Review

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Advances in Yeast Probiotic Production and Formulation for Preventative Health

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Abstract: The use of probiotics has been gaining popularity in terms of inclusion into human diets over recent years. Based on properties exerted by these living organisms, several benefits have been elucidated and conferred to the host. Bacteria has been more commonly used in probiotic preparations, in comparison to yeast candidates, however, yeast exhibit several beneficial properties such as the prevention and treatment of diarrhoea, production of antimicrobial agents, prevention of pathogen adherence to intestinal sites, maintain microbial balance, modulation of the immune system, resistant to some antibiotics, amongst others. This review details the use of yeast organisms as biotherapeutics and has a special focus on production considerations and their formulation into different delivery formats.

Keywords: probiotics; yeast; biotherapeutics; formulation

1. Introduction

Probiotics and their use for human health implications have been studied extensively, and in more recent years, their acceptance for use by the global population has seen a positive trajectory. According to the most widely accepted definition, a probiotic is known to be "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1].

Generally, only bacterial and yeast organisms have been classified as probiotics, with the most common being Lactobacilli, Bifidobacteria, Enterococci, Faecalibacterium, Clostridia and more recently Propionibacteria [2]. Upon application, these organisms have been found to prevent and treat various clinical diseases, improve the intestinal micro-environment, prevent physiological stress and the proliferation of pathogens, improve health of the intestinal epithelium, modulate immunological homeostasis amongst others [3]. Historically, negative perceptions plagued yeast cultures, as these organisms were generally referred to as pathogenic, disease-causing microbes. However, several studies centred on the use of non-pathogenic yeasts, that possess probiotic properties have emerged, which highlights their innate ability to influence physiology, metabolism, and immune homeostasis in the colon [4]. Yeasts have been studied and have proven to be effective starter cultures, and significant interest has been noted in their use in various biotechnological applications [5].

Yeasts make up <0.1% of the human microbiome Most yeast isolates that have been isolated from the human microbiome include *Candida albicans, Torulopsis glabratra, Candida tropicalis, Malassezia spp., and Saccharomyces spp* [6], [7]. Other probiotic yeast candidates include Cryptococcus spp. *Candida famata* [9], *C. tropicalis* [10] , *Debaryomyces hansenii* [11], *Issatchenkia orientalis* [10], *Kluyveromyces lactis* [12], *Kluyveromyces marxianus* [12], [13], [14], *Metschnikowia gruessii* [13], *Pichia jadinii* (Buerth et al. 2016), *Pichia kluyveri* [10], *Pichia kudriavzevii* [10], *Pichia pastoris* [15], *Pichia guilliermondii* [16], *Wickeramomyces anomalus* [16]. These organisms, due to their ability to resist low pHs, to produce digestive enzymes, bile salts, organic acids, make them ideal candidates to serve as probiotics [7].

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Yeasts that have been classified as generally regarded as safe (GRAS), have been shown to have several health implications on the human host. These influences may include but are not limited to being effective on gut microbiota dysbiosis, possess anti-inflammatory, anti-proliferative, anti-cancer and anti-allergenic properties [17], [18].

Saccharomyces boulardii, *Saccharomyces cerevisiae*, *Candida* spp, are the most common yeasts used as probiotics, as is used most for the treatment of *Clostridium difficile* diarrhoea [19]. *S. boulardii*, was first isolated from litchis in Indochina, and is not autochthonous in the microbiome [7]. However, this non-pathogenic yeast is known to have the following characteristics that advocates its use as a probiotic (Figure 1).

According to Arevalo-Villena et al. [20], when developing a yeast probiotic product, the following characteristics ought to be sought, in a candidate organism (Table 1). Other factors that may be considered include the assimilation of cholesterol assimilation, the deconjugation of bile salts, demonstration of antioxidant, haemolytic, cytotoxicity, activity, as well as ability to produce cytokines and phytase.

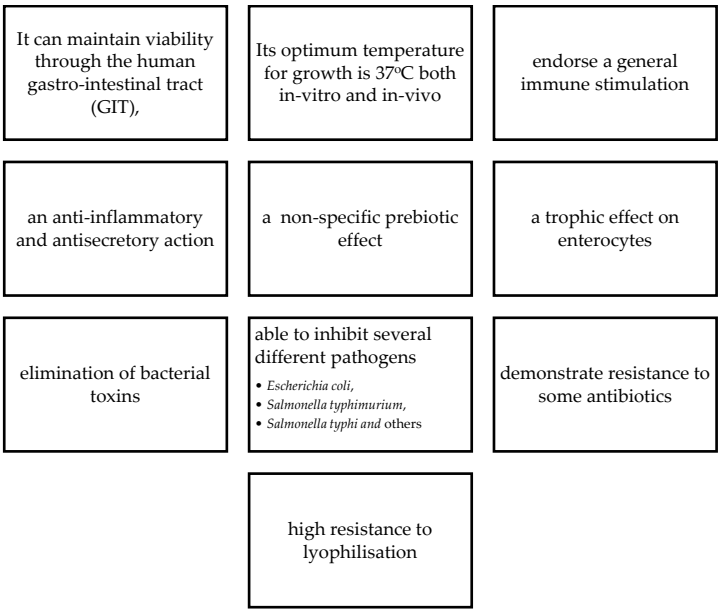

Figure 1. Probiotic ability of Saccharomyces boulardii [5].

Table 1. Assessment criteria used for the assessment of yeast probiotic potential**.**

Characteristic	**Rationale**	**Reference**
Hydrophobicity	For an organism to show functionality as a probiotic, it needs to display hydrophobicity. The organism of interest needs to demonstrate its ability to adhere/interact with the mucus present within the GIT to confer the probiotic effect.	[21]
Auto-aggregation	This is a characteristic wherein cells are able to self-aggregate and adhere to the mucus/mucosal lining in order to form a biofilm. A desirable level of auto-aggregation is ~30 to 60%	[22]
Biofilm formation	To show the ability of cells to adhere to each other and the host lining	[23]

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Adherence ability	To assess the ability of the probiotic cell to adhere to the mucosal lining and confer a probiotic	[24]
Survival	To assess the organism's ability to survive exposure to low pHs (gastric conditions) and the presence of bile salts (0.3%)	[25]
Antibiotic resistance	In the instances of yeasts intended for use as a probiotic, antibiotic resistance using a disk assay method will infer information pertaining to the ability of the organism to demonstrate antibiotic resistance.	[26]
Antimicrobial activity	To assess the yeast's ability to demonstrate anti-microbial activity, which is pertinent for the treatment of pathogens	[27]

2. Status of the Biomanufacturing of Yeast Probiotics

Yeast biomass production is a common practice across the globe [28]. During probiotic production, for the interest of this review, yeast probiotics; the primary focus of process optimisation is to maximise volumetric organism productivity [29].

2.1. Key Bioprocess Considerations

The main contributing factors to this key process indicator are composition and components present in the growth medium, operational parameters used in the process, such as temperature, pH, aeration, as well as mode of cultivations, either batch, fed batch or continuous.

Despite there being several commercial probiotics available, the focus of producers is to ensure that these products can be produced economically to increase market share [29]. At present, major producers of probiotics have to date, developed highly efficient, refined and vertically integrated microbial production systems [29]. Upon biomanufacturing of probiotics, the following characteristics are of vital importance, to promote the uptake of the technology (Figure 2).

