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Salmonella infection – prevention and treatment by antibiotics and probiotic yeasts: a review

Abraham Majak Gut,[1] Todor Vasiljevic,[1] Thomas Yeager[2] and Osaana N. Donkor[1,*]

Abstract

Global *Salmonella* infection, especially in developing countries, is a health and economic burden. The use of antibiotic drugs in treating the infection is proving less effective due to the alarming rise of antibiotic-resistant strains of *Salmonella*, the effects of antibiotics on normal gut microflora and antibiotic-associated diarrhoea, all of which bring a growing need for alternative treatments, including the use of probiotic micro-organisms. However, there are issues with probiotics, including their potential to be opportunistic pathogens and antibiotic-resistant carriers, and their antibiotic susceptibility if used as complementary therapy. Clinical trials, animal trials and *in vitro* investigations into the prophylactic and therapeutic efficacies of probiotics have demonstrated antagonistic properties against *Salmonella* and other enteropathogenic bacteria. Nonetheless, there is a need for further studies into the potential mechanisms, efficacy and mode of delivery of yeast probiotics in *Salmonella* infections. This review discusses *Salmonella* infections and treatment using antibiotics and probiotics.

INTRODUCTION

The global burden of morbidity and mortality from human enteric pathogenic bacteria, including *Salmonella* species, is immense, despite the presence of antibiotic drugs [1–3]. Research has estimated that *Salmonella* infection causes 2.8 billion cases of diarrhoea annually worldwide. *Salmonella enterica* serovar Typhi (*S.* Typhi), the causative agent of typhoid fever, is reported to cause 16–33 million infectious cases, with an estimated 500 000 to 600 000 deaths, while non-typhoidal *Salmonella* (NTS) infections account for 90 million cases and 155 000 deaths worldwide annually [4]. The incidence of *Salmonella* infections has been exacerbated by the high prevalence of human immunodeficiency virus (HIV) infections in Africa, and it has been reported that there are 2000–7500 *Salmonella* infection cases per 100 000 HIV-infected adults [5]. In Australia, 127 195 cases of *Salmonella* infection were reported to the National Notifiable Diseases Surveillance System (NNDSS) from 2000 to 2013; however, the real cases of salmonellosis were underestimated, as it has been assumed that for every case of *Salmonella* infection reported, there are seven cases of salmonellosis in the community that have not been reported [6]. In 2010, Australia reported 40 000 salmonellosis cases, 2100 hospitalizations, 6750 complications and 15 deaths [6]. In the USA, *Salmonella*

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Author affiliations: [1]Institute for Sustainable Industries and Livable Cities, College of Health and Biomedicine, Victoria University, Werribee Campus, PO Box 14428, Melbourne, Victoria 8001, Australia; [2]Institute for Sustainable Industries and Livable Cities, College of Engineering and Science, Victoria University, Werribee Campus, PO Box 14428, Melbourne, Victoria 8001, Australia.
***Correspondence:** Osaana N. Donkor, osaana.donkor@vu.edu.au
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Abbreviations: AAD, antibiotic-associated diarrhoea; AGA1, A-agglutinin anchorage subunit; AP, activator protein; B cells, lymphocytes from bone marrow; Caco-2, human colonic epithelial cell lines; cAMP, adenosine 3′, 5′-cyclic monophosphate; CD, cluster of differentiation; CED, cell death abnormality protein; CorA, magnesium transport protein CorA; CR3, complement receptor 3; DC, dendritic cell; EPS, extracellular polysaccharide; ERK 1/2, extracellular signal-regulated kinases; FAO, Food and Agriculture Organization; FIG2, factor-induced gene 2 protein; *fliC*, *Salmonella flagellin* gene; FLO, flocculation protein; G-CSF, granulocyte colony-stimulating factor; GIT, gastrointestinal tract; H antigen, flagella antigen; HIV, human immunodeficiency virus; IL, interleukin; IPEC, intestinal epithelial cell lines; JNK, c-Jun N-terminal kinases; kb, kilobase; Lg-FLO1, gene encoding flocculin; Lpf, long polar fimbriae; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MEL, mannosylerthritol lipids; MgtA, magnesium-transporting ATPase, P-type 1 for *S. typhimurium*; MgtB, magnesium-transporting ATPase, P-type 1 for *E. coli*; MSK1, mitogen- and stress-activated protein kinase-1; MUC1, mucin-like protein; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NNDSS, National Notifiable Diseases Surveillance System; NTS, non-typhoidal *Salmonella*; O antigen, capsular antigen; pH, potential hydrogen; PqsA, *Pseudomonas quinolone* signal gene A; *S.* Dublin, *Salmonella enterica* serovar Dublin; *S.* Enteritidis, *Salmonella enterica* serovar Enteritidis; *S.* Heidelberg, *Salmonella enterica* serovar Heidelberg; *S.* Newport, *Salmonella enterica* serovar Newport; *S.* paratyphi, *Salmonella enterica* serovar Paratyphi; *S.* Typhi, *Salmonella enterica* serovar Typhi; *S.* Typhimuriym, *Salmonella enterica* serovar Typhimurium; SAIF, *S. boulardii* anti-inflammatory factor; SAPK, stress-activated protein kinase; SCARF1, scavenger receptor class F member 1; SPI, *Salmonella* pathogenicity island; SREC, scavenger receptor from endothelial cells; T cells, lymphocytes that mature in thymus; T3SS, type III secretion system; Tafi, thin aggregative fimbriae; TcdA, *C. difficile* toxin B; TcdB, *C. difficile* toxin B; TLR, Toll-like receptor; TMP-SMX, trimethoprim/sulfamethoxazole; TNF, tumour necrosis factor; Trk, potassium uptake protein; *tvi*A, virulence polysaccharide biosynthesis protein for *S. paratyphi*; *tvi*B, virulence polysaccharide biosynthesis protein for *S.* Typhi; Ty21a, attenuated live *S.* Typhi vaccine; Vi, capsular polysaccharide vaccine for typhoid fever; Vi-rEPA, recombinant exoprotein A of *Pseudomonas aeruginosa* (Vi-rEPA)/*S.* Typhi vaccine; ZnuABC, zinc import ATP-binding protein.

is the leading cause of foodborne infections and associated medical costs amounted to $2.17 billion (for 1.4 million infections) in 2010 [7]. Bloodstream infections caused by *Salmonella enterica* in Asia accounted for 30 % of all community-acquired infections [8], while in Africa 29.1 % of community-acquired bloodstream infections were attributed to the same *Salmonella* species [9].

Antibiotics are becoming less effective against some bacterial pathogens, such as typhoidal *Salmonella* strains, and the rise of antibiotic-resistant bacteria means that there is a need for novel ways of preventing or treating infections caused by enteric pathogenic bacteria [10]. Studies on probiotics-based treatment/complementary treatment of *Helicobacter pylori* and *Clostridium difficile* have long been recognized as efficacious [11].

Probiotics are defined by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) as 'live micro-organisms which when administered in adequate amounts confer a health benefit on the host' [12]. Species of *Lactobacillus* and *Bifidobacterium* are the most commonly used probiotics in the treatment of infectious diseases, including antibiotic-associated and travellers' diarrhoeas. Other micro-organisms, including *Saccharomyces boulardii*), *Streptococcus thermophilus, Enterococcus faecium*, *Leuconostoc* species, *Escherichia coli* Nissle 1917 strain and *Bacillus* species, are being researched *in vitro* or in animals and human trials, or are being used in humans for prophylaxis or therapeutic purposes [10, 13–15].

Specific criteria have been set for micro-organisms to qualify as effective probiotics. These include adherence to host cells in the gastrointestinal tract (GIT), ability to exclude or reduce the adherence of pathogens to the GIT, stimulation of immunity and the ability to persist and multiply in the GIT (resistance to acidic gastric juice, basic pancreatic juice, lysozyme and bile salts). Furthermore, other criteria include the ability to produce acids, hydrogen peroxide and bacteriocins that are antagonistic to the growth of pathogens and the ability to co-aggregate to form a normal sustaining flora. They must possess some of these properties to qualify as probiotics. Moreover, probiotic micro-organisms should be non-invasive, non-carcinogenic and non-pathogenic [12, 16, 17].

The objective of this paper is to provide a critical review of *Salmonella* infections and current treatment of salmonellosis, and to understand the prophylactic and therapeutic potential of probiotic micro-organisms and their mechanisms of action in preventing and treating *Salmonella* and other enteric pathogens infections. In particular, this paper focuses on probiotic yeasts, although probiotic bacteria are also briefly discussed.

SALMONELLA: THE BACKGROUND

Salmonella is a genus of the family *Enterobacteriaceae*. It is a Gram-negative, non-spore-forming, rod-shaped and facultative anaerobic bacterium. *Salmonella* cells move by means of a peritrichous flagellum. They are 2–5 µm long by 0.5–1.5 µm wide and, depending on the serotype, the *Salmonella* genome ranges from 4460 to 4857 kb. The bacterium was first identified in a veterinary laboratory in the 19th century in the USA. *Salmonella* is a lactose fermenter (some sub-species) and a hydrogen sulfite producer, and is oxidase-negative and catalase-positive. It hydrolyzes urea, utilizes citrate and decarboxylates lysine as its sole carbon source [5, 7].

