Review

Probiotic yeasts: a developing reality?

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Review

Probiotic Yeasts: A Developing Reality?

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Abstract: Yeasts are gaining increasing attention for their potential health benefits as probiotics in recent years. Researchers are actively searching for new yeast strains with probiotic properties (i.e, *Debaryomyces hansenii*; *Kluyveromyces marxianus*; *Yarrowia lipolytica*; *Pichia hudriavzevii*; *Torulaspora delbrueckii*) from various sources, including traditional fermented foods, the human gut, and the environment. This exploration is expanding the pool of potential probiotic yeasts beyond the well-studied *Saccharomyces boulardii*. Research suggests that specific yeast strains possess properties that could be beneficial for managing conditions like inflammatory bowel disease, irritable bowel syndrome, skin disorders and allergy. Additionally, probiotic yeasts may compete with pathogenic bacteria for adhesion sites and nutrients, thereby inhibiting their growth and colonization. They might also produce antimicrobial compounds that directly eliminate harmful bacteria. To achieve these goals, the approach that uses probiotics for human health is changing. Next-generation yeast probiotics are emerging as a powerful new approach in the field of live biotherapeutics. By using genetic engineering, scientists are able to give these tools specialized capabilities. However, most research on these probiotic yeasts is still in its early stages, and more clinical trials are needed to confirm their efficacy and safety for various health conditions. This review could provide a brief overview of the situation in this field.

Keywords: yeast probiotics; promising benefit; probiotic development; genetic engineering

1. Introduction

Probiotics are live bacteria that are good for human and animal health, especially the digestive system, and can help restore the proper balance of microorganisms in the gut. Because they support gut health, these microbes are frequently referred to as "good" or "helpful" microorganisms. For a long time, the discussion around probiotics has centered on lactic acid bacteria (LAB) like *Lactobacillus* and *Bifidobacterium*. These bacteria have been extensively studied and documented for their role in promoting a healthy gut microbiome, offering benefits like improved digestion, immune function, and even mental health [1,2]. However, a new frontier was emerging in the world of probiotics – the rise of probiotic yeasts. Many studies suggested that certain yeast strains hold significant potential to join the ranks of established probiotic powerhouses [3].

2. Beyond Bacteria: Unveiling the Potential of Yeasts

In the last few decades, researchers have begun to explore the potential of yeast strains which have shown particular promise in supporting gut health [2]. Even though LAB will continue to play a significant role, these interesting new opportunities seem to have a positive impact on the future of gut health. Yeasts have been gaining increasing attention as potential probiotic candidates in recent years. While bacteria have dominated the probiotic market for a long time, research suggests that specific yeast strains possess properties that could be beneficial for human health [3]

Probiotic yeasts were generating excitement because they have interesting characteristics from an application point of view. 1) *Unique Advantages*: some probiotic yeasts might offer advantages over LAB in terms of survival. Studies suggest that certain yeast strains may be more resilient to the hostile environment of the stomach, reaching the intestines in higher numbers where they can exert their probiotic effects [2]. 2) *Diverse Functionality*: early research indicates that probiotic yeasts might have a broader range of functionality compared to some LAB strains. For example, some yeasts may produce compounds that not only benefit their own growth but also stimulate the growth of other

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beneficial bacteria in the gut [2]. This synergistic effect could lead to a more efficient and diverse gut microbiome. 3) *Appropriated Applications*: the discovery of various probiotic yeast strains opens doors for the development of more targeted probiotic interventions. Different yeast strains might be effective for addressing specific gut health concerns, offering a more personalized approach to probiotic supplementation [4].

