Buts and De Keyser 2006), inhibits pathogenic *E. coli*-mediated apoptosis of T84 colonic cell line in vitro (Dalmasso et al.

2006), and interferes with bacterial virulence factors (Wu et al. 2008).

S. cerevisiae UFMG A-905, isolated from the Brazilian distilled spirit cachaça made from sugarcane juice, also showed potential to be used as a probiotic (Martins et al. 2005), including a higher resistance to GIT simulated stresses than *S. boulardii* (Daniel Zamith-Miranda, manuscript in preparation). In mice treated with *S. cerevisiae* UFMG A-905 and later infected with *S.* Typhimurium and *C. difficile*, lower levels of inflammation were observed in the liver and colon, respectively (Martins et al. 2005). Treated mice also had a lower mortality rate than naïve controls challenged with *S.* Typhimurium (Martins et al. 2005; Martins et al. 2011). *S. cerevisiae* UFMG A-905 have shown adhesion to some enteropathogenic bacteria, such as *E. coli*, *S.* Typhi, and *S.* Typhimurium (Tiago et al. 2012), neutralizing the translocation of the latter from the GIT to the liver, spleen, and mesenteric lymph nodes (Martins et al. 2007). This probiotic yeast increased the production of IL-10 and sIgA in healthy mice (Martins et al. 2007; Generoso et al. 2010). In a mouse typhoid fever model, *S. cerevisiae* UFMG A-905 reduced both inflammation and IL-6, TNF-α, IFN-γ, and IL-10 levels, due to modulation of signaling pathways responsible for the expression of many inflammatory cytokines, such as NF-κB, AP-1, and MAPK pathways (Martins et al. 2011).

S. cerevisiae 982 was isolated from feta cheese and has immunomodulatory activity in the small intestine of mice, by stimulating the secretion of sIgA, TNF-α, IFN-γ, IL-5, IL-6, IL-10, and IL-12, dependent on toll-like receptors (TLR)-2, TLR-4, TLR-6, and TLR-9 expression (Kourelis et al. 2010). *S. cerevisiae* 982 appears to favor a Th1 response, while *S. boulardii* strain KK1, isolated from children feces, favors a Th2/Treg response, due to the inability of the latter to stimulate IFN-γ secretion and TLR-4 and TLR-9 expression (Kourelis et al. 2010). Another *S. cerevisiae* strain, LV02/CNCM I-3856, was able to induce secretion of TNF-α and IL-10 in human PBMCs in vitro and to decrease the level of serum IL-6 in a mouse colitis model (Foligné et al. 2010; Sivignon et al. 2015). In an in vitro model using porcine intestinal epithelial cell line (IPEC-1) and enterotoxigenic *E. coli*, *S. cerevisiae* LV02/CNCM I-3856 treatment inhibited the inflammatory mediators IL-6 and IL-8 induced by bacterial infection (Zanello et al. 2011). This probiotic strain modulated MAPK pathway and agglutinated *E. coli*, thus contributing to the anti-inflammatory phenotype observed. However, *S. cerevisiae* LV02/CNCM I-3856 did not prevent disruption of barrier integrity of IPEC-1 cell monolayer, indicating it does not act upon *E. coli* enterotoxins (Zanello et al. 2011). *S. cerevisiae* Sc47, a veterinary probiotic widely used in animal nutrition, also adheres to and neutralizes several *Salmonella* spp. (Pérez-Sotelo et al. 2005). Several *S. cerevisiae* strains isolated from kefir have shown potential to diminish inflammatory response in an intestinal epithelial cell model in vitro (Romanin et al. 2010).