The genus is classified into two species: *Salmonella enterica* and *Salmonella bongori*. Biochemical and genomic analysis of *Salmonella enterica* has led to further classification into subspecies, including *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica* [7, 18, 19]. The clinically important *Salmonella* species are classified under *Salmonella enterica*, which is further classified into more than 2,579 serovars on the basis of their antigenicity [7, 20].

Salmonella species are harboured in the intestinal tract of humans and farm animals. Reptiles and insects also act as *Salmonella* reservoirs. Moreover, eggs, poultry meat, pork, beef, dairy products, nuts, vegetables and water act as sources of *Salmonella*. The risk of infection is high in low- and middle-income countries or societies, with more than 100 infections per 100 000 people per year [6, 7, 21, 22]. Some *Salmonella* serotypes are host-specific, while others can infect more than one type of warm-blooded animal [5]. The *S.* Typhi and *Salmonella enterica* serovar Gallinarum serovars are restricted to human and poultry hosts, respectively, whereas *Salmonella enterica* serotype Dublin (*S.* Dublin) and *Salmonella enterica* serovar Choleraesus are adapted to cattle and pigs, respectively, but can infect other warm-blooded animals. However, other serovars, such as *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis), are generalists and are able to infect any warm-blooded animal [5].

The bacterium can be transmitted through faecal–oral routes, where susceptible hosts may acquire *Salmonella* through contaminated foods and water and therefore transmissions can be controlled through foods and water [6]. Moreover, infection with *Salmonella* from food or water can also be prevented with vaccination. *Salmonella* vaccines include killed whole-cell, Vi, live oral Ty2la and Vi-rEPA. The use of vaccine may reduce infections, but availability, efficacy, safety and cost are some of the issues that hamper its use and effectiveness [22, 23].

SALMONELLA PATHOGENESIS

After the ingestion of contaminated food or water, *Salmonella* colonizes the distal ileum and proximal colon [24, 25]. The infective dose for salmonellosis that is capable of establishing infection in the mucosa of the small intestine ranges from 10^5 to 10^6 cells [26]. *Salmonella* uses its flagella as a mode of movement as well as chemotaxis to target cells, the enterocytes. In humans, *Salmonella* cells use type I fimbriae, including long polar fimbriae (Lpf) and thin aggregative fimbriae (Tafi), to adhere to enterocytes. Type IV pili are used by *S.* Typhi to attach to host cells [27]. Once *Salmonella* has adhered to the host cells on the apical side of M cells or enterocytes, it uses *Salmonella* pathogenicity islands (SPIs) – encoded type III secretion systems (T3SSs) – to be phagocytized into the receptive macrophages [27]. *Salmonella* cells can then be exocytosed into the interstitial spaces of the lamina propria, where they are randomly picked by macrophages, dendritic cells and polymorphonuclear cells and distributed to the host efferent lymph in the mesenteric lymph nodes before being transported to the spleen and liver via the bloodstream [28]. The attachment of *Salmonella* to the receptive epithelial cells and internalization into lamina propria causes inflammatory responses, including the release of pro-inflammatory cytokines. Pro-inflammatory cytokines cause acute inflammatory responses which lead to diarrhoea, ulceration and the destruction of the mucosa cells [29].

Apart from the invasiveness of *Salmonella cells*, enterotoxin and cytotoxin have been identified across all of the *Salmonella* sub-species. These toxins are reported to be similar to cholera toxins. Some of them have been found to be either heat-labile or heat-stable, and they have been reported to be associated with diarrhoea [30–32]. Enterotoxin was reported to induce the accumulation of fluid in the ligated murine ileal loop and was also found to have cytotoxic activity [33]. Cytotoxin inhibits protein synthesis, and it has been reported that it is responsible for intestinal mucosal surface damage, as well as enteric symptoms and inflammatory diarrhoea [25]. *S.* Typhi toxin is reported to be associated with persistent infection and the signs and symptoms of typhoid fever [34]. On the other hand, another study reported that there were no differences in virulence between mutant *Salmonella* without toxin phenotypes and wild-type with toxin phenotypes [35].

O antigen lipopolysaccharide (LPS) plays a role in the pathogenesis of *Salmonella* infections. All parts of LPS are important in the pathogenesis of *Salmonella,* but the length, structure, composition and surface roughness of O side-chains can influence the virulence. Failure to produce a full length of chain decreases virulence. The length of the chain influences resistance to the lytic action of the complement cascade. Furthermore, smooth surface strains are more resistant to the lytic action of the cascade than rough surface strains, and this has been postulated to be due to steric hindrance of complement cascade binding to the *Salmonella* cell [25].

Salmonella pathogenesis is also influenced by the virulence plasmids, which contain virulence genes. *S.* Typhimurium, *S* *S.* Dublin and *S.* Enteritidis virulence plasmids have been reported to be responsible for systemic dissemination of infection in the mesenteric lymph nodes, spleen and liver. It has been reported that virulence plasmids are commonly found in *Salmonella* isolated from human or animal organs or blood, rather than in faeces, food, or environmental samples [25].

Salmonella also possesses other virulence factors (including flagella and flagellin), superoxide dismutase and ion acquisition systems [36]. Flagella increase invasiveness due to the motility of *Salmonella*, while flagellin has been reported to induce an inflammatory response. Bactericidal reactive oxygen species that have been produced against intracellular pathogens by the host can be inactivated by *Salmonella* superoxide dismutase. Moreover, *Salmonella* produces ion acquisition systems for the acquisition or transport of iron, magnesium, zinc and potassium, where their concentrations are low. *Salmonella* produces siderophores, including enterobactin and salmochelin. These siderophores are critical in accessing limited iron in the host. *Salmonella* also uses CorA, MgtA and MgtB systems to acquire limited magnesium. ZnuABC and Trk systems are used for zinc and potassium uptake, respectively. All of these ions are critical for the survival and pathogenesis of *Salmonella* [36].

DISEASES CAUSED BY *SALMONELLA* INFECTIONS

Infection of humans with *Salmonella* results in three main infectious diseases, namely typhoid fever, paratyphoid fever and NTS. Typhoid and paratyphoid fevers are caused by *S.* Typhi and *Salmonella enterica* serovar Paratyphi (*S.* Paratyphi), respectively, and are characterized by gastroenteritis and complications such as septicaemia, immunological symptoms, leukopenia and neurological sympotoms. These typhoidal and paratyphoidal complications account for deaths [7, 34]. On the other hand, *S.* Typhimurium, *S.* Enteritidis, *Salmonella enterica* serovar Newport (*S.* Newport) and *Salmonella enterica* serovar Heidelberg (*S. Heidelberg*) cause NTS infections, which are restricted to gastroenteritis (nausea, vomiting and diarrhoea) or occasional bacteraemia (dissemination of infection in the body), and are usually non-fatal [7].

LABORATORY DIAGNOSIS OF *SALMONELLA* INFECTION

Blood culture is the gold standard method for diagnosis of *S.* Typhi and *Salmonella* Paratyphi infections [37]. Blood volume, duration of illness, the presence of bacteraemia and antibiotic treatment commencement can impact on the reliability of the result obtained from blood culture [23].

Salmonella is serologically classified into six serotypes, which are detected on the basis of their antigenicity. The Widal test method, which detects the presence of *Salmonella* O and H antigens, is another method that can be used to diagnose *Salmonella* infections and is useful in areas where resources are limited. This method does not differentiate *Salmonella* species or serotypes and can cross-agglutinate with other non-*Salmonella Enterobacteriaceae* bacteria. False-negative Widal tests have been reported and false-positive results may also be expected in patients with malaria, dengue and disseminated tuberculosis [23]. The enzyme-linked immunosorbent assay (ELISA), which detects IgM and IgG antibodies against *Salmonella* surface molecules, is another useful tool in the diagnosis of *Salmonella* infection. The Typhidot ELISA kit detects both IgG and IgM. Its sensitivity and specificity have been reported as >95 %, and 75 %, respectively. Typhidot-M, which only detects IgM, has a sensitivity of 90 % and a specificity of 93 % [23].

Validated molecular biology methods are also employed in the diagnosis of *Salmonella* infections from blood, faeces, foods and environmental samples [25]. The nested multiplex polymerase chain reaction method (PCR), which targets the *Salmonella* flagellin gene (*fliC*), polysaccharide capsule gene and virulence (*vi*) genes (*tviA* and *tviB*), is reported to offer better specificity, sensitivity and turnaround times compared to the other methods discussed [38].

TREATMENT OF *SALMONELLA* INFECTIONS BY ANTIBIOTIC DRUGS

Antibiotic drugs are critical in the treatment of infectious diseases and have considerably improved quality of life, in addition to reducing the mortality associated with bacterial infections. The selectivity of antibiotic drugs against invading bacteria ensures minimal harm to the patients and at the same time guarantees maximum eradication of the target bacteria [10].