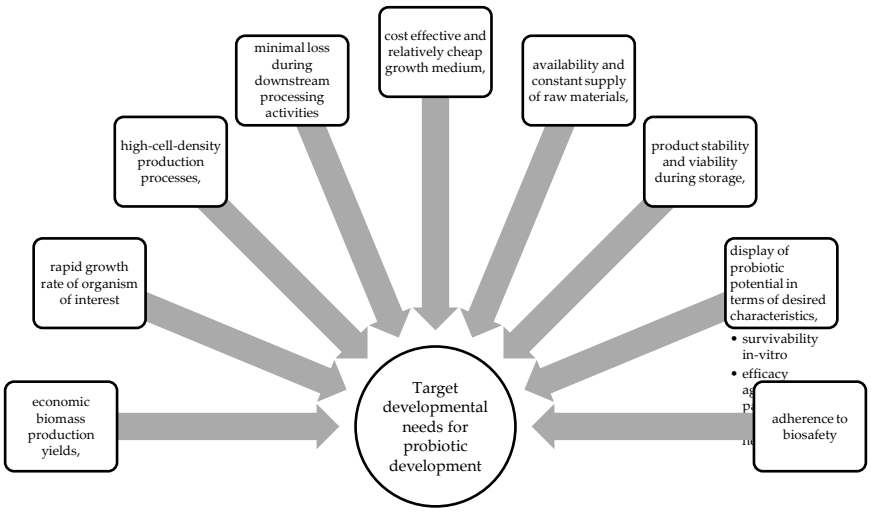

Figure 2. Factors to consider when developing a yeast probiotic using a classical biotechnological approach.

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2.2. Advances in Yeast Probiotic Manufacture

Conventional yeast production requires a cultivation medium that typically contains carbon, nitrogen, vitamins and trace metals [30]. As the growth medium for probiotic production is a major consideration in process development, other non-conventional fermentation feedstocks are being considered to produce probiotics to minimise production costs (Table 2). Agro-industrial residues such as molasses has been used for the cultivation of yeast probiotics [17]. Molasses is a viscous, sugar rich nutrient source, that contains ~34% sucrose, glucose, fructose and other minerals. In sugar producing countries, such as Brazil, and South Africa, ~10 million tons of sugar cane molasses is discharged [17], hence making it a suitable nutrient feedstock for the large-scale production and manufacture of probiotics.

Additionally, with the rise in food production, food waste volumes are also on the increase. Hence, the food waste that is accumulated, is rich in proteins carbohydrates and lipids, and therefore can be used as a suitable substrate to cultivate microorganisms (Sharma 2021). This circular economy or in some instances referred to as bioeconomy initiatives, are intended to reduce the economic, societal and environmental costs, and to drive waste to wealth activities (Sharma 2021). Probiotics are not usually the intended product produced using waste valorisation initiatives, however, there has been some successful attempts in demonstrating the concept in studies conducted by Patil et al., (2022) and [31]. These studies demonstrated the production of Kluyveromyces, Torula, Candida and *Saccharomyces* spp as well as *Saccharomyces boulardii* CCT 4308, using coffee pulp and sugar cane molasses, respectively. It is envisaged that more instances of successful waste valorisation are expected in this area of R&D in the coming years.

2.3. Challenges Associated with Yeast Probiotic Manufacturing

Less established entities that are interested in the use, manufacture and/or supply of probiotics may not have access to the skills, expertise and infrastructure required to produce these bio-based products. These entities include but are not limited to small and medium enterprise farmers, start-up biotech-based companies and the research and development community. Although these institutions and stakeholders may have a high degree of interest in probiotic development and manufacture, they may not necessarily have the high levels of competencies in probiotic development, manufacture and supply in comparison to the established players [29].

In these instances, niche probiotics may be developed, or novel candidate products may be identified through collaborative R&D partnerships, however the developmental pathway for these concepts to commercial scale are in some instances not clear and seemed to be filled with challenges and high risk. The disadvantage of these unexploited R&D initiatives, is that the value of research investments may not be realised, or the needs identified upon specific product development remain unmet [29].

Success in these instances has been attained with the creation of non-classical R&D pathways to commercialisation. Feedback loops initiated within product and process development, agile manufacturing, market and user testing, coupled to the intellectual property management and regulatory frameworks (if applicable) are necessary to rapidly develop and deliver products through the value chain [29]. This also entails the specification of production performance targets, final product adherence to end use specifications, market and financial data as well as production capacity to fulfil market demand. These factors are essential in de-risking the success if the probiotic product and its adoption for use.

2.4. Manufacturing Considerations to Produce Yeast Probiotics

During product and process development, it is a key development area to determine base line performance of the production process. Thereafter ancillary development steps may be taken to further retrofit production process, especially that of cost-sensitive unit operations. Simulations and process modelling offers a useful tool, termed – in-silico, to enable the optimisation of key process steps in the production process. With the use of this strategy, it is envisaged that significant time and

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expenses are saved by removing the need to conduct several actual cultivations at laboratory and pilot scales, which ultimately achieves economic impact.

Another important consideration for probiotic production is the techno-economic modelling and assessment. This exercise details the technical and economic details of the process and includes the following components:

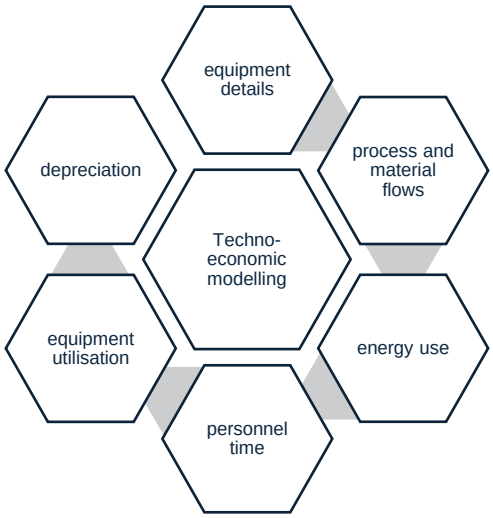

Figure 3. Aspects that contribute to the techno-economic feasibility of the production process.

2.5. Location of Known Producers and Global Manufacturers of Yeast Probiotics

A key component of successfully commercialising a probiotic, is the access to suitable manufacturing expertise, particularly in developing countries. Infrastructure requirements are capital intense and are limited in availability. Probiotic technologies require a production scale pipeline that facilitates technology development from small scale to manufacturing scale.

Large enterprises that have shown efficient production competencies have skills and infrastructure that is currently producing a wide variety of products. These production facilities are fully utilised, using tight production scheduling strategies that give them the edge of new market entrants, or smaller entities that lack vertical process integration.

Biomanufacturing entities on the African continent are limited, and scarcely available. The global probiotic market consists of various entities and are categorised into three tiers (BCC Market Research, 2022). These companies and their respective tiering are listed in Table 2. As can be seen, tier 1 companies that occupy ~40 to 45 % of the global market, are found in the United States of America, Europe and Japan, with most tier 2 companies presiding in these regions.

Table 2. Major global producers (tier 1) of probiotics (BCC Market Research 2022).