Genetic engineering and heterologous protein expression in *S. boulardii*

To date, several *S. cerevisiae* laboratory strains have been genetically engineered to express proteins of interest that have a wide range of biotechnological applications, including biofuels, pharmaceutical and food industry, and models for eukaryotic cell mechanisms and physiology research (Schneiter 2004; Latorre-García et al. 2008; Ardiani et al. 2010; Da Silva and Srikrishnan 2012; Reis et al. 2012). Strains have been optimized and genetic tools have been developed and characterized with the ultimate goal of achieving efficient and low cost production of desired biomolecules. Most of the genetically engineered *S. cerevisiae* strains are haploid, since it is more difficult to genetically modify polyploid yeasts. Also, some genetic manipulations require auxotrophic mutants, i.e., strains lacking the ability to synthesize an organic molecule, which can be restored upon transformation with a plasmid encoding both the protein of interest to be expressed and the genetic sequence of such organic molecule. However, as suggested elsewhere, genetic engineering of polyploid industrial yeast strains presents the advantage of higher growth and metabolic rates (Latorre-García et al. 2008). Although probiotic *S. cerevisiae* strains have been widely studied for their probiotic potential, very few works focused on their genetic transformation. Interestingly, in all those works, *S. boulardii* was the chosen strain. Genetically modified probiotic *S. cerevisiae* strains able to express foreign proteins with biotherapeutic potential could act against pathogens or disorders of the GIT. These strains would have a synergistically combined probiotic and therapeutic effect and would also represent safer therapeutic protein carriers when compared to probiotic bacteria. Different probiotic bacteria induce diverse immune responses due to different pathogen-associated molecular patterns (PAMPs) in their surface and have defined and well-established genetic transformation tools (Owen et al. 2013). However, the risk of sharing genetic material carrying antibiotic resistance with pathogenic bacteria is very high and might lead to a multidrug resistant bacterial species (Temmerman et al. 2003; Salyers et al. 2004; Cummins and Ho 2005; Mathur and Singh 2005; Czerucka et al. 2007). Thus, different probiotic *S. cerevisiae* strains should be preferred as therapeutic protein carriers, since they also stimulate different types of immune responses as discussed above and will not exchange genetic material with other microorganisms.

To the best of our knowledge, the first successful attempt to transform a *S. boulardii* strain was done by Edwards-Ingram et al. (2007). They transformed *S. boulardii* UL with pAG26,

a plasmid developed for *S. cerevisiae* transformations, which contains the hygromycin resistance gene (Goldstein and McCusker 1999). The scope was to recover *S. boulardii* following its administration to mice and allow its isolation from other microorganisms from intestinal microbiota. Although the expression of protein was not directly assessed, the survival of *S. boulardii* in the medium containing hygromycin indicates the probiotic yeast successfully expressed the heterologous protein hygromycin B phosphotransferase, which confers resistance to the referred antibiotic (Gritz et al. 1983; Goldstein and McCusker 1999). Moreover, they were the first to demonstrate that viably transformed *S. boulardii* strains can be recovered after being administered to animal models. More recently, we were also able to transform *S. boulardii* MYA-796 from American Type Culture Collection (ATCC) with pYC440, a plasmid designed and optimized for *S. cerevisiae* expression, carrying also the hygromycin B resistance gene (Reis et al. 2012), obtaining mutant probiotic yeasts able to grow in the presence of this antibiotic (Douradinha et al. 2014).

Latorre-García and colleagues, while trying to obtain *Saccharomyces* strains overexpressing glucoamylase in order to produce ethanol from starch, successfully transformed *S. boulardii* UL strain (Latorre-García et al. 2008). They were able to transform several laboratory and industrial *Saccharomyces* strains with plasmids encoding the glucoamylase sequence, under a *S. cerevisiae* galactose/cytochrome c hybrid promoter, CYC-GAL, which induces protein expression once galactose is added to the growth media. The plasmid used to transform industrial *Saccharomyces* strains also encodes the *S. cerevisiae* 2μ, a yeast replicating sequence, and the geneticin (G418) resistance gene. Although *S. boulardii* UL (also designated *S. cerevisiae* Y111 by the authors) had shown a low transformation rate, it was the second highest out of the 10 industrial strains tested. Transformed *S. boulardii* UL also showed a high level of glucoamylase activity, indicating successful expression of an active and functional glucoamylase protein.