NTS infections do not usually require treatment with antibiotic drugs, however complications such as meningitis and septicaemia do occur and require treatment with antibiotic drugs, including ciprofloxacin, ceftriaxone and ampicillin [22, 39]. Infections caused by *S.* Typhi and *S.* Paratyphi may involve serious complications and require treatment with antibiotics such as cefixime, chloramphenicol, amoxicillin, trimethoprim/sulfamethoxazole (TMP-SMX), azithromycin, aztreonam, cefotaxime or ceftriaxone to prevent death [23]. Dexamethasone is a corticosteroid drug and may be used when a complication such as delirium, obtundation, stupor, coma or shock occurs [23].

CURRENT ISSUES WITH THE USE OF ANTIBIOTIC DRUGS FOR TREATING *SALMONELLA* INFECTIONS

Bacterial infections have traditionally been treated with antibiotic drugs; however, certain bacterial species have developed resistance to current antibiotics. Bacteria with the ability to grow or survive in a concentration of antibiotic drug that is normally sufficient to be bactericidal or bacteriostatic are referred to as antibiotic drug-resistant bacteria, whereas antibiotic-susceptible bacteria are species that can be killed or have their growth inhibited by the recommended dose of antibiotic drug [40]. Resistance to an antibiotic drug may be innate or acquired through exposure of the bacteria to the antibiotic drug. Conjugation, transduction and transformation are the genetic mechanisms used by bacteria to acquire antibiotic-resistant genes. Conjugation involves the transfer of DNA on plasmids from one organism to another. In transformation, naked DNA is carried directly from one organism to another, while in transduction, the DNA is transferred by bacteriophage [40].

There is emerging resistance among *Salmonella* species to first-line antibiotic drugs, as well as to alternative medicines [21]. It was reported in Malawi in 2010 that 7 % of *S.* Typhi infection cases were multi-drug resistant, and in 2014 the figure increased to 97 % [41, 42]. In the USA, *S.* Enteritidis accounted for 50 % of ciprofloxacin-resistant infections, whereas *S.* Newport, *S.* Typhimurium and *S.* Heidelberg were reported to be responsible for 75 % of antibiotic-resistant infections, due to their resistance to ceftriaxone and ampicillin. The resistance of *Salmonella* species to antibiotic drugs has been shown to be serotype-specific according to metadata research [39].

The rise of antibiotic-resistance among pathogenic bacteria, including *Salmonella*, species is associated with a number of factors, including excessive use of antibiotic drugs as a result of easy access (over the counter and internet sales) in some countries [39]. The use of antibiotics for growth promotion in animal husbandry and for the protection of crops, together with poor hygiene practices, have also contributed to the overuse of antibiotic drugs, and hence resistance [10, 39, 40].

The inability to treat infectious bacterial diseases has resulted in high mortality and morbidity and substantial economic losses. It has been reported that in Europe, 25 000 people die and €1.5 billion is spent annually due to antibiotic-resistant infections, whereas in the USA, 23 000 deaths are reported and >$20 billion is spent on nosocomial antibiotic-resistant infections in hospitals in a year [40].

The effect of antibiotic drugs on the human microbiome is of great significance. Antibiotic drug use has been associated with interference with the normal flora, and as a consequence, disorders such as inflammatory bowel disease or allergies may happen due to the altered microbiome [10]. Furthermore, antibiotic-associated diarrhoea (AAD) is caused by changes to the microbiome resulting from the administration of antibiotics. This reduces carbohydrate digestion and short-chain fatty acid absorption and thus results in induced osmotic diarrhoea. Long hospital stay due to AAD contributes to the risk of nosocomial infections and is an increased economic cost [10].

PREVENTION AND ALTERNATIVE/ COMPLEMENTARY TREATMENTS OF *SALMONELLA* INFECTION BY PROBIOTICS

Probiotic micro-organisms exert their prophylactic and therapeutic properties against pathogenic micro-organisms in three main ways: they may modulate both innate and acquired immunity, act directly on the pathogens and produce antibiotic molecules [43]. These mechanisms of action are influenced by the probiotics metabolism, the cell surface molecules, the ability to secrete antibacterial molecules and the genetic makeup of the organisms [43].

Probiotic bacteria such as *Lactobacilli*, *Enterococci*, *Bifidobacteria*, *Pediococcus*, *E.coli, Streptococcus* and *Leuconostoc* species are normally found in the human GIT, where they form normal flora [44], and are commonly included in popular fermented functional foods to make their delivery easy [44–47]. Probiotic products can also be in the form of lyophilized capsules or powders or aqueous solutions [48]. Probiotic bacteria have been widely used in the treatment of infectious bacterial diseases and their efficacious application are summarized in Table 1. Apart from the treatment of infectious diseases briefly discussed below, these organisms confer other benefits, such as appropriate digestion, epithelial cell function, metabolism, enteric nerve function and angiogenesis to the host [10].

PROPHYLACTIC AND THERAPEUTIC EFFICACIES OF YEASTS

Yeasts are eukaryotes and are classified into two groups: ascomycetes and basidiomycetes [49, 50]. The ascomycetes division contains yeast species with probiotic potential, such as the genera *Saccharomyces*, *Schizosaccharomyces*, *Kluveromyces*, *Zygosaccharomyces* and *Devaryomyces* [49].

Studies have indicated that yeast can be used in the prevention and treatment of infectious bacterial diseases, including typhoid, paratyphoid and NTS. Currently, *S. boulardii* is the yeast strain being used as a probiotic [51–53], while other yeast species and strains have been proven to be efficacious in *in vitro* and animal trials [54]. In contrast to probiotic bacteria, which are affected by drugs that target enteric pathogenic bacteria, yeasts are not targeted when they are used as a complementary therapy [48]. Fig. 1 summarizes the antagonistic mechanisms of probiotics against bacterial pathogens. These mechanistic properties of probiotic yeasts against pathogens are discussed below and further studies are summarized in Table 2. Yeasts also have a wide range of other beneficial applications for humans, as illustrated in Fig. 2.

PROTECTION AND PRESERVATION OF TIGHT JUNCTIONS

Tight junctions are the apical epithelial layers that separate the interface lumen of the GIT and deep cell layers. It is composed of transmembrane proteins, cytoplasmic adaptors and the actin cytoskeleton. Tight junctions attach adjacent cells to each other and provide intercellular seals. They function as a physical barrier that prevents noxious objects, including pathogenic organisms, from entering into deeper layers within tissues. However, some micro-organisms, such as *Salmonella* species, have developed mechanisms to evade this barrier [55]. Probiotic micro-organisms, including yeast species, have been reported to not only maintain normal functions of the gut mucosa, but also protect it from toxins, allergens and pathogens. The protective effects of probiotics are attributed to cytoprotection, cell proliferation, cell migration, resistance to apoptosis, synthesis of proteins and gene expression [56]. *S. boulardii* is reported to inhibit pro-inflammatory cytokines such as IL-8 production by the host and prevent the activation of MAP kinases Erk1 /2 and JNK/ SAPK. *S. boulardii* anti-inflammatory factor (SAIF) was postulated to be responsible for tight junction protection and preservation. Furthermore, *S. boulardii* produce produces proteases that break down toxins produced by bacterial pathogens [57].

Inflammatory bowel diseases such as irritable bowel syndrome, gluten intolerance, gastroenteritis and *H. pylori* infections disrupt tight junctions and this can predispose the susceptible host to *Salmonella* and other enteric pathogen infections [56]. Mice with genetic and inducible colitis (hence disrupted tight junctions) were more prone to be colonized and infected by *S.* Typhimurium than mice without inflammatory diseases [58]. These inflammatory diseases are currently prevented and/or treated using *Saccharomyces* species [56] and this shows how yeasts may be used prophylactically in infection prevention with respect to enteropathogenic bacteria such as *Salmonella*. The infection rate was reduced in the yeast-treated group due to the protection of tight junctions through cytoprotection, cell proliferation, cell migration, resistance to apoptosis, synthesis of proteins and gene expression.

IMMUNOMODULATORY PROPERTIES

The immunomodulatory properties of probiotics are associated with their cell wall components, DNA and metabolites, and therefore their ability to elicit immunity may be independent of the viability of probiotics such as yeast cells [43]. The target host cells for immunomodulation by probiotics are enterocytes and gastrointestinal-associated immune cells. The sensitive cells can be stimulated due to the presence of β-glucan and mannose receptors for probiotic fragments or whole cells. The adhesion of probiotic organisms to sensitive cells or the production and release of soluble factors may modulate immunity or trigger signalling cascades in immune cells [43]. Yeast cell wall components, including mannoproteins and β-glucan, induce immunomodulatory responses when they interact with dentritic or other immune cells with receptors [59]. For example, the attachment of *S. boulardii* to dendritic cells (DCs) was reported to induce the secretion of immunoglobulins A and M and cytokines, including interleukin (IL)-1β, IL-12, IL-6, TNFα and IL-10. This immunomodulatory mechanisms was postulated to be due to tumour necrosis factor alpha (TNFα) and the transcriptional upregulation of C–C chemokine receptor type 7 mRNAs by yeast cells [60].

The cell wall components of *Saccharomyces cerevisiae*, including mannoprotein, act as nonspecific immune stimulators by interacting with macrophages through receptors. Yeast cell components, including β-glucan and mannoprotein, have adjuvant effects and can activate neutrophils, eosinophils, macrophages and complements [61].