3. *Saccharomyces boulardii*: A Pioneering Probiotic Yeast

As is known, *Saccharomyces cerevisiae* var. *boulardii* (often simply referred to as *S. boulardii*) has received the most scientific attention and is traditionally recognized as the probiotic yeast par excellence [3,5,6]. *S. boulardii* has been studied more extensively than other probiotic yeast strains. Research suggests that this yeast generally appears to be safe for most healthy individuals, and it may be effective in addressing issues like antibiotic-associated diarrhea, traveler's diarrhea, acute infectious diarrhea, and even inflammatory bowel disease [5,7]. This is crucial for establishing a probiotic strain as a viable option for widespread use. Recent advancements in genetic engineering techniques are opening up possibilities for developing next-generation probiotic yeasts with enhanced probiotic properties or targeted functionalities [8,9].

4. Probiotic Yeast Effects

Understanding how yeast strains exert their probiotic effects is critical for selecting the most beneficial ones and maximizing their potential health impact.

This includes investigating their interaction with gut microbiota, immune modulation capabilities, and potential for improving gut health [10,11].

4.1. Interactions with Gut Microbiota

Probiotic yeasts may compete with pathogenic bacteria for adhesion sites and nutrients, thereby inhibiting their growth and colonization. Additionally, they might produce antimicrobial compounds that directly eliminate harmful bacteria [8,12,13].

The specific types of compounds identified so far can be categorized into two main groups:

Killer toxins (or zymocins): these are proteinaceous compounds produced by certain probiotic yeast strains, particularly those belonging to *S. cerevisiae*. Killer toxins target specific receptors on other yeast and fungal cells, creating pores that lead to cell death [14].

Mycocins: these are another group of antimicrobial compounds produced by some probiotic yeasts. Mycocins are extracellular proteins with a broader target range, meaning they can inhibit β-glucan synthesis, affecting the growth of other yeasts and moulds [15]. In addition, mycocins produced by *Wickerhamomyces anomalus* (*Ascomycota, Saccharomycetes, Phaffomycetaceae*) exert antimicrobial activity against Gramnegative bacteria such *Acinetobacter baumannii* [16] and *Klebsiella pneumoniae* [17] and Grampositive bacteria, such as *Staphylococcus aureus* [18].

The exact mechanisms by which mycocins work are still being elucidated, but they are believed to disrupt the cell membranes of target organisms [19]. In addition, mycocins are studied in the development of vaccines and used as epidemiological markers [15].

It is crucial to remember that investigations are still being conducted to fully define and describe the range of antimicrobial substances that probiotic yeasts release. Additionally, the effectiveness of these compounds can vary depending on the specific yeast strain and the target organism.

Probiotic yeasts can interact synergistically with beneficial gut bacteria, promoting their growth and activity. Probiotic yeasts have the ability to work in concert with good gut bacteria to enhance their development and activity. Improved nutritional absorption may result from increased nutrient degradation. Additionally, this "teamwork" stimulates the synthesis of beneficial metabolites like short-chain fatty acids, which support gut health by nourishing the intestinal lining. [13,19–21].

Moreover, probiotic yeasts may influence the composition and diversity of gut microbiota, potentially enriching beneficial populations and reducing the abundance of harmful ones [19]. This shift in the microbial balance can significantly improve gut health and immune function [20,21].

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4.2. Immune Modulation Capabilities

Probiotic yeasts play a multifaceted role in regulating the immune system within the gut. Beyond their competition with harmful bacteria, they interact with immune cells like macrophages and dendritic cells residing in the gut lining [22–24]. This interaction stimulates the production of cytokines and other immune mediators, such as interleukin (IL)-10 and interferon (IFN)-γ, that enhance the body's overall defense mechanisms against invading pathogens [22,25].

In addition, probiotic yeasts may modulate the activity of immune cells involved in chronic inflammatory processes, potentially alleviating symptoms associated with inflammatory bowel disease (IBD) and other inflammatory gut disorders [20]. Studies suggest that probiotic yeasts can downregulate the production of pro-inflammatory cytokines like IL-1β and tumor necrosis factor-α (TNF-α), while promoting the production of anti-inflammatory mediators like IL-10 [22,25].

Probiotic yeasts seem to be also involved in allergy prevention. Early life exposure to certain *S.cerevisiae* strains might play a role in preventing food allergies and asthma in mice by influencing the development of the immune system [26,27]. Studies in animal models suggest that probiotic yeasts can regulate the balance between Th1 and Th2 immune responses, potentially turning the immune system towards a less allergic state [28]. However, more human trials are needed to confirm this benefit.