More recently, the ability to express molecules with biotherapeutic potential in *S. boulardii* was addressed (Pohlmann et al. 2013; Michael et al. 2013). Michael and colleagues successfully expressed mouse IL-10 through *S. boulardii* transformation with a plasmid encoding this interleukin sequence and using the promoter sequence of *S. cerevisiae* α-mating factor, the terminator sequence of the alcohol dehydrogenase 1 (ADH1), and resistance gene to G418 (aminoglycoside 3'-phosphotransferase) as selection marker (Michael et al. 2013). The aim was to use IL-10 expressing *S. boulardii* in a colitis mouse model, B6-*IL10*$^{-/-}$ (Kuhn et al. 1993), and evaluate its therapeutic action using noninvasive magnetic resonance imaging. IL-10 is an anti-inflammatory cytokine which would suppress the effector functions of regulatory T cells thus ameliorating the symptoms of colitis. Although the authors confirmed that IL-10 produced by transformed *S. boulardii* was functional in an in vitro assay, both transformed and untransformed probiotic yeast had the same levels of therapeutic effect in mice suffering from colitis. Since the observed effect could be derived from the instability of the IL-10 dimer in vivo conditions, the same authors expressed viral analogs of IL-10 in *S. boulardii* in the same conditions described above (Pohlmann et al. 2013). These IL-10 viral analogs, derived from Epstein-Barr virus (EBV) and human cytomegalovirus (HCMV), would be more stable than the mouse IL-10 and less prone to degradation by the host immune system, as a result of a viral adaptation to host molecules. Both viral analogs were successfully expressed in *S. boulardii* and their functionality confirmed by in vitro assays (Pohlmann et al. 2013), although no test in animal colitis or other conditions using these probiotic transformants was reported. In another work, Wang and colleagues successfully expressed antigens of an avian pathogen, *Eimeria tenella*, in the surface of *S. boulardii* (Wang et al. 2014). Using a surface display system based on the *S. boulardii* α-agglutinin proteins 1 and 2 (AGA1p and AGA2p, respectively), they obtained probiotic yeast transformants expressing *E. tenella* microneme-2 antigen (EtMIC2) and enhanced green fluorescent protein (EGFP) in the outer membrane of the yeasts. The *S. boulardii* strain used was derived from the French company Biocodex, and the plasmid contained *S. cerevisiae* glucose-induced promoter sequences of 3-phosphoglycerate kinase 1 (PGK1) and translational elongation factor 1 (TEF1) and *S. boulardii* AGA1p and AGA2p sequences. The vaccination potential of the EtMIC2 expressing probiotic yeasts in animal models was not reported.

The isolation of successfully transformed microorganisms is generally mediated by selective markers encoded by the plasmids, as performed by the aforementioned research groups. However, some transformation strategies require auxotrophic mutant strains, which lack the ability to synthesize an organic molecule, e.g., an amino acid, which can be restored upon transformation with a bicistronic plasmid encoding both a protein which will produce per turn that organic molecule and a desired foreign sequence to be expressed. So far, no natural auxotrophic mutants of *S. boulardii* have been reported and only recently auxotrophic strains of this probiotic yeast were obtained by UV mutagenesis (Hamedi et al. 2013; Hudson et al. 2014). Hamedi and colleagues elegantly described the first *URA3 S. boulardii* mutants (Hamedi et al. 2013), obtained by mutagenesis of the wild-type strain *lyo* derived from DiarSafe, produced by the British company Wren Laboratories, Ltd. The authors further transformed the ura3^{-} *S. boulardii* with a commercial plasmid pGEM-Teasy (Promega) where they inserted the *URA3* sequence of *S. boulardii*, thus restoring its ability to produce uracil. In vitro assays showed that the obtained mutants also conserved the probiotic abilities to resist the stresses found in the GIT, such as bile salts and acidic pH. In another independent work, Hudson and colleagues, using the

S. boulardii strain MYA-797 from ATCC, also obtained ura3^{-} mutants from the probiotic yeast and confirmed the results obtained by Hamedi and colleagues regarding stress resistance and complementation with an URA3 plasmid (Hudson et al. 2014). They also showed that these mutants could grow in anaerobic conditions, as they would face in GIT, and successfully transform them with a GFP sequence encoding plasmid, which was functionally expressed by ura3^{-} *S. boulardii* mutants, as determined by flow cytometry. Moreover, they demonstrated that viable and GFP-expressing ura3^{-} *S. boulardii* cells can be recovered after oral administration from mice Peyer's patches, lymphoid organs found in the small intestine.

A major concern shared by all potential platforms of biomedical and veterinary use is the toxicity levels and consequent nefarious effects (Gottardi and Douradinha 2013). As for any biotherapeutic approach, transformed probiotic yeasts should be subjected to extensive tests in order to confirm if transformation and expression of foreign genes would either lead to any level of toxicity or to the impairment of their probiotic potential. Initial tests in vitro and in animal models will indicate any potentially toxic side effects that would prevent moving forward to human trials. In fact, in some of the aforementioned works, transformed *S. boulardii* was administered to animal models and neither undesirable side effect nor loss of probiotic effects were reported (Edwards-Ingram et al. 2007; Michael et al. 2013; Hudson et al. 2014). Thus, human trials with transformed probiotic yeasts are most likely to occur with no setbacks.