The immunomodulatory properties of pathogenic fungal species are postulated to be due to the presence of β-glucan receptors on a susceptible host [62]. Beta-glucan is also

Table 1. Prophylactic and therapeutic properties of probiotic bacteria

Probiotic micro-organisms	Indicator enteric pathogens and/or animal models	Treatment mechanisms and outcomes	References
L.casei 11578, *Lactobacillus delbrueckii* ssp. *bulgaricus* 11 842 (*L. bulgaricus*), *Lactlbacillus fermentum* 1493 (*L. fermentum*) and the commercial probiotic product, PROB	Infection of neonatal broiler chicks with *S.* Enteritidis	Significant reduction of *S.* Enteritidis in the chick faeces in a time-dependent manner; feeding 24 h prior to infection was efficacious	[111]
L. casei, L. bulgaricus, Lactobacillus cellobiosus (L. cellobiosus), Lactobacillus helveticus (L. helvetticus) and *L. fermentum*	Infection of 1-day-old broiler chicks with *S. Enteritidis*	Reduced colonization of chicks' gastrointestinal tract	[112–114]
L. casei, L. bulgaricus, L. cellobiosus, L. helveticus and *L. fermentum*	Infection of neonatal broiler chicks with *S.* Enteritidis and *S.* Typhimurium	Caecal tonsils load of *S.* Enteritidis was reduced by 60–70 %, while the *S.* Typhimurium load in caecal tonsil was reduced by 89–95 % as a result of treatment with probiotic compared to control	[115]
Commercial probiotic – floraMax	Infection of chicks and poults with *S.* Heidelberg	Reduced colonization and hence lower recovery of *S.* Heidelberg from caecal tonsil from both treated chicks and poults compared to control chicks and poults	[116]
Lactobacillus rhamnosus (*L. rhamnosus*) GG (ATCC 53103) and *B. longum* 46 (DSM14583)	*V. cholerae*	Removed 68 and 59 % enterotoxin in an aqueous solution, respectively	[117]
Bifidobacterium longum subsp. *longum/infantis*	*E.coli* 0157: H7	Prevented the production of toxin in the caecum and translocation of toxin from the GIT to the blood stream and hence reduced mortality	[118]
Lactobacilli, *Bifidobacterium bifidum* strains Bb12 and *Lactobacillus kefir*	*S.* Typhimurium	Secrete molecules that prevent invasion of epithelial cells	[43]
Lactobacillus acidophilus (L.acidophilus)	*In vitro* trial using human colonic adenocarcinoma cell line infected with *S.* Typhimurium	Attenuation of inflammatory response triggered by *S.* Typhimurium infection	[119]
E.coli Nissle 1917	Stimulation of intestinal epithelial cell line	Suppression of TNF-α induced IL-8 transcription and production Only viable *E.coli* Nissle 1917 showed immunomodulation	[120]
L. fermentum ME-3 and ofloxacin antibiotic, *L. plantarum* cell-free extract with co-trimoxazole	*S.* Typhimurium	Prevented invasion of organs and completely eradicated *S.* Typhimurium	[121, 122]
E.coli Nissle 1917 (EcN)	Infection of Caco-2 cells with *C. perfringens*	IL-1 β, IL-6, G-CSF and GM-CSF production was significantly increased in the absence of EcN, but decreased in the presence of EcN	[123]
Lactobacillus rhamnosus G and *Bifidobacterium lactis* Bb12.	*E. coli and S.* Typhimurium *in vitro* experiment	Significant co-aggregation of pathogens with probiotic bacteria	[124]
E.coli Nissle 1917 and L. acidophilus	*E.coli* 0157:H7 and cell lines	Suppressed production of pro-inflammatory cytokines and inhibited *E.coli* 0157:H7 virulence	[125]
E.coli Nissle 1917	*S.* Typhimurium, *Yersinia enterocolitica*, *Shigella flexneri*, *Legionella pneumophila* and *Listeria monocytogenes*	The ability of these probiotic bacteria to inhibit invasion is not dependent on direct contact with the pathogen; rather it is due to the production of not-yet-identified molecules	[126]
Bifidobacterium longum Bar33 and *B. lactis* Bar30	Infection of Caco-2 cells with *S.* Typhimurium and *E. coli H10407*	Displaced pathogenic bacteria from attachment site of CaCo-2	[127]
E.coli Nissle 1917	*C. difficile* and *C. perfringens*	Inhibited production of and deactivated toxins	[123]
L. plantarum 299 v, *L. rhamnosus* GG, *Bifidobacterium lactis* Bb12 and *L. rhamnosus* LGG	Infection of human mucusa cells with enteropathogenic *E. coli*, *S.* Typhimurium ATCC 12028 and *Clostridium histolyticum* DSM 627	Competition for the same receptor in the GIT and stimulation of mucin production by probiotic resulted in inhibition of pathogenic bacteria adhesion to the GIT; probiotics also degrade carbohydrate receptors for pathogens, exclude pathogens by establishing biofilms, produce receptor analogue for pathogens to bind to instead of binding to host cells and prevent binding of pathogens by producing surfactants	[43, 128]
Genetically engineered *L. lactis*	*C. difficile* and *H. pylori* in mice	Elicited immunity by expressing non-toxic fragments of TcdA and TcdB and produced *H. pylori* lipoprotein Lpp20, which elicited immunity *in vivo* and therefore prevented or treated *H. pylori* infections	[129]
Single-strain *Lactobacillus* species	*E. coli, Enterococci faecalis, Enterococcus faecium, Enterpbacter cloacae, Streptococcus salivarius, Listeria monocytogenes, S. aureus, Proteus mirabilis, P. aeruginosa* and *Bacteroides thetaiotaomicron* in *in*	Inhibited growth due to antibacterial metabolites other than hydrogen peroxide because of inhibition in anaerobic condition	[130]

Table 1. cont.

Probiotic micro-organisms	Indicator enteric pathogens and/or animal models	Treatment mechanisms and outcomes	References
	vitro experiment		
Bifidobacterium breve (*B. breve*) strain Yakult	*E. coli* (STEC) O157: H7 in mouse model	*B. breve* inhibited stx gene production by STEC cells	[131]
Clostridium butyricum strain MIYAIRI	Enteropathogenic *E.coli* (EHEC) 0157: H7 in mouse model	Inhibited toxin expression by producing butyric and lactic acid and reduced viability of EHEC *E.coli* 0157: H7 by producing butyric acid	[132]
Lactobacillus strains, three *Pediococcus* strains and four *Bifidobacterium* strains	*E.coli* (EHEC) 0157: H7 in *in vitro experiment*	All probiotics inhibit toxin production due to the production of organic acid, which resulted in low pH	[133]
Bifidobacterium thermophilum RBL67	Human colonic fermentation model using HT29-MTX cell lines; infection with *Salmonella* and *in vitro* trial	Probiotic prevented invasion and protected epithelial lining; probiotic also prevented expression of virulence factors by *Salmonella*	[134, 135]
Feed-grade lactobacilli (TGI)	Poultry (broiler) infection with *Salmonella*	Consumption of probiotic increased liveability in *Salmonella*-infected broilers compare to the control	[136]
L. plantarum MTCC5690	An animal trial using mice infected with *Salmonella*	Consumption of probiotic in fermented milk stimulated immunity and prevented GIT colonization by *Salmonella* and hence prevented infection	[137]
L. salivarius 59 and *Enterococci faecium* PXN33	An animal trial using poultry infected with *Salmonella*	Prevented colonization of Git by *S.* Enteritidis when used as multi-strain probiotic	[138]
L. rhamnosus GG (2×10^9 organisms per day)	A human trial involving 400 adult travellers	Reduced traveller's diarrhoea to 3.9 % in the treatment group compared to 7.4 % in the placebo group	[139]
Genetically engineered *E. coli* Nissle 1917	Animal trial using turkey infected with *Salmonella*	Ninety-seven per cent lower carriage of Salmonella in the GIT in the treated group compared to the control group, postulated to be due to the production of antimicrobial molecules by *E. coli* Nissle 1917	[140]
Genetically modified non-pathogenic *S.* Typhimurium	*S.* Typhimurium and murine model	Protected murine model due to competition for nutrients with pathogenic strains	[141]

found in probiotic yeast species such as *S. cerevisiae* (in the cell wall) and therefore a non-pathogenic yeast species may have the potential to modulate cell-mediated and humoral immunity in a host with its receptors [60].