Name	**Country**
ADM,	USA
Abbott,	USA
Asahi Group Holdings Ltd.,	Japan
Chobani LLC,	USA
Chr. Hansen Inc.,	Denmark

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DSM	Netherlands
Danone Inc., IFF,	France
Kerry,	Ireland
Estee Lauder Inc.,	USA
Morinaga Milk Industry Co. Ltd.,	Japan
NESTLÉ,	Switzerland
Yakult Honsha Co. Ltd.	Japan

3. The Use of Genetically Modified Organisms (GMO) as Probiotics.

Conventionally, wild type organisms have been applied as probiotics, however, the advances in genome editing and associated tools have unlocked the possibility of being able to engineer probiotics to deliver customised therapeutics [32]. Ma et al. [33] has provided an extensive review on the theoretical basis for probiotic gene editing technology, as well as the use of these engineered probiotics for the treatment of diseases. These diseases range from inflammatory bowel disease, cancer, obesity and diabetes amongst others, and have been tested both in human and animal models. Interestingly, only 8% of the genetically modified organisms listed in the review were yeast probiotics, with the remaining belonging to their bacterial counterparts. It is envisaged that the advent of genome editing may impart a variety of benefits to human health especially in the treatment of specific diseases. To date, there has been significant hesitation and consumer resistance for use of genetically modified organisms as probiotics, however, this aversion towards its use may be reduced with the progression of several clinical studies that are currently ongoing [34].

4. General Routes of Administration of Yeast Probiotics

The practise of using probiotics has become widely accepted as a natural means to stimulate health for humans. Today, probiotics are used as health supplements in food, as pharmaceuticals or chemical supplements [35]. If a probiotic is classified as pharmaceutical or drug for treatment of a disease or disorder, stricter requirements are necessary to substantiate the claims stated by the manufacturer. It must be proven safe and effective for its intended use through clinical trials and be approved by the Food and Drug Administration (FDA) before it can be sold. Depending on the intended use of a probiotic, if to be used as a drug or a dietary supplement or a nutraceutical, regulatory requirements differ [36]. According to the definition provided by the FDA; a drug is an article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of dis-ease [37].

With regards to nutraceuticals, these are known as pharmaceutical alternatives that exert physiological benefits, used to promote health and support the structure or function of the human body [38]. Nutraceuticals are regarded as safe and less likely to have side effects, as such, probiotics are generally classified as nutraceuticals. The safety of probiotics is apparently due to the absence of toxicity in their populations. Additionally, most probiotics form part of the natural microbiome of the human host and therefore are applied back to a known environment (WHO, 2002).

In most instances, the optimal concentration of active probiotic cells, required to confer a positive benefit to the host is not known. What is known, is that the probiotic needs to be in high enough concentration to survive some of the physiological barriers upon transit to the target site. Another important consideration is the method selected for use to deliver the probiotic to the intended site.

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5. Conventional Pharmaceutical Methods Used to Administer Probiotics

5.1. Oral Delivery Systems

The most common delivery of probiotics is oral administration. This infers that the final step in the probiotic production process is the formulation and packaging of a probiotic into a delivery system that will be able maintain functionality of the probiotic through the harsh gastric environment. Conventionally used delivery systems include tablets, capsules, hydrogels, granules and others as described in Figure 4.

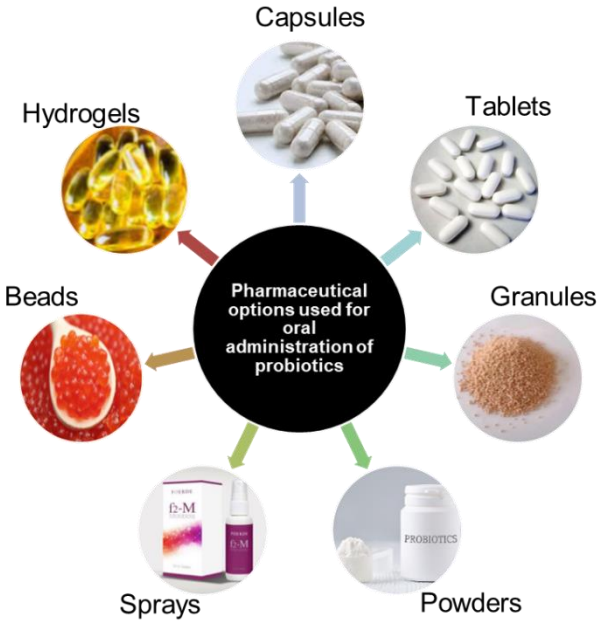

Figure 4. Different oral delivery systems used to administer probiotics.

Oral administration of the probiotic product is a widely accepted channel for the delivery of drugs and probiotic microorganisms in several disease and treatment applications. Consequently, it presents the biggest challenges in administering probiotics, as the live cell preparations found in final formulated products, need to ultimately survive the gastric environment in the stomach which can reach a pH below 3 [39]. Most authors that have deduced upon application of probiotics, that when conferred in adequate amounts, these organisms are able to confer health benefits to the host. This infers that for every step in the probiotic production process, viability needs to be maintained, and cell losses need to be kept to a minimum, since it is envisaged that a certain component of the probiotic population will die upon exposure to the gastric environment.

Table 3. Some of the benefits that are offered from oral administrations of probiotics and addition in food products.

Benefits	Organisms of interest	Reference
Inhibition of Cd absorption	*L. Plantarum*	[40]
Protection of the intestinal barrier – by alleviation of Cd-induced oxidative stress		
Enhancement of antimicrobial activity	*L. paracasei* and L. casei	[41]

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Reduction of hypertension effects	*S. cerevisiae*	[42]
Modification of the fecal resistome during *Helocobacter pylori* treatment – reduction of antibiotic resistance	*S. boulardii*	[43]
Potential in removal of toxins	*S. cerevisiae* W13 and *S. boulardii* ATCC MYA-796	[44]
Improvement of glycaemic indices in type II diabetic patients.	*S. cerevisiae*	[45]
Inhibition and reduction of *Gardnerella vaginalis* biofilms in mice	*S. cerevisiae* CNCM I-3856 and *L. rhamnosus* ATCC 53103	[46]
Cholesterol reduction	*Pichia fermentans* BY5 *Pichia kudriavzevii* BY10 *Pichia kudriavzevii* BY15 *Yarrowia lipolytica HY4*	[47]
Better sensory properties with lower ethanol content	*Meyerozyma caribbica 9D*	[48]
Production of alcohol-free and low-alcohol products	*S. boulardii*	[49]

5.2. Transdermal Delivery Systems

According to Chen et al. [50]; methods that are simple and effective for delivery of probiotics into the dermis for the regulation are lacking. Yeast organisms, such as *S. boulardii* has been used for the treatment of skin ailments, including acne, due to its anti-inflammatory properties. However, in this instance, the probiotic product was administered dermally, as one would expect, but rather as an oral supplement. Upon ingestion, the anti-inflammatory effects of the organisms are exerted towards the skin. S*. boulardii* demonstrated the ability to produce acetic acid which is known to exert antifungal and antimicrobial effects. This preparation, when used topically, was shown to reduce the bacterial load responsible for causing infections or skin conditions such as acne, rosacea, seborrheic dermatitis (scalp eczema), and eczema. In this instance, yeast by-products and not the organism itself was used as a topical treatment. All the *S. boulardii* strains tested in this study, secreted an antimicrobial agent that demonstrated an inhibitory effect on *E. coli* and bioactivity against *Candida albicans* hyphae [50].