4.3. Overall Impact on Gut Health

Probiotic yeasts extend their influence beyond immune modulation, impacting various aspects of gut health. An important function by probiotic yeasts is that they can enhance the gut barrier, a complex system that acts as a physical and functional layer that protects the body from harmful substances and pathogens [29]. This can improve gut health and reduce the risk of infections. Probiotic yeasts can strengthen this barrier in several ways: by stimulating the production of tight junction proteins, which are essential for maintaining a strong and impermeable gut lining [30]. This tighter barrier reduces the risk of harmful substances and pathogens leaking from the gut into the bloodstream. Probiotic yeasts might promote the production of mucus by goblet cells in the gut lining [31]. This mucus layer acts as a lubricating and protective barrier, further enhancing gut defense mechanisms. Studies suggest that probiotic yeasts might be beneficial in preventing or alleviating symptoms of various gastrointestinal disorders, including diarrhea, constipation, and inflammatory bowel disease. Probiotic yeasts, specifically strains of *S. boulardii*, have demonstrated potential in mitigating the duration and intensity of diarrhea associated with antibiotic use [32–34]. They may also be beneficial in managing traveler's diarrhea [35] and might improve bowel movement frequency in individuals with constipation [36]. As was previously mentioned, probiotic yeasts have the ability to regulate immune responses, which may lessen the inflammation linked to inflammatory bowel disease (IBD) [37]. Nevertheless, further investigation is required to ascertain their efficacy in the management of IBD.

By breaking down complex carbohydrates (sugars) that our bodies might find difficult to digest on their own, like those found in grains and legumes, and by producing digestive enzymes like lactase and β-glucosidases, probiotic yeasts can improve nutrient absorption and contribute to efficient digestion [3,21,38,39]. Moreover, probiotic yeasts ferment dietary fibers in the gut, leading to the production of beneficial **short-chain fatty acids (**SCFAs) like butyrate, acetate, and propionate [40]. These SCFAs serve as an energy source for gut cells, promoting gut lining health and nutrient absorption [41].

By thoroughly investigating these areas of functional characterization, researchers can gain valuable insights into the specific mechanisms by which different yeast strains exert their probiotic effects. This knowledge is essential for selecting the most promising candidates with unique functionalities for further development and ensuring their targeted application for optimal gut health benefits.

5. Yeast "New" Strains as Probiotics: Myth or Reality?

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While research into probiotic yeasts is ongoing, there are not any definitively established "new" probiotic yeast strains yet [3]. However, the exciting part is that researchers are looking beyond *S. boulardii*. They are investigating the potential of various yeast genera like *Debaryomyces*, *Kluyveromyces*, *Yarrowia*, *Pichia*, and *Torulaspora*, isolated from fermented foods, traditional beverage, human microbiota, and natural sources [3,42–44].

These “new” probiotic yeast strains might offer a wider range of functionalities compared to existing probiotic options. They could potentially target specific gut health concerns or work synergistically with bacteria to enhance overall gut health [3].

Isolated from a variety of fermented foods and natural sources, strains of *Debaryomyces* yeast have been shown to have potential benefits, including tolerance to bile and stomach acid, which enables them to enter the intestines, and the generation of compounds that support gut microbiota [3]. Though intriguing, *Debaryomyces* as a validated probiotic is still in its early stages because it has not been as well researched as more well-known probiotics like *S. boulardii*.

To validate its particular health benefits in humans, more research is required [3]. So, what is real and what is myth? As of right moment, neither. Probiotics like *Debaryomyces* strains have potential, but further study is required to make that potential a reality. Furthermore, distinct *Debaryomyces* strains may have differing probiotic capabilities, similar to bacteria. Finding the most effective strains is therefore essential [3].