Among the host receptors that recognize β-glucan are complement receptor 3 (CR3), dectin-1, scavenger receptor class F member 1 (SCARF1), cluster of differentiation 36 (CD36) and cell death abnormality protein 1 (CED1) [which is found in nematodes and is similar to human scavenger receptor from endothelial cells (SREC)] [62, 63]. CR3 is an integrin dimer and is expressed by immune cells such as monocytes, macrophages, DCs, neutrophils and natural killer cells. Dectin-1 is primarily expressed by macrophages, dendritic cells and neutrophils, while SCARF1 is expressed on macrophages. The binding of stimulators such as β-glucan to the above receptors on immune cells elicits immune responses. Some of these immune responses have been found to include phagocytosis, oxidative burst, neutrophil degranulation, fungal killing and the production of inflammatory lipid mediators, cytokines and chemokines. Chemokines recruit and coordinate the activation of other immune cells, including T cells, B cells and natural killer cells [60, 62]. CD36 is a sensor for β-amyloid, modified low-density lipoprotein, bacterial diacylated lipoproteins and lipoteichoic acid. These receptors mediate the binding of *Candida albicans* and *Cryptococcus neoformans* to mammalian cells via β-glucan and induce inflammatory cytokines and chemokines. However, collaboration with Toll-like receptor 2 (TLR2) is needed in order for these receptors to induce immune responses [62].

Mannose receptor is expressed by activated macrophages. Mannose is also recognized by langerin and dectin-2 and these also act as its receptors. Stimulation of mannose receptors can lead to pro-inflammatory or anti-inflammatory responses. Langerin (also known as cluster of differentiation 207) is a receptor on Langerhans cells, whereas dectin 2 is a receptor for mannan on a fungal cell wall. The type of response is dependent on the yeast cell wall components (the presence of β-glucan and mannoproteins) and the host cell receptors. Additionally, dectin 2 has an affinity for α-glucan, while langerin has an affinity for chitin [64].

The immunomodulatory properties of yeasts was demonstrated in *S. boulardii*, which has strong immunomodulatory properties; it induced the production of immunoglobulin A (IgA), tumour necrosis factor-alpha (TNF-α) and many ILs, including IL-1β, IL-5, IL-6, IL-10 and IL-12, as well as downregulating the production of IL-8 expression by acting on the NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway in uninfected enterocytes and on mitogen-activated protein kinases (MAPKs) and AP-1activator protein-1 (AP-1) in *S.* Typhimurium-infected enterocytes [54, 65]. *S. boulardii* was shown to reduce the production of pro-inflammatory

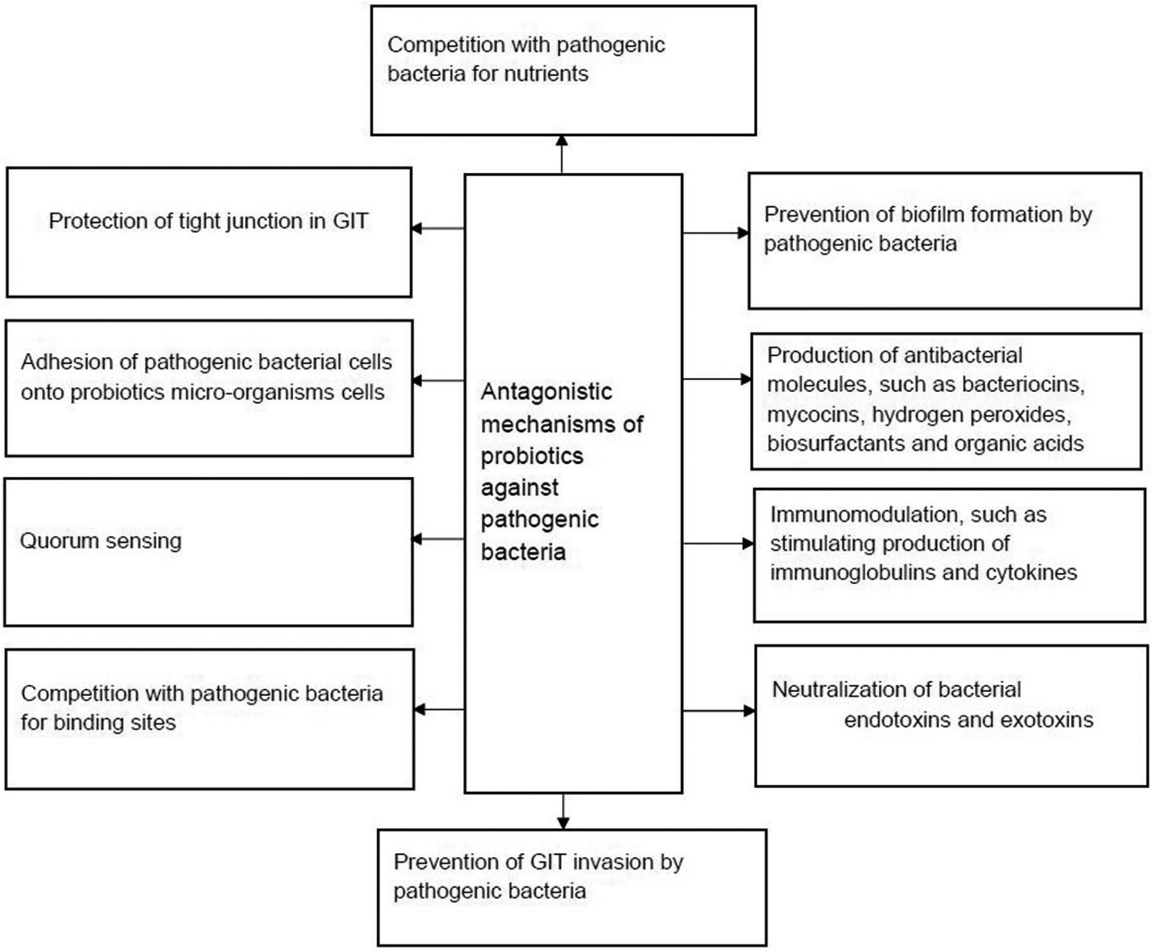

Fig. 1. Antagonistic mechanisms of probiotics against pathogenic bacteria [3, 52, 54, 70, 74, 86, 89, 92].

immune factors, including IL-6 and TNF-α, in a pathogenic *E. coli* infection colitis and it prevented *E. coli*-mediated apoptosis of T84 colonic cell lines [54]. In contrast to the above findings, the ability of *S. boulardii* to modulate immunity in healthy mucosa was reported to be minimal in research conducted on a murine model [59].

Yeast genera (including *Saccharomyces*, *Kluyveromyces* and *Issatchenkia*) isolated from kefir milk showed downward regulation of intestinal epithelial innate immune responses when cells were subjected to TLR ligands such as *Salmonella* flagellin and *E. coli* LPS. *Kluyveromyces marxianus* inhibited the expression of TNF-α and IL-1β cytokines by enterocytes when stimulated by LPS and flagellin. This yeast strain was also shown to block the NF-kB pathway and therefore inhibited pro-inflammatory cytokines, chemokines and the release of TNFα. The immunomodulatory ability of yeast species (especially *S. cerevisiae* CIDCA8112 and *Kluyveromyces marxianus*) isolated from kefir was shown to be dose-dependent. The viability of yeast cells was found to be a deciding factor in the downregulation of the innate response by human colonic epithelial cell lines (Caco-2). The inactivation of yeast strains by heat and UV irradiation completely destroyed the immunomodulatory effects [66, 67].

BINDING OF PATHOGENIC BACTERIA ONTO YEAST CELL WALLS

Cell adhesion is defined as a process whereby cells attach to each other or to a foreign surface with the aid of adhesins. In this context, foreign surfaces may include other biotic or abiotic structures [68]. In yeasts, adhesins are protein mosaics on the surface of cell walls which are involved in development, symbiosis and pathogenesis [69]. Currently, eight *S. cerevisiae* adhesins have been identified and these include FLO1, FLO5, FLO9, FLO10, FLO11 (or MUC1), FIG2, LgFLO1 and AGA1. The expression of these adhesins is determined by genetic factors, such as yeast species or environmental growth conditions, including growth medium, aeration or acidity [68, 69].