6. Other Probiotic Delivery Systems

It is known that the most common and effective delivery route for probiotics is orally, as the intended destination for the product is the GI tract of the host [51]. However, during processing of pharmaceutical products in general, such as tablets, high processing temperatures may be used to obtain aesthetically pleasing final products, which may kill the probiotic microorganisms of interest.

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This occurrence may be applicable to many formulations that contain viable organisms and proves to be the biggest challenge that needs to be overcome in terms of final product processing.

In order to circumvent this significant challenge, fermented also known as functional foods are used as a delivery system, and do not require high temperature processing. Instead, indigenous microorganisms already present in the ingredient mix, are activated and thereby replicate to higher concentrations. This is termed as non-conventional delivery methods, despite it being consumed by several populations dating back to early human civilisation.

Fermented foods derived from animals and plants are vital in human diets as they contain beneficial microorganisms and compounds including organic acids, ethanol or antimicrobial compounds [52]. These fermented foods are termed functional foods, which are foods that offer benefits that are more than their nutritional value and are divided into dairy and non-dairy options.

6.1. Functional Foods as a Source of Probiotics

6.1.1. Dairy - Based Probiotics

In the early 1990s, Nobel Laureate, Elie Metchnikoff (1845–1916) whilst working in Bulgaria noticed how a certain population in the country had lived a longer life span than others. Upon further investigation, the researcher discovered that this population consumed a fermented drinking yoghurt daily [53]. This preliminary research laid a foundation for the study of probiotics detailing the use of functional foods as a dietary supplement. These foods contained beneficial microorganisms; either bacterial or yeast cultures or a combination of both. *S. boulardii* a known yeast probiotic, has been isolated from dairy products including milk, yoghurt, cream, cheese and kefir.

Yeasts have shown the ability to produce enzymes that synthesise milk proteins. However, this property is only activated once lactic acid bacteria (LAB), breakdown the lactose present in dairy-based foods, into glucose and galactose. Once this conversion is done, both the yeasts and LAB cultures, use the available sugar as a carbon source to grow and replicate [54]. [55] added that yeasts such as *S. boulardii* should be included in products solely as a probiotic as it offers no benefits to some dairy products such as yoghurts, however in cheese making, yeasts have a more important contributions to the process of cheese maturation. These yeasts contribute to the development of the flavour of the cheese, as well as texture due to proteolysis, lipolysis and utilisation of lactic acid [55], [56], [57].

Dairy products have been the most utilized carrier/ formulation of bacterial probiotics with limited applications for yeasts strains [58]. Upon assessing the literature landscape, it was found that the *Saccharomyces*, *Pichia*, *Candida*, *Meyerozyma, Debaryomyces and Kleuveromyces* genera found in different types of fermented vegetables, cheese, and kefir [59], [60], [61], [62]. Additionally, *Wickerhamomyces*, *Torulaspora, Yarrowia* and *Metschnikowia* are the other yeast genera present in fermented fish, legumes and meat products [58], [63]. The use of dairy-based food as a means for probiotic delivery is largely affected by poor shelf-life. As a result, non-dairy based probiotic formulations have been evaluated for their potential as an alternative solution.

6.1.2. Non-Dairy Based Probiotics

It has been found that in most instances, yeasts such as *S. boulardii* does not naturally occur in food and usually added as a supplement. *S. boulardii* is commonly added into cereals and legumes to stabilise nutrients using its enzymes [53]. One of the nutrients that is present and broken down by the organisms is phytates or phytic acid which is the primary storage compound for phosphorus in seeds. This compound binds to metals rendering them insoluble and thus inaccessible as nutritional components. *S. boulardii* is added to synthesise the phytates using phytases which in turn enhance the bioavailability and absorption or important essential minerals such as iron, zinc, magnesium and phosphorus [53], [64], [65].

Lazo-Velez et al. [66] proposed that *S. boulardii* be supplemented with cereal based or low water activity foods to be used as vehicles to administer this probiotic yeast. Additionally, the application

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of *S. boulardii* has been successfully added to fermented drinks which include beers, grain drinks, malts and fruit/veggie juices [67], [68]

7. Advancements in Probiotic Delivery Systems

The administering probiotics has evolved into various methods based on the need. Typically, probiotic administration has been done through oral ingestion, however, new advances include the application of probiotics into the nostrils using nasal sprays, applications through the vagina or as topical application, termed transdermal applications.

An additional mechanism of oral supplementation includes the sublingual routes, whereby the probiotic is applied under the tongue where it is absorbed rapidly. This route of administration is currently being researched, along with the advent of rectal Suppositories or probiotic based enemas. Probiotic enemas contain a solution of probiotics, which is administered through the rectum and colon, and has been gaining popularity as an alternative remedy for gut health, immune system support, and some diseases of the digestive tract.

Other avenues being explored for probiotic administration indicate that meat and meat products are emerging as potential routes when supplemented with probiotics. Members of the Lactobacillus and Bifidobacterium genus, are the most commonly used probiotics in meat and yeasts such as *S. boulardii* may be explored in future research. The application of probiotics in meat products have been predominantly used as bioprotective cultures against harmful and pathogenic bacteria. This probiotic effect is mainly centred on the production of bacteriocins which aid the host [69].

8. Formulation Techniques Used for Yeast Probiotics

Probiotics administered orally transit through the mouth, stomach, small intestine, and the colon where they are subjected to saliva, acidic conditions, pancreatic juices, bile acids and digestive enzymes as well as competition with host microbiota for nutrients and adhesion sites [70]. As a result, these probiotics are shown to lose viability upon transit. This is attributed to their high sensitivity to gastrointestinal conditions, extrinsic factors such as processing techniques and storage conditions as well as intrinsic factors which include water activity, antimicrobial components and redox potential in the product matrix [71]. As stated above, a probiotic must maintain its viable activity to confer a therapeutic effect to the host. Typically, the intended viable population is targeted to be within the range of ~1.0 × 10^{06} as a minimum, up to a maximum of ~1.0 × 10^{09} CFU.ml^{-1} viable cells to be considered effective [72].

The challenges associated with poor survival of probiotics during processing and passage to the GIT have been studied extensively [71], [73], [74], [75], [76], [77], [78]. According to Sehrawat et al. [79], before the introduction of new technologies, there was even more challenges in the use of probiotics as starter cultures, as these preparations were used in liquid form, which was associated with low shelf-life, high risk of bacteriophage infections as well as high production and transportation costs [71], [80]. Therefore, the increased demand for use of probiotics in food and pharmaceutical industries based on their demonstrated efficacy on health and nutritional benefits has prompted the intensive research being conducted on solving these production and viability hurdles.

8.1. Immobilization

Immobilization has shown to be an effective method for the preservation of yeasts. It is typically used as a method of entrapment of bioactive materials in protective matrices, and several reports have indicated its suitability to enhance viability of many probiotic bacterial [81] and yeast cultures [82]. However, in some instances, yeast probiotics cells were found to be not entirely protected, as a small percentage of the immobilized material is still exposed to the external environment at the surface of the carrier and may as a consequence, be deemed inefficient [81].