Many transformation techniques have been developed and successfully used in *S. cerevisiae*: lithium acetate, electroporation, biolistic, the spheroplast, and the glass beads techniques (reviewed in Kawai et al. 2010). In the works referenced above, transformation of *S. boulardii* was only achieved with the lithium acetate protocol (Pohlmann et al. 2013; Michael et al. 2013; Douradinha et al. 2014; Hudson et al. 2014; Wang et al. 2014) and the electroporation approach (Hamedi et al. 2013; Hudson et al. 2014). It is presently unknown if the other referred transformation techniques would either be effective or if they would increase the transformation rate of probiotic *S. cerevisiae* strains. Also, transformation of probiotic *S. cerevisiae* strains other than *S. boulardii* has not yet been reported. Equally, transformation aiming integration in the genome of any of the aforementioned probiotic yeasts has also not been achieved at the present.

Probiotic *S. cerevisiae* strains available genetic information

The genomes of some probiotic *S. cerevisiae* strains have been made public recently and all have 95 % or higher homology with the non-probiotic *S. cerevisiae* strain s288c (Khatri et al. 2013; Batista et al. 2014; Douradinha et al. 2014). Since there are innumerous techniques, plasmids, and other DNA sequences optimized and used successfully in genetic manipulation of laboratorial non-probiotic *S. cerevisiae* strains (Da Silva and Srikrishnan 2012), the similarity among different strains at the genetic and protein level justifies the application of these genetic tools for probiotic *S. cerevisiae* transformation. In fact, most of the aforementioned works concerning genetic manipulation of *S. boulardii* used either genetic sequences fully (Latorre-García et al. 2008; Pohlmann et al. 2013; Michael et al. 2013; Douradinha et al. 2014; Hudson et al. 2014) or partially (Wang et al. 2014) derived from non-probiotic *S. cerevisiae* strains. Only two works used *S. boulardii* homologous sequences (Hamedi et al. 2013; Wang et al. 2014). The ideal approach to ensure efficient and successful genetic manipulation of the probiotic yeasts would be the use of genetic tools carrying promoters, transcription terminator, or other sequences derived from the target strain, as already pursued by some (Wang et al. 2014). It is now possible to use such approaches since the genomes of some *S. boulardii* strains are available online (Khatri et al. 2013; Batista et al. 2014; Douradinha et al. 2014) and the genome of the probiotic strain *S. cerevisiae* UFMG A-905 has just been made accessible (Thiago Mafra Batista, personal communication).

It is noteworthy to mention that we have identified differences between *S. cerevisiae* s288c and *S. boulardii* potential genetic tools, such as the low level of δ transposon sequences and resistance to canavanine, both in the former (Douradinha et al. 2014). However, as mentioned above, *S. boulardii* exhibits higher copy numbers of some genes and aneuploidy. Thus, the differences observed concerning δ transposon sequences number and resistance to canavanine might derive from these genetic dissimilarities and probably are present in other likewise aneuploid *S. cerevisiae* strains, regardless or not if they possess probiotic potential.

Future directions

S. cerevisiae genetic manipulations have helped us to unravel many of the physiological and metabolic processes of the eukaryotic cell, and have been routinely use to produce molecules of biotechnological relevance (Ardiani et al. 2010; Da Silva and Srikrishnan 2012; Reis et al. 2012). More recently, transformation of *S. boulardii* showed it is possible to use this probiotic yeast to also produce molecules with industrial (Latorre-García et al. 2008) and biotherapeutic potential (Pohlmann et al. 2013; Michael et al. 2013; Wang et al. 2014). For instance, probiotic strains could be engineered to express antigens for specific intestinal disorders, such as mucin MUC-1 for colon cancer (Byrd and Bresalier 2004) or adhesion protein intimin for pathogenic *E. coli* O157:H7 (Gedek 1999; Oliveira et al. 2012). *S. cerevisiae* strains with