Table 2. Prophylactic and therapeutic properties of yeasts

Probiotic micro-organisms	Indicator enteric pathogens and/ or animal models	Treatment mechanisms and outcomes	References
S. boulardii	Human trials	Improved tolerance to number of calories per day, reduced incidence of diarrhoea, reduced number of treatment days and reduced duration of diarrhoea	[74]
S. boulardii	*Salmonella* and *E.coli* in rat model	Neutralized LPS and therefore reduced its toxicity in the rat model; inflammatory lesions and necrotic bodies were seen in the control's liver and heart	[98]
S. cerevisiae UFMG A-905 from Brazilian distilled spirit cachaça, *S. cerevisiae* 982 from cheese and *S. boulardii* from chicken faeces	PBMCs (peripheral blood mononuclear cells) and mouse model	Reduction of inflammation and IL-6, TNF-α, interferon gamma (IFN-γ) and IL-10 by *S. cerevisiae* UFMG A-905 production, and stimulation of type 1 T helper (th1) response by *S. cerevisiae* 982 Induced TNF-α and IL-10 production Reduced the serum level of IL-6 in a mouse colitis model. Immunomodulatory properties through reduction of inflammation and IL-6, TNF-α, Interferon gamma (IFN- γ) and IL-10 production	[54]
S. cerevisiae	*Salmonella* species in *in vitro* experiment	Viable yeast bind better to *Salmonella* than non-viable yeast	[71]
Pichia kudriavzevii RY55	*E. coli*, *Enterococcus faecalis, Klebsiella* sp., *S. aureus*, *Pseudomonas aeruginosa* and *Pseudomonas alcaligenes* in *in vitro* experiment	Mycoccins inhibited the growth of pathogenic bacteria	[86]
Candida krusei isolated from fermented vegetables	*E. coli, S.* Typhimurium, *S. aureus* and *Bacillus cereus* in *in vitro* experiment	The killer toxin produced by yeast inhibited the growth of pathogenic bacteria	[88]
Yarrowia lipolytica	*Bacterial species* in *in vitro* experiment	Produces organic acids, including a-ketoglutaric, a pyruvic, citric and isocitric acid, which may have bactericidal or bacteriostatic effects on bacterial growth	[142]
S. cerevisiae	*In vitro* experiment on *Enterobacteriaceae* and lactic acid bacteria	Bactericidal or bacteriostatic effects due to production of carbon dioxide, sulfur dioxide, a high concentration of ethanol and secretion of organic acids which in turn reduce pH	[142, 143]
S. boulardii	*V. cholerae*, *C. difficile* and *C. perfringens* toxins in mouse model	Minimized the effects of toxin fluid secretion, and decreased mucosal permeability, mucosal damage and the release of inflammatory cytokines when administered to mice before they they were given the cholera toxin, and deactivated or inhibited production of toxins by *C. difficile* and *C. perfringens*	[74]
S. cerevisiae and *C. albicans*	*Pseudomonas*, *Staphylococcus epidermidis* and *Burkholderia pseudomallei* in *in vitro* experiment	Quorum-sensing molecules (farnesol) prevented biofilm formation by *Pseudomonas* and *Staphylococcus epidermidis* and enhanced the efficacy of B-lactams against *Burkholderia pseudomallei*	[3, 100]
S. boulardii	Human trial in children	Decreased severity and duration of infectious diarrhoea in children and shortened acute diarrhoea by almost a day in a clinical trial	[10]
S. cerevisiae IFST062013 isolated from fruit juice	*In vitro* experiment on Gram-negative and -positive bacteria	Significant antibacterial effects in gram-negative than gram-positive bacteria compared to antibiotic doxycycline, while cell lysate was more potent than whole cells or supernatants; induced pro- and anti-inflammatory mediators simultaneously and as a result enhanced the maintenance of balance between Th1- and Th2-type cytokines	[89]

Using *S. cerevisiae* as a model, yeast cells have been found to form six different communal structures. Sessile non-adhesive cells that do not produce adhesins on a solid surface exposed to air can form non-adhesive colonies, especially on laboratory agar media. However, in a liquid medium, non-adhesive yeast cells exist as individual cells in a planktonic form that makes the media look turbid. Yeast cells can produce self-adhesion genes and therefore auto-aggregate without aggregating to foreign biotic or abiotic surfaces. Alternatively, yeast cells can produce adhesins for self-aggregation as well as an adherent to foreign surfaces and thereby form a biofilm. Furthermore, adhesin-producing yeast cells in liquid media can form flocs on the bottom or flor on the surface. Lastly, yeast cells can develop filaments when they produce adhesins and adhere to the bottom in liquid substrates [68].

Intimate binding of *S.* Typhimurium pili to yeast *S. boulardii* has been demonstrated by transmission electron microscopy [70]. The underlying mechanism of binding is postulated to be due to the presence of mannose-specific

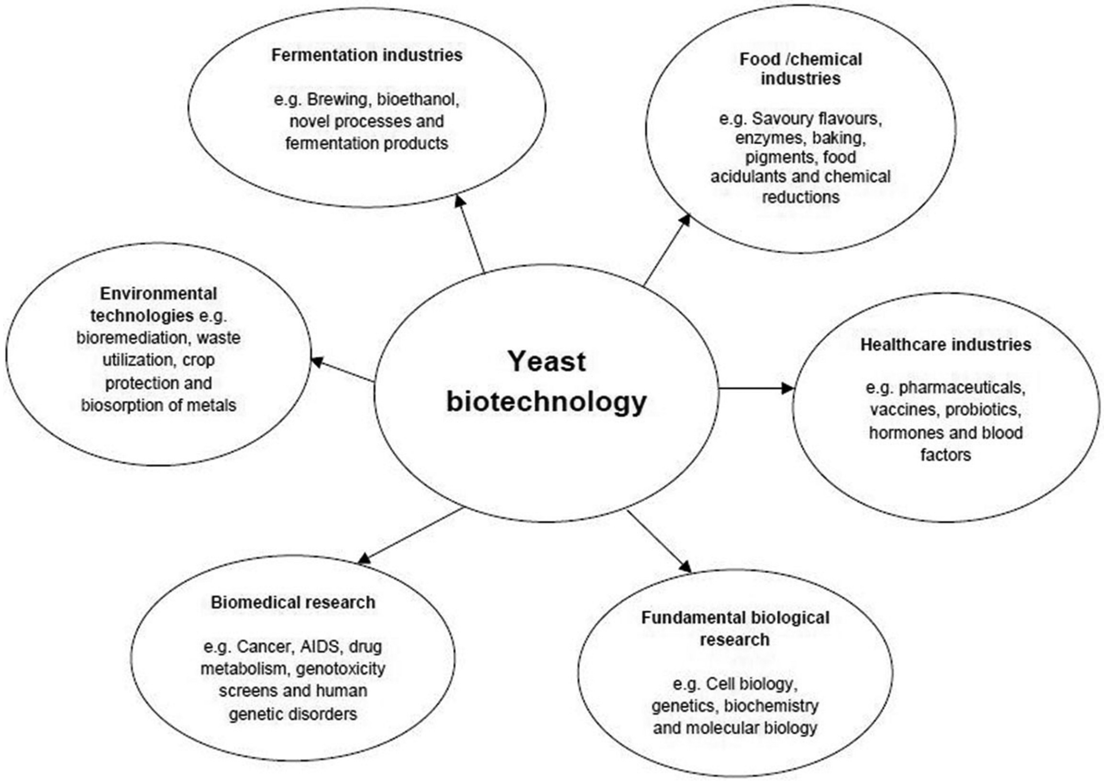

Fig. 2. Biotechnology applications of yeast [49].

adhesins/receptors such as fimbriae on bacteria cell walls that can bind to mannose on yeast cell walls. *S. boulardii* cell walls possess high mannose content and hence the capacity to bind bacteria pathogens with mannose-binding fimbriae [70, 71]. Bacterial pathogens, including *Salmonella* species, have been reported to bind better to probiotic yeasts than to parabiotic yeasts [71]. Moreover, adhesions of pathogenic bacterial cells onto yeast cell walls were found to be prominent when yeast growth was at the stationary phase compared to other growth phases [72]. The presence of sugars (including mannose, glucose and maltose media), and to some extent bile salts, in aqueous solutions was found to inhibit the binding of *S. boulardii* to pathogenic bacteria, including *Salmonella* species [70, 72]. Therefore, to improve the binding of *S. boulardii* to pathogenic bacteria, the consumption of foods or drinks rich in these sugars should be limited when yeast is used prophylactically or therapeutically.

Some bacteria, including *Salmonella* species, do show variation in the expression of fimbriae and therefore specific binding of yeast to the *Salmonella* may vary depending on the strains and/or genetic mutations [73]. Consequently, the efficacy of adhesion as a prophylaxis can be influenced by strains or genetic mutations that may occur over time.

Enteropathogenic bacteria, including *Salmonella* species and pathogenic *E.coli,* have been shown to preferentially and irreversibly bind to surfaces of *S. boulardii* [52, 71, 72]. The binding of pathogenic bacteria onto yeast cell walls limits their infectivity, since *S. boulardii* does not bind to the GIT; the bound bacterial cells pass transiently through the GIT and are excreted in the faeces [74].

The ability of *S. boulardii* to bind enteropathogenic bacteria is independent of viability; both probiotic and para-probiotic yeasts were shown to bind pathogenic bacterial cells [71, 75]. Interestingly, yeast species were reported not to bind to bacteria normally found in GIT, with the exception of the *S. cerevisiae* UFMG 905 strain, which bound *Bacteroides fragilis* [72]. *S. boulardii* has been reported to significantly reduce the internalization of *S.* Typhimurium in a human T84 cell monolayer when both yeast and the pathogen were applied together in an *in vitro* experiment [70]. Furthermore, *Pichia pastoris* X-33 and *S. boulardii* have been reported to reduce the binding of *S.* Typhimurium to human colorectal HCT-116 cells (by 47 and 37%, respectively) [76].

Mice infected with *S.* Typhimurium showed colonization along the GIT, but when the infected mice were administered with *S. boulardii*, the bacterial cells clustered around the yeast cells, which was indicative of the adherence of *S.* Typhimurium onto *S. boulardii* cells [65].

GROWTH INHIBITION

The growth inhibitory properties of probiotics, especially yeasts, against bacteria have been proposed to include the production of a high concentration of ethanol, the synthesis of killer toxins, pH changes, organic acid production and competition for nutrients [77].

Competition for nutrients is considered to be the most important antagonistic property of yeast against other fungi in the context of postharvest fungal pathogens in fruits; yeast species have the capacity to quickly deplete glucose, fructose and sucrose, and therefore suppress the growth of other micro-organisms [77]. Moreover, some yeast species possess iron sequestering molecules that give them a competitive advantage to deplete iron, which is needed for growth and pathogenesis by many pathogens [77].