8.2. Encapsulation

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Encapsulation is one of the most utilized methods for protection of probiotics from harsh conditions and is defined as a process that involves packaging of live probiotic cells in a food-grade material such as polymers, proteins, and fats [47], [83]. In this process, encapsulated cells are contained within the coating material, which is formed continuously around an inner core matrix [81]. Additionally, these techniques improve the bioavailability of encapsulated probiotics by facilitating controlled release at the target site, the large intestine [84], [85]. Encapsulation is categorized into two classes based on particle size such as microencapsulation (3-800 μm) and nanoencapsulation (10-1000 nm). Since microbes are the size of a micron, microencapsulation is the only possible technique for encapsulating all probiotics including yeasts. Microencapsulation techniques such as extrusion, emulsion, spray drying, spray chilling, fluidized bed, freeze drying, spray-freeze drying, coacervation, and electrospraying are currently utilized to formulate probiotics [72], [85], [86].

In general, encapsulation is carried out in three steps. The initial step involves incorporation of the microbial cells into a solid or liquid matrix. The second step includes spraying and dispersion of the solid and liquid matrix, respectively. In the third phase, stabilization of the system is carried out either through physical (evaporation, solidification as well as coalescence) or chemical (polymerization) and gelation processes.

Encapsulation was suitably demonstrated for yeast probiotic applications in a study conducted by Patarroyo et al. [87] whereby *Kluyveromyces lactis* was encapsulated in cross-linked gelatin hydrogels, which a commercially available and relatively inexpensive material that will easily allow for industrial scale-up. The encapsulation enhanced rigidity of the final probiotic product as cell viability levels were enhanced by 50% under simulated GIT conditions [87].

Alginate, starch, k-carrageenan, chitosan, xanthan gum and cellulose acetate phthalate, gelatin, and milk proteins are some of the known polymers used in encapsulation of probiotics to date [88], [89], [90]. The encapsulating materials are selected based on their ability to stabilize the final product, non-toxicity, protective effect to the cells and possess a satisfactory control in the release of the bioactive material in the intestinal tract [85], [86]. Extrusion, spray drying, coacervation, liposomes, and emulsions are encapsulation techniques that are conventionally used in the food industry.

8.3. Extrusion

Although extrusion is largely employed in encapsulation of bacterial cells, it is a low-cost, easy technique that is carried out under mild conditions and results in high viability of encapsulated probiotics. As described by Rodrigues et al. Rodrigues et al. (2020), extrusion involves the use of hydrocolloid solutions containing microbial cultures. The mixture is then extruded through a nozzle in crosslinking solution which provides instant transition of the hydrocolloid solution to gel which results in the formation of beads [86]. These beads are stable at low pH levels and deform under alkaline conditions. In a study by Graff et al. Graff et al. (2008), *S. boulardii* was encapsulated with alginate microspheres coated with chitosan by extrusion. This report revealed that after 120 min at pH 1.1, encapsulated yeast cells remained entrapped in the microspheres whereas 99% of the non-encapsulated probiotic survived was lost. The authors further stated that exposure to pH 6.8 resulted in the release of viable yeast cells, demonstrating the effectiveness of this technique [91], [92], [93], [94].

8.4. Spray Drying

In spray drying, hot gas is used to atomize a liquid product into powder, instantly. It is a cost-effective and rapid microencapsulation method which results in high productivity. Spray-drying is the most common process in the food industry [85], [95]. However, the harsh conditions such as high temperature, dehydration, osmotic stress, and pressure applied during the process also pose detrimental effects to the probiotics being processed. These conditions result in alteration of cell membrane components such as fatty acids, proteins, and lipids which eventual cause cell death [96]. Improvement of cell viability during spray drying has been achieved through optimization of the

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process conditions and the use of lower temperature has proved to be effective as a result of reduced heat damage [86], [97].

8.5. Spray Chilling

Spray chilling is similar to spray drying as small droplets are also produced in this technique. The matrix (formed by lipids) and the encapsulated agent are dispersed by atomization in a cold air chamber which enables solidification of the particles [86]. Although it is less exploited, this process is an excellent alternative for encapsulation of probiotic due to its cost-effectiveness and applicability at industrial scale [98], [99]. In a study by Arslan-Tontul & Erbas [100], encapsulation of *S. boulardii* by spray drying and spray chilling using gum Arabic and b-cyclodextrin as an encapsulation material resulted in enhanced heat and survivability in the gut system [100].

8.5. Emulsions

During the preparation of emulsions, two immiscible liquids are dispersed in the presence of a stabilizing agent. An additional solidifying agent is used to separate the dispersed droplets. The emulsion is referred to as a water-in-oil (W/O) if the dispersed phase is aqueous whereas the opposite is named oil-in-water (O/W) or reverse phase. Simple emulsions are formed by two phases and addition of another phase results in double emulsions such as water-in-oil-in-water (W/O/W) or oil-in-water-in-oil (O/W/O) [85]. This technique improves solubility, activity as well as stability of immiscible compounds and it is widely employed in the food and pharmaceutical industries. This system, particularly, the dispersed aqueous phase, is mostly used in encapsulation of probiotics due to hydrophilic properties of microbial cells. In a study by Suvarna et al. [92], effects on encapsulation using emulsification were reported on four probiotic yeasts such as *Pichia barkeri* VIT-SJSN01, *Yarrowia lipolytica* VIT-ASN04, *Wickerhamomyces anomalus* VIT-ASN01 and *Saccharomyces cerevisiae* VIT-ASN03. This resulted in enhanced survival during storage and in simulated GIT conditions [92].

8.7. Fluidized Bed Drying

The fluidized bed drying technology is carried out through atomization of a coating over solid particles in suspensions. It is mainly used for coating, granulation and drying. Fluidized bed drying is a rapid, low-cost process that has high productivity [83]. This process is attractive as it allows for use of various encapsulating materials such as lipid, proteins and polysaccharides. The particles to be encapsulated are kept in constant motion due to air flow in a heated chamber. The coating particle size is reduced, forming a solid homogenous layer [86]. The ability of this technique to protect yeast cells along with the use of Hongqu rice peptides as a microencapsulation during thermal processing was investigated by [101]. it was found that the drying rate and yeast viability was significantly in comparison to free cells [101]. Another potential probiotic yeast, *Meyerozyma guilliemondii* Lv196 and stable granulated prototypes were reported with 0.2% loss of viability over 15 months of storage at room temperature [102], [103], [104], [105].

8.7. Supercritical Technology

Other microencapsulation techniques include supercritical technology and freeze-drying. Supercritical technology employs the use of supercritical fluids. These are solvents which describe the state of a material above its critical point at which its vapour/liquid phase equilibrium can exist. Above these conditions, the liquid-gas phase transition disappears and the properties, such as diffusion coefficient and density, continuously change with variation in pressure or temperature. Supercritical processes result in micro- or even nanoparticles with narrow size distribution and can also be used to achieve microencapsulation and surface coating of probiotics [96]. Supercritical carbon dioxide ($scCO_2$) is one of the most used supercritical fluid due to its environmental compatibility and low reactivity and low critical parameters. in supercritical technique, the probiotic cells are first immobilized during the process of interpolymer complex formation in $scCO_2$ and then the probiotic

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microcapsule is obtained by gasifying the $scCO_2$ through depressurizing [106], [107], [108]. This technique has mainly been applied in encapsulation of probiotic bacteria.