Ochangco *et al*. [45] investigated the potential probiotic benefits of various *D. hansenii* strains that were isolated from cheese and fish gut. The results indicated that the various traits of each strain might result in a range of probiotic effects. Although none of the strains exhibited the same level of stress resistance as *S. boulardii* strains, one strain survived well in the hostile gastric environment. Compared to *S. boulardii*, two strains produced a stronger anti-inflammatory response in immune cells, but one strain distinguished out as the most promising probiotic candidate because of its ability to adhere to gut cells, survive in hostile conditions, and produce anti-inflammatory effects [45].

Kluyveromyces, *Yarrowia*, and *Torulaspora* are all exciting possibilities in the realm of new probiotic yeasts, but like *Debaryomyces*, they are still in the early stages of exploration.

Strains of *Kluyveromyces*, in particular *K. marxianus*, have been isolated from fermented foods, including kefir grain, fermented traditional dairy products, sewage from sugar businesses, and natural environments, such as plants and sisal leaves, showing promise for probiotic applications [3,46]. Early research suggests that *K. marxianus* produces a broad range of distinct metabolites that may be useful to the food and biotechnology sectors and that this yeast might be tolerant to stomach acid and bile, allowing it to survive the digestive tract and reach the intestines. [3,46].

Similar to *Debaryomyces*, *Kluyveromyces* strains have not been extensively studied in humans. However, early research suggested that *K. marxianus* has potential benefits for gut health and immune function [47]. Recently, Nag *et al*. [48] observed in vitro that this yeast improved insulin sensitivity and reduced fat storage in fat cells, suggesting benefits for type 2 diabetes and obesity. Furthermore, this yeast showed cytotoxicity against colon cancer cells, suggesting anti-tumoral activity. Hence, these authors suggested that *K. marxianus* could have therapeutic potential. However, more research is needed to confirm their specific health benefits and identify the most effective strains [3,47,48].

Yarrowia is a relatively new genus being explored for potential probiotic properties [49]. Research for probiotic applications is even more limited compared to *Kluyveromyces*. Extensive investigation is needed to understand their safety and efficacy in humans [3]. For the past 20 years, the yeast *Y. lipolytica* has been used in industry to produce docosahexaenoic acid and eicosapentaenoic acid while adhering to good manufacturing procedures [50]. It has drawn notice recently for innovative biotechnological uses, like as animal feed addition with functional properties. The productive and immunological characteristics of the animals given *Y. lipolytica* were improved, and their microbioma, fatty acid content, and biochemical profiles were also enhanced [51]. Some strains might possess characteristics like adhesion to gut lining and antimicrobial activity against harmful gut pathogens [49,52]. Research has shown that this yeast is probiotic and beneficial to fish, birds, mammals, crabs, and mollusks. It is also harmless [51,53–55].

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Pichia sp. This genus has a probiotic potential among non-*Saccharomyces* yeasts [56,57]. *Pichia* probiotics are mostly obtained from food fermentation and have been shown to be able to thrive in gastrointestinal tracts. In addition to its probiotic functions as an antioxidant, the species *P. kudriavzevii* lowers cholesterol, has biological effects on the binding capacities of heavy metals, and improves the nutritional value of food [52,58,59].

Early research suggests *Torulaspora* strains, particularly the species *T. delbrueckii*, has potential as a probiotic showing benefits like improving gut barrier function and modulating the immune system [3]. Studies suggest *T. delbrueckii* may promote the growth of beneficial bacteria and inhibit harmful ones, leading to better digestion, reduced inflammation, and a stronger immune system [60]. Even if very little is known about its specific effects on gut health in humans, some *Torulaspora* strains exhibit an additional benefit, such as antibacterial and antifungal activity against like *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica* [3,61].