proven probiotic potential could be used as delivery agents of desired biomolecules: they can better withstand the host temperature of 37 °C and stresses found in the GIT (Fietto et al. 2004; Martins et al. 2005; Van der Aa Kühle et al. 2005; Diosma et al. 2014; Perricone et al. 2014) and present immunomodulatory properties (Martins et al. 2007; Czerucka et al. 2007; Foligné et al. 2010; Generoso et al. 2010; Romanin et al. 2010; Kourelis et al. 2010; Martins et al. 2011; Zanello et al. 2013; Martins et al. 2013; Smith et al. 2014; Plaza-Diaz et al. 2014). Interestingly, some of the probiotic *S. cerevisiae* strains elicit different immune responses (Kourelis et al. 2010). Such potential could be used to transform certain strains with desired therapeutic molecules and use their immunomodulation potential to favor an immune response which would be more beneficial to the particular pathology addressed. However, as referred above, to take full advantage of the potential of these yeasts as delivery vectors for such biomolecules, genetic tools and strategies should be adapted to the *S. cerevisiae* strain used. Due to the growing interest in using *S. boulardii* as an expression vector for molecules with biotechnological potential, we have no doubt that studies using other *S. cerevisiae* probiotic strains will be pursued and, consequently, the genetic information made available. We believe that probiotic *S. cerevisiae* strains have the potential to become effective delivery agents, especially for malaises occurring at the GIT level.

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Conflict of interest The authors declare that they have no competing interests.

References

Ardiani A, Higgins JP, Hodge JW (2010) Vaccines based on whole recombinant *Saccharomyces cerevisiae* cells. FEMS Yeast Res 10: 1060–1069

Batista TM, Marques ETA, Franco GR, Douradinha B (2014) Draft genome sequence of the probiotic yeast *Saccharomyces cerevisiae* var *boulardii* strain ATCC MYA-796. Genome Announc 2:e01345–14

Brandão RL, Castro IM, Bambirra EA, Amaral C, Fietto LG, Tropia MJM, José M, Dos Santos RG, Gomes NCM, Nicoli R, Amaral SC, Jose M (1998) Intracellular signal triggered by cholera toxin in *Saccharomyces boulardii* and *Saccharomyces cerevisiae*. Appl Env Microbiol 64:564–568

Buts J-P, De Keyser N (2006) Effects of *Saccharomyces boulardii* on intestinal mucosa. Dig Dis Sci 51:1485–1492. doi:10.1007/s10620-005-9016-x

Byrd J, Bresalier R (2004) Mucins and mucin binding proteins in colorectal cancer. Cancer Metastasis Rev 23:77–99

Cummins J, Ho M-W (2005) Genetically modified probiotics should be banned. Microb Ecol Heal Dis 17:66–68. doi:10.1080/08910600510044480

Czerucka D, Piche T, Rampal P (2007) Review article: yeast as probiotics—*Saccharomyces boulardii*. Aliment Pharmacol Ther 26:767–778

Da Silva NA, Srikrishnan S (2012) Introduction and expression of genes for metabolic engineering applications in *Saccharomyces cerevisiae*. FEMS Yeast Res 12:197–214

Dalmasso G, Loubat A, Dahan S, Calle G, Rampal P, Czerucka D (2006) *Saccharomyces boulardii* prevents TNF-alpha-induced apoptosis in EHEC-infected T84 cells. Res Microbiol 157:456–465. doi:10.1016/j.resmic.2005.11.007

Diosma G, Romanin DE, Rey-Burusco MF, Londero A, Garrote GL (2014) Yeasts from kefir grains: isolation, identification, and probiotic characterization. World J Microbiol Biotechnol 30:43–53. doi:10.1007/s11274-013-1419-9

Douradinha B, Reis VCB, Rogers MB, Torres FAG, Evans JD, Marques ETA (2014) Novel insights in genetic transformation of the probiotic yeast *Saccharomyces boulardii*. Bioengineered 5:1–9

Edwards-Ingram LC, Gent ME, Hoyle DC, Hayes A, Stateva LI, Oliver SG (2004) Comparative genomic hybridization provides new insights into the molecular taxonomy of the *Saccharomyces* sensu stricto complex. Genome Res 14:1043–1051

Edwards-Ingram L, Gitsham P, Burton N, Warhurst G, Clarke I, Hoyle D, Oliver SG, Stateva L (2007) Genotypic and physiological characterization of *Saccharomyces boulardii*, the probiotic strain of *Saccharomyces cerevisiae*. Appl Env Microbiol 73:2458–2467

Feizizadeh S, Salehi-Abargouei A, Akbari V (2014) Efficacy and safety of *Saccharomyces boulardii* for acute diarrhea. Pediatrics 134:e176–e191. doi:10.1542/peds.2013-3950

Ferraretto LF, Shaver RD, Bertics SJ (2012) Effect of dietary supplementation with live-cell yeast at two dosages on lactation performance, ruminal fermentation, and total-tract nutrient digestibility in dairy cows. J Dairy Sci 95:4017–4028. doi:10.3168/jds.2011-5190