Killer toxins, also called mycocins, are extracellular proteins, glycoproteins or glycolipids that are produced by yeast species against other yeast species with receptors for the toxins. The toxins genes are carried on extra-chromosome elements, including double-stranded RNA virus and double-stranded linear DNA, or on a chromosome [77]. The toxins kill susceptible yeasts but do not affect the producer. The mechanism of action of killer toxins involves the inhibition of beta-glucan synthesis or the hydrolysis of beta-glucan in the cell wall of the target yeast, the inhibition of DNA synthesis in the target yeast, the cleavage of tRNA, the inhibition of calcium uptake and the leakage of ions due to the formation of channels on the cytoplasmic membrane [77]. Killer toxins are large glycoprotein molecules and consequently have the potential to induce unwanted immune responses in the host [78], and therefore further studies on molecular size and possible modification are needed with regard to antigenicity and toxicity before these toxins are used therapeutically[79]. Several yeast genera, including *Saccharomyces*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, *Torulopsis*, *Williopsis* and *Zygosaccharomyces*, can produce killer toxins [77].

Yeast metabolites such as sulfur dioxide, carbon dioxide and ethanol have been postulated to have antagonistic effects on enteropathogenic bacteria. Sulfur dioxide, which can be produced by yeasts during fermentation, when dissolved in aqueous medium, produces sulfuric acid, which lowers the pH and therefore exerts its bactericidal or bacteriostatic effect. Furthermore, sulfuric acid is postulated to block microbial enzyme activity through the reduction of disulfide linkage, resulting in an antagonistic property against micro-organisms [80]. Moreover, the antibacterial property of carbon dioxide produced by yeast during fermentation is attributed to its dissolution in aqueous solution, which lowers the pH [81, 82]. Ethanol, a product of yeast metabolism, disrupts bacterial cell membranes through the denaturation of proteins and the dissolution of lipids, subsequently causing the lysis of bacterial cells in an *in vitro* experiment [83]. Concentrations of carbon dioxide and ethanol that are bactericidal may also be harmful to host cells. Ethanol has been reported to affect red blood cells physically and biochemically. Ethanol-induced membrane fluidization, decreased haemogloblin content and concentration in the cytoplasm have been reported [84]. Furthermore, it has been reported that ethanol has negative effects on neurons, hepatocytes and enterocytes [85], and therefore further studies are needed before potential therapeutic application.

A study on *Pichia kudriavzevii* RY55 found that mycocins produced by this yeast species have growth inhibition effects on potential bacterial pathogens, including *E. coli*, *Enterococcus faecalis*, *Klebsiella spp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Pseudomonas alcaligenes.* However, the optimum temperature and pH for the toxins were lower and higher, respectively, than in the normal human gut environment. The maximum activity of the enzyme was observed at 30 $^{\circ}$C and pH 5 [86]. Moreover, a killer toxin produced by *Candida krusei* that was isolated from fermented vegetables showed growth inhibition towards *E. coli*, *S.* Typhimurium, *S. aureus* and *Bacillus cereus* [87]. It has been reported that the killer toxin produced by *Williopsis Saturnus* shows a lack of bactericidal activity against *Streptococcus pneumoniae* [88].

S. cerevisiae IFST062013 isolated from fruit juice demonstrated moderate antibacterial activity compared to antibiotic doxycycline; the antagonistic effect was more pronounced against Gram-negative than Gram-positive bacteria. Moreover, a comparison of the effects of whole cells, cells lysates and supernatants indicated that cell lysates were more potent, which may be indicative of the antibacterial properties coming from the cell components rather than extracellular secretions. Nonetheless, the yeast species was reported to produce killer toxin and siderophore, and showed strong inhibition of bacterial biofilm formation [89].

PREVENTION OF INVASIVENESS AND SYSTEMIC INFECTION

The attachment of enteric bacterial pathogens, especially *Salmonella*, to receptive epithelial cells leads to internalization and hence infection, leading to symptoms and signs, including diarrhoea, ulceration and the destruction of the mucosa cells [29]. One of the mechanisms that has been proposed to explain how probiotics prevent invasion is competitive exclusion. This is defined as the ability of normal flora or probiotics, including yeast species, to limit the colonization of GIT, competing with invading pathogens by creating a restrictive physiological environment due to the production of antagonistic molecules and competition for binding sites and nutrients [90].

Lactobacillus kefiri CIDCA 8348, *L. plantarum* CIDCA 8327 and *Kluveromyces marxianus var. marxianus* CIDCA 8154 isolated from cheese whey fermented with kefir grain reduced the invasiveness of Caco-2/TC7 cells by *S.*

Enteritidis CIDCA 101. The precise mechanism and which of the probiotic micro-organisms (if it was not a synergistic effect) is responsible for the prevention of enterocyte invasion could not be explicitly identified in the research, as the three probiotic micro-organisms were used together [91]. *S. boulardii* prevented the invasiveness of *S.* Typhimurium and subsequent translocation to the spleen and liver in treated mice compared to untreated control mice, which had high bacterial counts in these organs [65].

BIOFILM FORMATION INHIBITION

Biofilms are defined as communities of micro-organisms attached to biotic or abiotic surfaces [68]. Bacterial biofilm formation occurs in stages, including the reversible attachment of bacterial cells on abiotic or biotic surfaces using forces such van der Waal forces. This is followed by hydrophilic/hydrophobic interactions between bacterial flagella, fimbriae, LPS or adhesive proteins with the receptive surfaces. When the bacteria have been irreversibly attached, the production of extracellular polysaccharide (EPS) and extracellular DNA proliferation occur. The final stage involves the maturation of the biofilm and subsequent dispersal for establishment at another site [92].

Biofilm formation in the GIT and other associated organs such as the liver is one of the virulence factors of bacterial pathogens, including enteropathogenic strains. It has been reported that biofilms account for more than 60 % of microbial infections in humans, and these infections are difficult to treat because of the antibiotic-resistant nature of micro-organisms in biofilms [92]. Typhoidal *Salmonella* infection, persistence and the asymptomatic carrier state are associated with biofilm formation in the gallbladder [93]. About 2–5 % of typhoid patients developed persistence and the asymptomatic carrier state as a result of biofilm formation [94].

Alpha-amylase, an enzyme produced by yeast cells, has been reported to prevent bacterial pathogen biofilm formation [92]. Moreover, other mechanisms, such as the creation of restrictive physiological environment by probiotics, result in competition for binding sites and nutrients, which also prevents biofilm formation [90].

It has been reported that at 10, 20 and 100 $\mu g\ ml^{-1}$ doses of alpha-amylase decreased *S. aureus* biofilm formation by 72 %, 89 and 90% respectively, while it was able to reduce matrix formation by 82 % in an *in vitro* experiment [92]. *S. cerevisiae* and *Saccharomyces kluyveri* produce alpha-amylase [95], and so yeast probiotics may potentially be used to produce this enzyme to inhibit biofilm formation and thus prevent carrier stage development in patients infected with *S.* Typhi.

EFFECTS ON BACTERIAL TOXINS

Enteropathogenic bacteria, including *Clostridium perfringens* , *S. aureus*, *Vibrio cholerae* , *Shigella dysenteriae*, *C. difficile* and *E. coli* (Shiga toxin-producing), as well as *Salmonella* species, produce toxins in the gastrointestinal tract. The expression of the *Salmonella* enterotoxin (*stn*) gene, which encodes a 29 kDa protein, is a hallmark of *S.* Typhimurium virulence. The toxin is responsible for symptoms that include nausea, vomiting, abdominal pain, fever and diarrhoea [33, 96].

V. cholerae pathogenesis involves the activation of adenosine 3′, 5′-cyclic monophosphate (cAMP). Likewise, adenylate cyclase in the cytoplasmic membrane in enterocyte activation is mediated by *Salmonella* enterotoxins which lead to a high concentration of adenosine monophosphate [25]. This high concentration of adenosine monophosphate causes a loss of intestinal fluid. *S. boulardii* is reported to inhibit cholera toxin-stimulated chloride secretion through the reduction of cAMP [97], and therefore this ability of *S. boulardii* to inhibit chloride secretion and subsequent fluid loss due to *V. cholerae* toxin may well have similar effects on *Salmonella*-associated diarrhoea, since *Salmonella* toxin is genetically, immunologically and functionally similar to *V. cholerae* toxin [25, 97].

*S. boulardi*i has been reported to deactivate or inhibit the production of toxins by *C. difficile* and *C. perfringens. S. boulardii* produces serine protease with proteolytic activity against *C. difficile* toxins [74]. Furthermore, *S. boulardii* minimized the effects of toxin fluid secretion, decreased mucosal permeability, decreased mucosal damage and decreased the release of inflammatory cytokines when administered to mice prior to them being given the *V. cholerae* toxin [74].

