Multi-Strain Probiotics

Subjects: Microbiology

Contributor: Iliya Kwoji

Multi-strain probiotics are composed of more than one species or strains of bacteria and sometimes, including some fungal species with benefits to human and animals' health. The mechanisms by which multi-strain probiotics exert their effects include cell-to-cell communications, interactions with the host tissues, and modulation of the immune systems.

Keywords: antibiotics ; probiotics ; cell-cell communication ; synergy ; antagonism

1. Introduction

The Food and Agriculture Organization/World Health Organization (FAO/WHO) working committee on probiotics defined probiotics as "live microorganisms which when administered in adequate amounts confer health benefits on the host" [1]. The use of probiotics is increasing due to consideration as a suitable option following restrictions on antibiotics as growth promoters in the livestock industries by many countries [2]. There are different forms of probiotics preparations, and sometimes, their efficacy depends on whether they are single- or multi-strain preparations [3]. Compared to single-strain preparations, multi-strain probiotics contain more than one strain of the same species, genera, or multiple genera and sometimes including both bacteria and fungi (*Saccharomyces* species) [4]. Some single-strain probiotics are beneficial in alleviating gastrointestinal-tracts-associated diseases [5]. However, previous *in-vitro* studies showed that some multi-strain probiotics could exhibit better inhibitory effects on entero-pathogens [6] and enhanced benefits by combining effects of different strains compared to their single-strain preparations [7].

2. Mechanisms of Action of Probiotics

The mechanism of probiotics' actions is the various means by which they exert their beneficial effects on the host, including immune modulation, stimulation/modulation of gut microbiota, stimulation of digestive enzymes, displacement of pathogens, and production of bioactive compounds [8][9][10]. The gut-associated actions are the principal effects of probiotics, also regarded as the basis of other health benefits [11] as summarized in Figure 1.

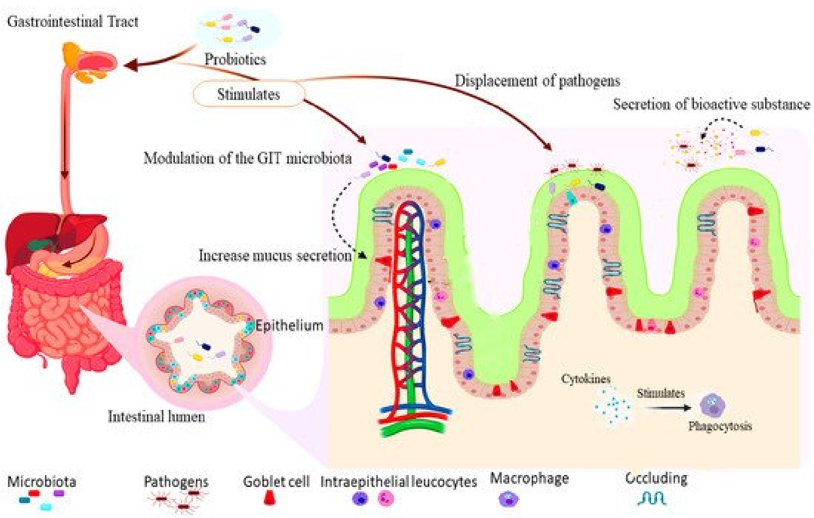

Figure 1. shows the mechanism of actions of probiotics: the intake of probiotics stimulates an increase in the secretion of mucus by goblet cells, mobilization of intraepithelial leucocytes, and tightening of the tight junctions to protect against the invasion of pathogens. The increase in mucus secretion and improvement of gut microbiota enhances competitive displacement and inhibition of pathogens adhesion to the gut epithelial surface. Furthermore, the action of bioactive substances such as lysozyme and cytokines stimulate phagocytosis by macrophages.