6. Fermentation Process: Boosting Nutrition and Flavor

Globally, interest in probiotic yeasts has surged in recent years and this is a rapidly evolving field. These yeasts are frequently associated with fermented food production, a traditional practice found worldwide [46]. The fermentation process not only enhances the taste and aroma of foods but also increases their nutritional value. Studies suggest that these yeasts might contribute to this enrichment by increasing B vitamins that are essential for energy production and metabolism; enhancing mineral bioavailability, making minerals more readily absorbed by the body; breaking down complex carbohydrates, aiding digestion [62–64]. Indonesia is one of main country in the world that utilizes probiotics isolated from fermented foods and animal digestive tracts. Research, mainly in Indonesia, has explored incorporating probiotic yeasts into functional foods for poultry, improving their gut health, nutrient absorption, and overall well-being [43]. However, research on probiotic yeasts specifically for human applications seems less prevalent when compared to poultry [43].The potential does not stop there. Studies suggest these yeasts might also serve as therapeutic agents for humans and animals suffering from dysbiosis, an imbalance of gut microbiota [2,65].

7. Commercial Formulas with Yeast Probiotics

As it is known probiotics are live microorganisms that offer health benefits when consumed. They work by promoting the growth of good bacteria in the gut and inhibiting the growth of harmful bacteria. The applications of yeasts in human foods and animal feeds as well as in agriculture and other sectors are increasing and market demand is providing motivation to continue or even increase research and development in this field [66]. Probiotics are widely used by healthy people and in clinical settings, but there can be side effects. With new strains and uses in vulnerable groups, clear instructions are needed for safe and effective use. An international group met to discuss potential risks, including those for vulnerable people, and the importance of high-quality probiotics for these groups. They also stressed the need for reporting side effects and using whole genome sequencing to check probiotic safety. This will help scientists and physicians determine how safe probiotics really are [67].

While *D. hansenii* has potential as a probiotic, it is not yet widely used in commercial formulas, especially for humans. *Torulaspora*, similar to *D.hansenii*, it is not yet a common ingredient in commercially available formulas. This is mainly due to:

a) *Limited research in humans*. Most research on *D. hansenii* as a probiotic has been conducted on animals. While promising, human trials are needed to confirm its effectiveness and safety [68,69].

b) *Focus on established strains*. Commercially available probiotic formulas often include well-studied strains like *Lactobacillus* and *Bifidobacterium* with a longer track record of safety and efficacy in humans.

c) *Formulation challenges*. *D. hansenii* might require specific processing or formulation techniques to ensure viability and delivery of its potential benefits. This yeast is sensitive to various environmental factors like drying, high temperatures, and low pH. These factors can damage the cells and reduce their viability during processing and storage [70].

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Things can be different in the future. *D. hansenii* may show up more frequently in commercial probiotic formulations as research continues. Alternative scenarios should be taken into consideration. These include combination formulas, where *D. hansenii* may be combined with established probiotic strains for a wider range of benefits, and/or specialized formulas, where *D. hansenii* may be included for specific gut health needs once research validates its benefits in those areas [70].

While *Torulaspora*, and especially *T. delbrueckii*, exhibits probiotic potential comparable to that of *D. hansenii*, it is not yet frequently found as an ingredient in commercially available formulations. Improved intestinal health is one possible advantage [71].

8. Synergy with Bacteria: A Powerful Duo

Also very interesting could be the study of the synergy between bacteria and yeasts. Combining probiotic yeasts with existing bacterial strains in supplements could create a synergistic effect, enhancing the overall health benefits [42]. For instance, certain yeasts might produce compounds that promote the growth of beneficial bacteria. This collaboration can offer several advantages [72].

(1) *Enhanced Microbial Growth*. Certain probiotic yeasts, like *S. boulardii*, may produce specific compounds such as prebiotics. These prebiotics act as food for beneficial bacteria strains like *Bifidobacteria* and lactobacilli, stimulating their growth and colonization in the gut [3].

(2) *Improved Barrier Function.* Numerous investigations demonstrated a correlation between *S. boulardii* and a decreased level of Firmicutes and Proteobacteria in the gut microbiota and a greater proportion of Bacteroidetes. Additionally, by increasing the synthesis of short-chain fatty acids and inducing proinflammatory immune responses, this yeast can reduce inflammation [29]. Furthermore, *S.boulardii* have been shown by Kunyeit *et al*., to dramatically decrease the adherence of the multidrug-resistant species *C. auris* to the abiotic surface, suggesting that this would be a useful strategy for managing this yeast [73].