Fietto JLR, Araújo RS, Valadão FN, Fietto LG, Brandão RL, Neves MJ, Gomes FCO, Nicoli JR, Castro IM (2004) Molecular and physiological comparisons between *Saccharomyces cerevisiae* and *Saccharomyces boulardii*. Can J Microbiol 621:615–621

Foligné B, Dewulf J, Vandekerckove P, Pignède G, Pot B (2010) Probiotic yeasts: anti-inflammatory potential of various non-pathogenic strains in experimental colitis in mice. World J Gastroenterol 16:2134. doi:10.3748/wjg.v16.i17.2134

Gedek BR (1999) Adherence of *Escherichia coli* serogroup 0 157 and the *Salmonella Typhimurium* mutant DT 104 to the surface of *Saccharomyces boulardii*. Mycoses 42:261–264

Generoso SV, Viana M, Santos R, Martins FS, Machado JAN, Arantes RME, Nicoli JR, Correia MITD, Cardoso VN (2010) *Saccharomyces cerevisiae* strain UFMG 905 protects against bacterial translocation, preserves gut barrier integrity and stimulates the immune system in a murine intestinal obstruction model. Arch Microbiol 192:477–484

Girardin M, Seidman EG (2011) Indications for the use of probiotics in gastrointestinal diseases. Dig Dis 29:574–587. doi:10.1159/000332980

Goldstein AL, McCusker JH (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast 15: 1541–1553

Gottardi R, Douradinha B (2013) Carbon nanotubes as a novel tool for vaccination against infectious diseases and cancer. J Nanobiotechnol 11:30. doi:10.1186/1477-3155-11-30

Gritz L, Davies J, Biogen SA, Acacias R (1983) Plasmid-encoded hygromycin B resistance: the sequence of hygromycin B

phosphotransferase gene and its expression in *Escherichia coli* and *Saccharomyces cerevisiae*. Gene 25:179–188

Hamedi H, Misaghi A, Modarressi MH, Salehi TZ, Khorasanizadeh D, Khalaj V (2013) Generation of a uracil auxotroph strain of the probiotic yeast *Saccharomyces boulardii* as a host for the recombinant protein production. Avicenna J Med Biotechnol 5:29–34

Hudson LE, Fasken MB, McDermott CD, McBride SM, Kuiper EG, Guiliano DB, Corbett AH, Lamb TJ (2014) Functional heterologous protein expression by genetically engineered probiotic yeast *Saccharomyces boulardii*. PLoS One 9, e112660

Kawai S, Hashimoto W, Murata K (2010) Transformation of *Saccharomyces cerevisiae* and other fungi: methods and possible underlying mechanism. Bioeng Bugs 1:395–403

Khatri I, Akhtar A, Kaur K, Tomar R, Prasad GS, Ramya TNC, Subramanian S (2013) Gleaning evolutionary insights from the genome sequence of a probiotic yeast *Saccharomyces boulardii*. Gut Pathog 5:30. doi:10.1186/1757-4749-5-30

Kourelis A, Kotzamanidis C, Litopoulou-Tzanetaki E, Papaconstantinou J, Tzanetakis N, Yiangou M (2010) Immunostimulatory activity of potential probiotic yeast strains in the dorsal air pouch system and the gut mucosa. J Appl Microbiol 109:260–271. doi:10.1111/j.1365-2672.2009.04651.x

Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75:263–274

Latorre-García L, Adam AC, Polaina J (2008) Overexpression of the glucoamylase-encoding STA1 gene of *Saccharomyces cerevisiae* var. *diastaticus* in laboratory and industrial strains of *Saccharomyces*. World J Microbiol Biotechnol 24:2957–2963

Martins FS, Nardi RMD, Arantes RME, Rosa CA, Neves MJ, Nicoli JR (2005) 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 51:83–92

Martins FS, Rodrigues ACP, Tiago FCP, Penna FJ, Rosa CA, Arantes RME, Nardi RMD, Neves MJ, Nicoli JR (2007) *Saccharomyces cerevisiae* strain 905 reduces the translocation of *Salmonella enterica* serotype Typhimurium and stimulates the immune system in gnotobiotic and conventional mice. J Med Microbiol 56:352–359

Martins FS, Dalmasso G, Arantes RME, Doye A, Lemichez E, Lagadec P, Imbert V, Peyron J-F, Rampal P, Nicoli JR, Czerucka D (2010) Interaction of *Saccharomyces boulardii* with *Salmonella enterica* serovar Typhimurium protects mice and modifies T84 cell response to the infection. PLoS One 5, e8925