8.9. Freeze Drying

Freeze drying is one of the well-established processes in probiotic processing. The technology involves freezing of microbial cells at extremely low temperature and drying though sublimation under high vacuum [96]. In comparison to spray drying, the operating conditions are less harsh and usually results in high survival rates. However, this process formation of extracellular crystals which results in high osmotic pressure cause cell damage. Therefore, the use of cryoprotective agents is generally applied to protect the cells. These cryoprotectants can be low molecular weight sugars such as glucose, lactose, mannose, trehalose and sorbitol or high molecular weight polysaccharides and proteins [96], [109]. This technique has been successfully applied in encapsulation of the commercially available yeast probiotic, *S. boulardii*. In a study by Thomas et al. [110], *S. boulardii* was encapsulated using layers of chitosan and dextran sulphate., whereby, the coated cells were subsequently frozen in liquid nitrogen before freeze-drying. This resulted in enhanced viability and the permeability of the encapsulated cells [110]. Illustration of the commonly used microencapsulation techniques described above as well as other methods such as refractive window drying, electro-spraying and electrospinning are outlined in Figure 5 [76].

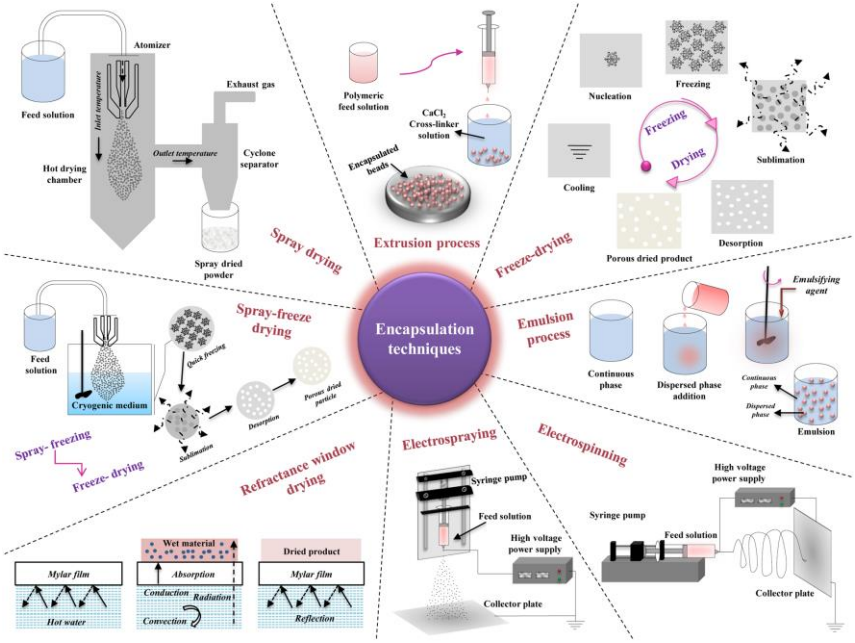

Figure 5. Diagrammatic representation of the different methods of encapsulation typically used for probiotic applications [76].

8.10. New Advances in Probiotic Formulations

Co-encapsulation of probiotics with prebiotics and use of duocaps are emerging technologies applied to further enhance survivability and probiotic efficacy. Co-encapsulation improves the oral delivery of viable cells towards the target site. As reviewed by Rashidinejad et al. [75], various studies have been reported and several polymers and polysaccharides such as inulin, fructo-oligosaccharides and lactulose have been used. As illustrated in Figure 6, co-encapsulation provides a protective layer to probiotic cells while enhancing viability through the coexistence with a prebiotic enhances its self-proliferation [75]. Co-encapsulated particles are referred to as synbiotics. However, to the best of our knowledge, there has not been any reports on co-encapsulation of yeast probiotics.

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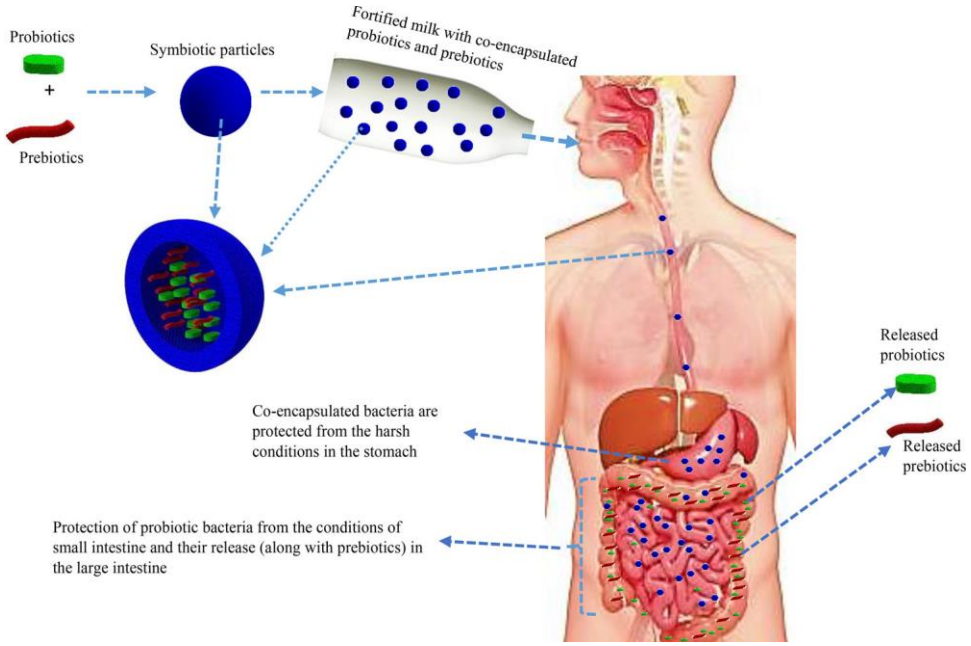

Figure 6. Illustration of co-encapsulated probiotics, prebiotics in fortified milk product and advantages offered by the technique [75].

9. Application of probiotics for preventative health benefits

9.1. Gut Microbiome Initiatives

Since the discovery of microorganisms in the 17th century; technologies and knowledge in this field have advanced rapidly, consequently resulting in microbiome mapping initiatives becoming a reality in the 21st century. Arnold et al. [111] stated that , microbiome research is an intrinsically multidisciplinary field, that has been able to reap the benefits of technological advancements in systems and synthetic biology, biomaterials engineering, and traditional microbiology. Prior to microbiome mapping, DNA technology and improvements thereof; paved the way for the advancements in whole genome sequencing and microbial population study shifts in the human body. Further advancements have resulted in the knowledge on how specific microbial compounds and activities result in health benefits, which has been developing area of research and developed [112].