(3) *Immune Modulation*. The combined effects of probiotic yeasts and bacteria could have a positive impact on the immune system. Studies suggest that this synergy might help regulate the inflammatory response and potentially reduce the risk of allergies or inflammatory bowel disease [11,29,42].

9. The Future

"New" probiotic yeasts could be incorporated into fermented foods or probiotic supplements, offering interesting possibilities for expanding the range of health benefits we can achieve through our diet and gut health [29,42]. Traditionally, probiotics have been associated with fermented dairy products like yogurt. Probiotic yeasts could be introduced into a wider range of fermented foods, like kimchi, kombucha, or even sourdough bread [74]. This would create an increased variety of fermented foods and more options for people with lactose intolerance or those who simply prefer different flavors. Further possibilities are represented by developing specific fermentation processes using various probiotic yeast strains. This could lead to fermented foods designed to target certain health concerns, like gut inflammation or immune function [75–77]. Moreover, probiotic yeasts might offer advantages when it comes to delivering beneficial microbes to the gut. Some yeast strains may be more resilient to stomach acid and bile, allowing them to reach the intestines in higher numbers. Hence, a wider range of health benefits could be achieved. For example, a) Probiotic strains can improve gut health, helping to maintain a healthy balance of gut microbiota and potentially reducing digestive issues like diarrhea or constipation. b) Some yeasts might stimulate the immune system, potentially reducing susceptibility to infections. c) Probiotic yeasts could target specific health concerns, like allergies, skin conditions, or even mental health, developing more targeted benefits.

To achieve these goals, the approach that uses probiotics for human health, to treat gut imbalances, is changing. Next-generation yeast probiotics are emerging as a powerful new approach in the field of live biotherapeutics [78,79]. These modified yeast strains go beyond the fundamental

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idea of probiotics. By using genetic engineering, scientists are able to give these tools specialized capabilities. Compared to conventional probiotics, this enables them to more effectively target particular health issues. For the time being, *S. boulardii* is one of the most promising next-generation yeast probiotics. Although this strain is currently utilized as a conventional probiotic to treat diarrhea, scientists are working to engineer it for additional uses [8,9,79]. Yeasts can be engineered to produce specific molecules that benefit the gut environment. For instance, they can be programmed to synthesize short-chain fatty acids that promote gut health or modifications can be made to allow the yeasts to reach specific areas of the gut where they can exert their desired effects. The hostile environment of the gut can be challenging for probiotics; hoewever, scientists are engineering yeast strains that are better able to pass them through the digestive system and establish themselves in the gut. Probiotics derived from next-generation yeast may revolutionize the way gastrointestinal disorders are treated and perhaps even other medical conditions. Though the field is still in its early stages, ongoing research and clinical trials are paving the way for the development of more effective and targeted treatments.

10. Conclusions

Because the discovery of probiotic yeasts constitutes an important development in the probiotics field, the future of these probiotics is promising. Further research is needed to fully understand the specific health benefits these yeasts offer to humans and explore their applications in various food and therapeutic contexts. Several insights are necessary to move forward: 1) *Mechanism Elucidation*: Understanding how probiotic yeasts interact with the gut microbiome and exert their health effects will pave the way for targeted applications. 2) *Strain Specificity*: Not all strains within each genus will have the same probiotic potential. Identifying the most beneficial strains is essential. 3) *Clinical Trials*: Studies in humans are needed to confirm the safety and effectiveness of yeast strains in promoting gut health.

This could lead to the development of novel probiotic supplements and functional foods promoting gut health and overall well-being for people around the world. However, the future of incorporating probiotic yeasts into fermented foods and supplements is definitely one to watch. It has the potential to revolutionize how we approach gut health and achieve a wider range of health benefits through diet and targeted interventions.

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