Martins FS, Elian SDA, Vieira AT, Tiago FCP, Martins AKS, Silva FCP, Souza ELS, Sousa LP, Araújo HRC, Pimenta PF, Bonjardim CA, Arantes RME, Teixeira MM, Nicoli JR (2011) 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 301:359–364

Martins FS, Vieira AT, Elian SDA, Arantes RME, Tiago FCP, Sousa LP, Araújo HRC, Pimenta PF, Bonjardim CA, Nicoli JR, Teixeira MM (2013) Inhibition of tissue inflammation and bacterial translocation as one of the protective mechanisms of *Saccharomyces boulardii* against *Salmonella* infection in mice. Microbes Infect 15:270–279

Mathur S, Singh R (2005) Antibiotic resistance in food lactic acid bacteria—a review. Int J Food Microbiol 105:281–295. doi:10.1016/j.ijfoodmicro.2005.03.008

McFarland LV (2010) Systematic review and meta-analysis of *Saccharomyces boulardii* in adult patients. World J Gastroenterol 16:2202. doi:10.3748/wjg.v16.i18.2202

Michael S, Keubler LM, Smoczek A, Meier M, Gunzer F, Pöhlmann C, Krause-Buchholz U, Hedrich H-J, Bleich A (2013) Quantitative phenotyping of inflammatory bowel disease in the IL-10-deficient mouse by use of noninvasive magnetic resonance imaging. Inflamm Bowel Dis 19:185–193

Mumy KL, Chen X, Kelly CP, McCormick BA (2008) *Saccharomyces boulardii* interferes with *Shigella* pathogenesis by postinvasion signaling events. Am J Physiol Gastrointest Liver Physiol 294:G599–G609. doi:10.1152/ajpgi.00391.2007

O'Horo JC, Jindai K, Kunzer B, Safdar N (2014) Treatment of recurrent *Clostridium difficile* infection: a systematic review. Infection 42:43–59. doi:10.1007/s15010-013-0496-x

Oliveira AF, Cardoso SA, Almeida FBDR, de Oliveira LL, Pitondo-Silva A, Soares SG, Hanna ES (2012) Oral immunization with attenuated *Salmonella* vaccine expressing *Escherichia coli* O157:H7 intimin gamma triggers both systemic and mucosal humoral immunity in mice. Microbiol Immunol 56:513–522

Owen JL, Sahay B, Mohamadzadeh M (2013) New generation of oral mucosal vaccines targeting dendritic cells. Curr Opin Chem Biol 17:918–924. doi:10.1016/j.cbpa.2013.06.013

Pérez-Sotelo LS, Talavera-Rojas M, Monroy-Salazar HG, Cuarón-Ibargüengoytia JA, Montes R, Jiménez DO, Vázquez-Chagoyán JC (2005) In vitro evaluation of the binding capacity of *Saccharomyces cerevisiae* Sc47 to adhere to the wall of *Salmonella* spp. Rev Latinoam Microbiol 47:70–75

Perricone M, Bevilacqua A, Corbo MR, Sinigaglia M (2014) Technological characterization and probiotic traits of yeasts isolated from Altamura sourdough to select promising microorganisms as functional starter cultures for cereal-based products. Food Microbiol 38:26–35. doi:10.1016/j.fm.2013.08.006

Plaza-Diaz J, Gomez-Llorente C, Fontana L, Gil A (2014) Modulation of immunity and inflammatory gene expression in the gut, in inflammatory diseases of the gut and in the liver by probiotics. World J Gastroenterol 20:15632–15649. doi:10.3748/wjg.v20.i42.15632

Pohlmann C, Thomas M, Forster S, Brandt M, Hartmann M, Bleich A, Gunzer F (2013) Use of engineered intestinal microorganisms as in situ cytokine delivery system. Bioengineered 4:1–8

Pothoulakis C (2009) Review article: anti-inflammatory mechanisms of action of *Saccharomyces boulardii*. Aliment Pharmacol Ther 30:826–833. doi:10.1111/j.1365-2036.2009.04102.x

Reis VCB, Nicola AM, de Souza Oliveira Neto O, Batista VDF, de Moraes LMP, Torres FAG (2012) Genetic characterization and construction of an auxotrophic strain of *Saccharomyces cerevisiae* JP1, a Brazilian industrial yeast strain for bioethanol production. J Ind Microbiol Biotechnol 39:1673–1683