The human body hosts complex microbial communities, wherein the combined membership of these organisms outnumbers our own cells by at least a factor of 10. The total number of microorganisms in the human body can reach ~100 trillion. The cells are responsible for awarding us with crucial traits which include our reliance on them to aid in nutrition, resist pathogens, and educate our immune system [113]. In comparison to other parts of the body, the human gut has the largest number of microbes, as both the gut and skin are immensely immersed with microbiota. It is estimated that the skin has about 10^{12} cells while the gut accounts for 10^{14} cells [114], [115].

The subsequent sections will focus on the gut and skin microbiome, which interestingly, share astoundingly similar characteristics as they are highly analogous to each other, both in terms of purpose and functionality [116]. According to O'Neill et al. [117], both organs are highly innervated and vascularised. Both these organs are essential for immune and neuroendocrine function. Furthermore, the inner surface of the gut and the outer surface of the skin are both covered by epithelial cells (ECs) which have direct contact with the exogenous environment [118].

According to Thursby and Juge [119], the human gastrointestinal (GI) tract represents one of the largest interfaces (250–400 m^2) between the host, environmental factors and antigens in the human body. In an average lifespan, around 60 tonnes of food pass through the human GI tract. This includes an abundance of microorganisms from the environment which poses a major threat to gut integrity. The digestive process starts after the ingestion of food in the mouth where the food is grinded by

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teeth into smaller particles which are then emptied into the mouth. Due to the harsh environment in the stomach the microbial community that exists is at a low concentration of ~10^2 cells. Once food is passed from the stomach, the contents called chyme are emptied slowly into the small intestine. In the small intestine, the duodenum, jejunum, and ileum; food is mixed with digestive juices from the pancreas, liver, and intestine, and push the mixture forward for further digestion. In the small intestine the microbial community can reach between 10^4 to 10^6 cells (Figure 1). Thereafter, all non-absorbed nutrients and waste matter that was not absorbed or used; is passed into the colon where there are ~between 10^{12} to 10^{14} cells (Figure 7) [120].

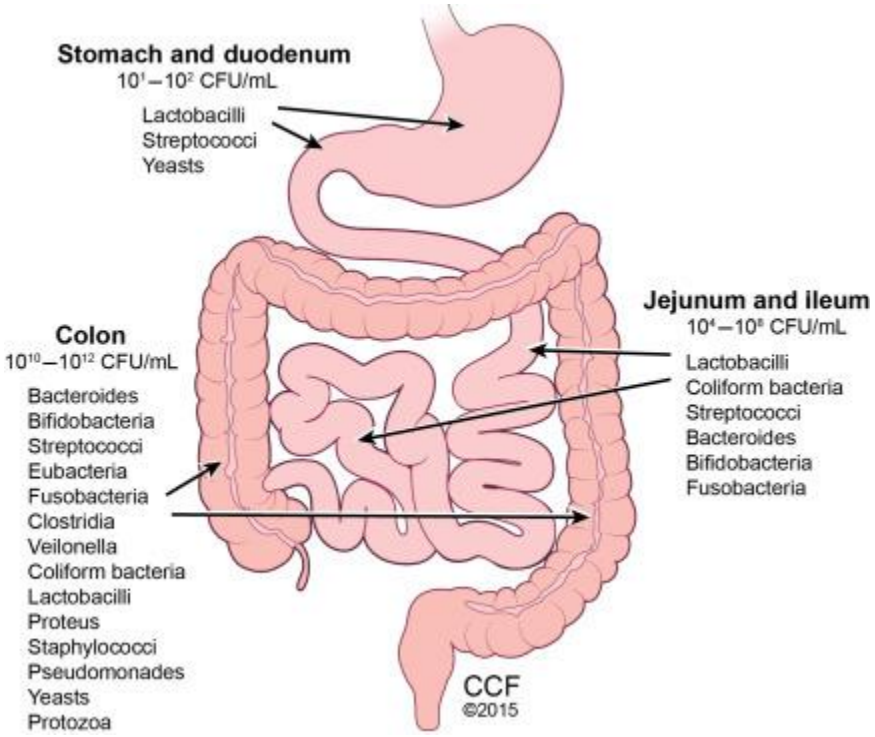

Figure 7. General composition of the human gut microbiome (Cresci & Izzo, 2018).

The microorganisms, bacteria, archaea and eukarya, that colonise the GI tract may exert countless benefits through a wide array of physiological functions. These may include but are not limited to improving gut integrity or shaping the intestinal epithelium, protecting against pathogens, harvesting energy, and regulating host immunity. However, there is potential for these mechanisms to be disrupted because of an altered microbial composition, known as dysbiosis [121], [122], [123], [124], [125].

Thursby and Juge, (2017) also added the role of gut microbiota in human health has gained increasing attention. Recent studies has shown that diverse groups of bacteria species colonise the gut, and the composition is strongly linked to every person's individual GI health. Additionally, there is growing evidence that indicates that by administering probiotics, the microbial ecosystem is modified, thereby exerting a variety of health benefits including a prevention and/or treatment of diseases (Gareau et al. 2010).

Microbial colonization of the GI tract mucosal tissue starts from infancy, these early life events have a long-standing consequence to the development of the human body and how it responds to diseases. During development from infancy, the developing microbiome is responsible for facilitating tolerance to environmental exposures or contributing to the development of disease, including inflammatory bowel disease, allergy, and asthma. Recent studies conducted by Gensollen et al.[126] stated that there is a critical period during early development wherein the disruption of optimal host-commensal interactions can lead to persistent and in some cases irreversible defects in the development and training of specific immune subsets.

The role of the microorganisms that form the microbiome is to facilitate metabolism, such as breaking down indigestible complex polysaccharides into essential nutrients such as vitamin K and

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B12, butyrate, and propionate [127]. The latter has been found to have a positive effect on the epithelial barrier integrity, which plays a crucial role in protecting microbiota from pathogenic microorganisms and avoiding inflammation in the gut [128]. Researchers that focus on the composition of the human microbiome have found that the most abundant genera of fungi in descending order of abundance in the human gut are Saccharomyces, Malassezia, and Candida [129]; with eight out of 15 genera comprising ascomycetes and approximately 5–65% comprising of Saccharomyces.

In recent microbiome studies, *Saccharomyces* strains have been observed in up to 96.8% of samples [130], [131], [132]. Since fungi are harboured in the gut environment, it follows that some resident species might provide a symbiotic benefit to the human host. The role of microbiome in the GI tract and its influence on human health has unlocked a significant area of interest, and further investigation, particularly the profiling of the African microbiome, is vital for further discovery in the development of niche treatment technologies for the global population.

9.2. Skin Microbiome

The skin is the largest and most external barrier of the body with the outer environment; therefore, it is considered the external interface between the body and the environment [133]. The skin is richly perfused with immune cells and heavily colonized by microbial cells, which in turn, trains immune cells and determines the well-being of the host. Also, it is worth noting that despite the skin covering many areas of the human body, the population and microbial concentration differs per area. It has also been found that a shift in population can also shift depending on the external environment, disease and diet [134].

The skin epidermis, including sweat and sebaceous glands, provide a total skin surface of about 25 m^2 and forms one of the largest epithelial surfaces for interaction with microbes [116]. Epithelial cells cover the surfaces of the body such as skin, airways, or the intestinal tract and provide an important link between the outside environment and the body interior [135].