The ability of yeast to bind or neutralize bacterial toxin is possibly probiotic strain-specific. *S. cerevisiae* LV02/CNCM I-3856 provided no protection when porcine IPEC-1 (intestinal epithelial cell lines 1) was infected with enterotoxigenic *E.coli*. The integrity of the IPEC-1 barrier was disrupted, which indicates that this strain does not act on the *E.coli* toxin [54].

LPS, an endotoxin of *Salmonella* and *E.coli*, is associated with sepsis, which can be life-threatening [96]. Alkaline phosphatases, an enzyme produced by *S. boulardii*, was shown to neutralize LPS and reduce its toxicity in a rat model, as well as reducing inflammatory lesions and necrotic bodies in the liver and heart of the treatment group compared to the control group [98].

EFFECTS OF QUORUM SENSING ON PATHOGENS

Micro-organisms produce extracellular compounds that measure microbial population density in the surrounding area and, as a result, regulate their population. This phenomenon is referred to as quorum sensing [99]. Quorum sensing in poly-microbial populations has both synergistic and antagonistic effects. When quorum sensing compounds such as farnesol, *N*-Acyl homoserine lactones, tyrosol and dodecanol are produced in sufficient quantities they cause the expression of genes within the population. Genes expression results in microbial growth mode, virulence gene expression, biofilm formation or morphological changes [3].

The quorum-sensing molecules produced by micro-organisms not only affect poly-microbial communities, but also the hosts. The immunomodulatory properties of farnesol have been documented, including stimulation of the NF-κB pathway through MEK1/2-ERK1/2-MSK1-dependent phosphorylation of p65, which leads to the production of cytokines, namely IL-6 and IL-1α [3]. However, on a negative note, the alteration of monocytes to dendritic cells by farnesol has been reported. In brief, the effects of farnesol on immune cells lead to reduced ability to recruit and activate T cells and hence compromised immunity [3].

Farnesol, an alcohol derivative produced by *S. cerevisiae* or *C. albicans*, has been shown to prevent bacterial biofilm formation [3, 100]. Farnesol was reported to antagonize the production of quinolone signal via the inhibition of *Pseudomonas* quinolone signal gene A (PqsA). Furthermore, farnesol has the potential to be used as a complementary therapy for bacterial infections. It was shown to increase the susceptibility of *S. aureus* to antibiotics and had synergistic effects on the efficacy of nafcillin and vancomycin in the prevention of biofilm formation by *Staphylococcus epidermidis*. Additionally, farnesol enhanced the efficiency of B-lactams against *Burkholderia pseudomallei* [3].

An*in vitro* experiment in murine showed that macrophage cell line RAW264.7 acted in synergy with farnesol and yeast cell walls to increase the expression of pro-inflammatory cytokines [3].

ANTIBACTERIAL PROPERTIES OF YEASTS BIO-SURFACTANTS

Bio-surfactants, also referred to as glycolipids, are compounds made up of one or two sugar molecules, especially glucose or galactose residues in alpha or beta configuration on a lipid backbone. Bio-surfactants are found in bacteria, fungi, plants and animal cell membranes such as glycosylceramides, diacylglycerolglycosides and sterylglycosides [101]. Bio-surfactants are classified as rhamnolipids, sophorolipids, trehalolipids and man-nosylerythritol lipids. These bio-surfactants are produced by micro-organisms, some of which are probiotic bacteria or yeasts [102, 103]. These bio-surfactants have been reported to be functional in bioactive compounds such as glycosylceramides, sphingolipids, glycosphingolipids, sphingosines and ceramides. Their bioactivity has been associated with anti-proliferative responses, such as the inhibition of cell growth, proliferation, differentiation, interruption of the cell cycle, signal transduction, senescence transformation, inflammation and apoptosis [101].

Phytosphingosine, an endogenous bioactive molecule in fungi, plant and human skins, has been shown to inhibit Gram-positive bacteria growth and also has anti-inflammatory properties. Moreover, sphingolipids such as cerebrosides and gangliosides have antibiotic properties, in that they can bind pathogens or their toxins and remove them from the GIT [101].

Biosurfactants have been reported to prevent pathogenic bacteria adhesion from infection sites as well as biofilm formation. *Candida sphaerica* UCP 0995 biosurfactant, also known as lunasan, has anti-adhesive properties against some gram-positive bacteria, including *S. aureus* and *Streptococcus agalactiae*, while the polymeric biosurfactant produced by *Candida lipolytica* UCP 0988 has anti-adhesive properties against *S. aureus, Lactobacillus casei* , *Streptococcus mutan* and *E.coli* [101]. Mannosylerthritol lipids (MEL) and cellobiose lipids produced by fungi have antibacterial activities through the disruption of cell membranes, which leads to cell lysis. MEL types A and B produced by *Candida antarctica* and *Schizonella melanogramma* have antagonistic properties against gram-positive and gram-negative bacteria [101].

PROBLEMS ASSOCIATED WITH THE PROPHYLACTIC AND THERAPEUTIC USE OF PROBIOTICS

The safety of probiotic products is an important aspect that needs consideration before they are used. *S. boulardii* is generally safe when used in a healthy population; however, in 2012, 100 cases of fungaemia were reported worldwide in individuals with gastrointestinal track issues and those who were immunocompromised [51]. *Saccharomyces* fungaemia is critically severe in patients with gastrointestinal diseases [51]. Moreover, an allergic reaction from the administration of *S. boulardii* was been reported in an infant who had previously been diagnosed with food protein-induced enterocolitis syndrome [104].

Candida species have also been reported to possess virulence factors, including glycosidases, proteases, haemolysin, lipases and phospholipases [105]. The ability of yeast species to exist in a dimorphic form (e.g. through the formation of hyphae) has been reported to be one of their virulence factors, and both the *Saccharomyces* and *Candida* species have been shown to form hyphae [106]. The formation of hyphae was found to be triggered by nutrient deficiency, as well as the presence of 0.5 % isoamyl alcohol [107]. This is of great significance when kefir is used as a probiotic. Kefir is a probiotic low-content alcoholic drink [46], and therefore the potential for yeast species to develop hyphae is a safety risk and needs further research. Moreover, yeasts also have negative impacts on humans, including being food spoilers [52].

Prophylactic and therapeutic use of probiotic bacteria in infectious diseases caused by pathogens such as *Salmonella* has some drawbacks due to the risk of multi-drug resistance gene acquisition [45]. Antibiotic-resistant genes have been detected in *Enterococci* and *Lactobacillus lactis* [74]. Both *Bacillus subtilis* and *E.coli* Nissle 1917 are known to be susceptible to most antibiotic drugs and therefore pose no risk of antibiotic resistance, and so are safe to use as probiotics in prophylaxis, however their susceptibility to antibiotics makes these bacteria unsuitable for complementary therapy in infectious bacteria treatment [108, 109]. Furthermore, probiotic bacteria have been implicated in sepsis and endocarditis in patients who are immunosuppressed or predisposed to translocation and systemic dissemination of bacteria [110]. These issues associated with probiotic bacteria make their use less attractive in infectious bacterial diseases and hence there is a need for alternative probiotic micro-organisms.

EFFECTS OF PROBIOTIC PRODUCT FORMULATION ON EFFICACY

Probiotics are commonly included in popular fermented functional foods, such as yoghurt, milk, cheese, soybean, fruits, sourdough, kefir and vegetable products, making their consumption easier and more enjoyable, while at the same time providing prophylactic and therapeutic benefits to consumers [44–46]. Probiotic products can also be in the form of lyophilized capsules or aqueous solutions. The survival of probiotics in lyophilized form during delivery in *in vivo* experiments has been reported to be higher than that observed in the aqueous suspension form [48]. However, 7–16 days at the optimum temperature (between 15–25 °C) is needed to resuscitate lyophilized yeasts cells. These requirements do not fit the temperature in the human GIT or the time period that substances stay in there, which may make the lyophilized yeast probiotic products less effective [48].

Furthermore, studies have shown that *S. boulardii* exhibited different revival rates in lyophilized form (between 50 and 60 %), whereas *S. cerevisiae* was found to have an even lower revival rate of about 20 % in aqueous solutions. These differences in the revival rates could be due to the different freeze-drying methods used by different manufacturers. Previous studies on *S. boulardii* and other *Saccharomyces* species that examined survival and recovery from different preserved forms showed diverse kinetics, such as viability for long storage times, revival and survival in the GIT. Despite this variability, lyophilization is the preferred method of preservation [48].

CONCLUSION AND FUTURE PERSPECTIVES

Probiotic bacteria and yeasts are currently used for prophylaxis and complementary therapy against infectious and non-infectious diseases. The rise of antibiotic resistance and the potential of probiotic bacteria to carry antibiotic-resistant genes, coupled with opportunistic pathogens, has increased the need for alternative biotherapeutic drugs. Yeast species isolated from various sources have antagonistic properties against enteric bacterial pathogens. The antagonistic mechanisms have been reported in many *in vitro* experiments and a few animal trials. The use of yeasts in humans as s probiotic is very limited. Currently, *S. boulardii* is the only probiotic yeast used for prophylaxis and therapies in various ailments, but it has been implicated in fungaemia and allergic reactions. Other yeast species with prophylactic and therapeutic potential with respect to infectious diseases such as *Salmonella* need further research.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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