Rodrigues ACP, Cara DC, Cunha FQ, Vieira EC, Nicoli JR, Vieira LQ (2000) *Saccharomyces boulardii* stimulates sIgA production and the phagocytic system of gnotobiotic mice. J Appl Microbiol 89:404–414

Romanin D, Serradell M, González Maciel D, Lausada N, Garrote GL, Rumbo M (2010) Down-regulation of intestinal epithelial innate response by probiotic yeasts isolated from kefir. Int J Food Microbiol 140:102–108. doi:10.1016/j.ijfoodmicro.2010.04.014

Salyers AA, Gupta A, Wang Y (2004) Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol 12:412–416. doi:10.1016/j.tim.2004.07.004

Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE (2006) Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. Lancet Infect Dis 6:374–382. doi:10.1016/S1473-3099(06)70495-9

Schneiter R (2004) Genetics, molecular and cell biology of yeast. Université di Fribourg Suisse, Fribourg

Sivignon A, de Vallée A, Barnich N, Denizot J, Darcha C, Pignède G, Vandekerckove P, Darfeuille-Michaud A (2015) *Saccharomyces cerevisiae* CNCM I-3856 prevents colitis induced by AIEC bacteria in the transgenic mouse model mimicking Crohn's disease. Inflamm Bowel Dis 21:276–286

Smith IM, Christensen JE, Arneborg N, Jespersen L (2014) Yeast modulation of human dendritic cell cytokine secretion: an in vitro study. PloS Onene 9, e96595. doi:10.1371/journal.pone.0096595

Soares RL (2014) Irritable bowel syndrome: a clinical review. World J Gastroenterol 20:12144. doi:10.3748/wjg.v20.i34.12144

Sougioultzis S, Simeonidis S, Bhaskar KR, Chen X, Anton PM, Keates S, Pothoulakis C, Kelly CP (2006) *Saccharomyces boulardii* produces a soluble anti-inflammatory factor that inhibits NF-kappaB-mediated IL-8 gene expression. Biochem Biophys Res Commun 343:69–76. doi:10.1016/j.bbrc.2006.02.080

Temmerman R, Pot B, Huys G, Swings J (2003) Identification and antibiotic susceptibility of bacterial isolates from probiotic products. Int J Food Microbiol 81:1–10

Tiago FCP, Martins FS, Souza ELS, Pimenta PFP, Araujo HRC, Castro IM, Brandão RL, Nicoli JR (2012) Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by *Saccharomyces* probiotics. J Med Microbiol 61:1194–1207

Van der Aa Kühle A, Skovgaard K, Jespersen L (2005) In vitro screening of probiotic properties of *Saccharomyces cerevisiae* var. *boulardii* and food-borne *Saccharomyces cerevisiae* strains. Int J Food Microbiol 101:29–39. doi:10.1016/j.ijfoodmicro.2004.10.039

Vieira AT, Teixeira MM, Martins FS (2013) The role of probiotics and prebiotics in inducing gut immunity. Front Immunol 4:445. doi:10.3389/fimmu.2013.00445

Wang T, Sun H, Zhang J, Liu Q, Wang L, Chen P, Wang F, Li H, Xiao Y, Zhao X (2014) The establishment of *Saccharomyces boulardii* surface display system using a single expression vector. Fungal Genet Biol 64:1–10

Whelan K (2007) Enteral-tube-feeding diarrhoea: manipulating the colonic microbiota with probiotics and prebiotics. Proc Nutr Soc 66: 299–306. doi:10.1017/S0029665107005551

Wu X, Vallance BA, Boyer L, Bergstrom KSB, Walker J, Madsen K, O'Kusky JR, Buchan AM, Jacobson K (2008) *Saccharomyces boulardii* ameliorates *Citrobacter rodentium*-induced colitis through actions on bacterial virulence factors. Am J Physiol Gastrointest Liver Physiol 294:G295–G306

Zanello G, Berri M, Dupont J, Sizaret P-Y, D'Inca R, Salmon H, Meurens F (2011) *Saccharomyces cerevisiae* modulates immune gene expressions and inhibits ETEC-mediated ERK1/2 and p38 signaling pathways in intestinal epithelial cells. PLoS One 6, e18573. doi:10.1371/journal.pone.0018573

Zanello G, Melo S, Berri M, Inca RD, Auclair E, Salmon H (2013) Effects of dietary yeast strains on immunoglobulin in colostrum and milk of sows. Vet Immunol Immunopathol 152:20–27