Like those in our gut, skin microorganisms have important functions in the protection against invading pathogens, the teaching of our immune system and the breakdown of natural products [136], [137], [138], [139]. According to Byrd et al. [140], several skin microbiome assessment surveys have to date, used amplicon sequencing, however in recent years; major technical breakthroughs have occurred, which uses shotgun metagenomic sequencing. The advantage of using the latter approach is that it does not sequence specific target regions. This technique simultaneously captures all genetic material in a sample, including human, bacterial, fungal, archaeal and viral microorganisms, providing vital information on the microbial composition.

9.3. Case Studies Assessing the Use of Yeast Probiotics and Its Impact on the Host Microbiome

The microbiome of a healthy individual consists of balanced populations of both beneficial and harmful microorganisms [141]. These play a major role in providing the host with physiological, metabolic, and immune functions useful in warding off pathogens and any imbalance results in increased levels of harmful microbes. There is a mutual relationship between the gut and human flora. The colon harbors the highest population, however, only < 0.1% of these are fungi and *Saccharomyces* and *Candida* are the dominant genera [142], [143], [144]. As the most commercialized probiotic yeast, *S. boulardii* is widely used in treatment of gut-related diseases such as Traveler's diarrhea, AIDS-associated syndrome, irritable bowel syndrome and Crohn's disease. Oral administration of *S. boulardii* alone or in combination with other probiotics has proven to induce changes in the gut microbial combinations in various clinical reports [143], [145], [146], [147]. S. *boulardii* influences the host microbiome by direct inhibition of pathogenic intestinal microbes and normalizing the pH of the gastrointestinal tract, this is achieved by reducing the pathogenicity of toxic microorganisms [144].

A recent study representing the effect of the use of *S, boulardii* on the gut microbiota was reported by Yang et al. Yang et al. (2022). The potential of this probiotic yeast in treatment of non-alcoholic steatohepatitis (NASH) in mice through gut-liver axis was demonstrated. NASH is a non-alcoholic

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fatty liver disease associated with inflammation, damage, and presence of excess fat in the liver. Yang et al. [149], fed NASH-inducing diet [Methionine-choline-deficient (MCD)] to all test mice and the control group was given normal chow diet (NCD). Florastor®, a commercial product containing lyophilized *S. boulardii* CNCM I-745 as a main component was also administered by gavage to random mice (both on MCD diet and the control group) five days a week. After 8 weeks, the mouse fecal genomic DNA was extracted, sequenced, and analyzed. The positive effect of administering *S. boulardii* to MCD diet-fed mice was evident (summarized in Table 4) by the microbial composition presented at family level [148]. It was concluded that this probiotic played a role in restoring the gut microbiome diversity that was reduced by the diet. Additionally, the MCD diet resulted in changes in the mycobiome, dominated by *Pichia* and *Trichosporon*. This was an indication of the robustness of these fungal genera under severe conditions in the gut [148]. Furthermore, the positive impacts of the gut mycobiota on regulating functions of other human organs such as brain, pancreas, liver, and kidney as well as overall host immunity towards intestinal and extraintestinal diseases has been comprehensively reviewed by [150].

Table 4. Dominating microbial families in mice fed with normal chow diet, MCD only as well as MCD and *S. boulardii* at family level.

Normal chow diet	**MCD diet**	**MCD plus *S. boulardii***
Muribaculaceae	***Akkermansiaceae***	***Lachnospiraceae***
Ruminococcaeceae	***Erysipelotrichaceae***	***Atopobiaceae***
Lactobacillaceae	***Tannerellaceae***	***Ruminococcaceae***

Co-supplementation of multi-strain probiotic has also shown to have even outstanding benefits. The World Health Organisation stated that "mental health is critically important for everyone, everywhere" (WHO, 2002), the positive impacts of probiotics in cognitive performance were reported by Bloemendaal et al. [152]. This was determined by the increase in population of plant fibre degrading bacteria that produce short-chain fatty acids which are known for their beneficial effect on gut and brain health [152].

In another study, benefits of co-supplementation of bacterial (*Lacticasebacillus rhamnosus*) and fungal (*S. boulardii*) probiotics protected the gut microbiome post antibiotic administration *in vitro* [153]. Here, the human intestinal ecosystem was simulated using SHIME model. Three regions of the gastrointestinal tract were represented, upper part, proximal and distal colon. Mucin-covered mucosms were included in the proximal colon to simulate luminal mucus-associated microbiota and the parameters in the reactors were stabilized for two weeks. The study involved two healthy human adults who consented to give feacal samples. After inoculation, baseline conditions were established and then a 5-day antibiotic (amoxicillin and clavulanic acid) treatment was initiated. The study was conducted in parallel where one set was dosed with probiotics (*L. rhamnosus* and *S. boulardii*). Composition of the gut microbiota was then profiled. Although, the overall population was donor-dependent, there was a clear protective impact of the yeast probiotic towards *L. rhamnosus* against antibiotics. Furthermore, the presence of each or both probiotics significantly enhanced abundance of other *Lactobacillaceae*, *Bifidobacteriaceae* and *Lachnospiraceae*. This demonstrated the ability of probiotics to restore, stimulate and strengthen the composition as well as functionality of the microbial community negatively impacted by the use of antibiotics [153].

Functionality of yeasts as probiotics are not only limited to their use as whole cells therapeutics. A review conducted by Saber et al. [153], indicated that their metabolic by-products, such as folic acid and β-glucan may have an effect on cancerous cells, by being able to affect pathogenic bacteria, inactivate carcinogenic compounds particularly those derived from food, being able to improve

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intestinal barrier function, modulate host immune responses, antitoxic functions, apoptosis and anti-proliferative effects [4].

9.4. The Use of Yeast Probiotics in Skin Applications

Further to the limited instances of the use of yeasts as probiotics, employed as food supplements and/or additives, there are lesser studies that focus on the use of these organisms for skin applications. This scantity could be due to the fact that, *S. boulardii* the most commonly studied yeast probiotic, is mostly active in the colon and can grow at low pH levels (2.0-3.0); whereas the skin pH is 5.5. Other yeast genera such as *Candida*, *Cryptococcus*, *Epidermophyton*, *Hortaea*, *Malassezia*, *Microsporum* and *Trichophyton* are well-known for causing vaginal yeast infections, athlete's foot, jock itch, ringworm or thrush owing to their ability to ability to penetrate tissues [154], and limited report of beneficial yeasts are limited in contrast.

10. Conclusionary Remarks and Future Prospects

The use of probiotics has gained significant momentum in terms of advocation for use amongst the global population. Advent of genetic engineered probiotics may be more effective, cheaper production costs, higher stability and specificity for the treatment of a plethora of human ailments and disorders [3]. Yet, the application of these GMOs faces significant hurdles, particularly in terms of biosafety considerations upon ingestion by the host. Several clinical trials have been conducted to date; however, their effect cannot be guaranteed to achieve their intended effect, and therefore prevents effective deployment. With more in depth understanding into the human microbiome and its relation to disease mechanisms, the safety and endorsement of engineered probiotics, both bacterial and yeast, may gain acceptance for use, particularly, when conventional health strategies prove ineffective [3]. Additionally, in terms of advances in probiotic production and formulation, significant strides have been made to deliver highly efficacious probiotic treatments for the treat of several human metabolic disorders.

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