3. Applications and Biological Functions of Multi-Strain Probiotics

3.1. Treatment of Diseases

Different randomized control clinical trials revealed that some specific probiotics are useful in the therapeutic management of gastrointestinal (GI) illnesses like inflammatory bowel disease (IBD) [12], irritable bowel syndrome (IBS), and pouchitis [13][14]. The administration of multi-strain probiotics containing different *Lactobacilli* species, *Streptococcus* and *Bifidobacterium,* to patients who have systemic sclerosis alleviates the symptoms of gastrointestinal reflux and increased microbial alpha diversity group [14] (Table 1).

Table 1. The use of multi-strain probiotics in disease treatment.

Probiotics Mixture	Conditions	Mechanism of Actions	References
B. bifidum W23, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *Lactococcus lactis* W19 and *L. lactis* W58	Endotoxins	Improvement of endothelial barrier, inhibition of mast cell, activation of proinflammatory cytokines, and decrease endotoxin	[15]
L. acidophilus, *L. casei*, *B. bifidum*, and *L. fermentum*	Cognitive function in Alzheimer's disease		[16]
L. paracasei DSM 24,733, *L. plantarum* DSM 24,730, *L. acidophilus* DSM 24,735, and *L. delbrueckii* subspecies *bulgaricus* DSM 24,734), Bifidobacteria (*B. longum* DSM 24,736, *B. breve* DSM 24,732, and *B. infantis* DSM 24,737), and Streptococcus (*S. thermophilus* DSM 24,731)	Systemic sclerosis-associated gastrointestinal disease	Improvement of GI reflux and intestinal microbiota alpha diversity	[17]
L. acidophilus LaVK2 and *B. bifidum* Bbvk3	Dextran sodium-sulphate salt-induced ulcerative colitis in mice	Reduction in myeloperoxidase activity, levels of TNF-α, IL-6, and IFN-γ	[18]
L. bulgaricus 151 and *S. thermophilus* MK-10	Dextran sodium-sulphate salt-induced colitis	Modulation of intestinal microbiota, decrease the content of putrefactive short-chain fatty acid, enhanced production of cytokines.	[19]
B. bifidum (KCTC 12199BP), *B. lactis* (KCTC 11904BP), *B. longum* (KCTC 12200BP), *L. acidophilus* (KCTC 11906BP), *L. rhamnosus* (KCTC 12202BP) and *S. thermophilus* (KCTC 11870BP)	Irritable Bowel Syndrome (IBS)	Alleviation of IBS symptoms and improvement of intestinal microbiota	[20]
B. longum and *L. casei* strain Shirota	Treatment of obesity	Decreased weight and triglyceride in rats fed with the high-fat diet.	[21]
S. boulardii, *L. acidophilus*, *L. plantarum*, *B. lactis*	IBS associated with bacterial overgrowth and constipation	Improvement in bloating, and pain associated with constipation	[22]
L. plantarum, *B. breve*, and *L. fermentum*	high-dietary fat-induced obesity and *E. coli* challenged	Causes reduced Lipopolysaccharide and IL-1β, improved the structure of intestinal flora and increased the fecal short-chain fatty acid (SCFA) content	[23]

3.2. Inhibition of Pathogens

A multi-strain probiotic containing different *Lactobacillus* strains hinders the adhesion of *E. coli* and *E. faecalis* to the bladder cell lines, unlike the single-probiotics preparations [24] (Table 2).

Table 2. Multi-strains probiotics against pathogenic microbes.

Multi-Strain Probiotics Isolates	Pathogenic Bacteria	Host	References
B. subtilis and *L. mesentroides*	*Vibrio cholereae*	In-vitro agar diffusion test	[25]
L. plantarum F44, *L. paracasei* F8, *B. breve* 46 and *B. lactis*	*Clostridium difficile*	Mice	[26]

Multi-Strain Probiotics Isolates	Pathogenic Bacteria	Host	References
S. oralis and *S. salivarius*	Biofilm (*S. aureus*, *S. epidermidis*, *S. pneumoniae*, *S. pyogenes*, *Propionibacterium acnes* and *Moraxella catarrhalis*	Dogs	[27]
L. acidophilus LAP5, *L. fermentum* P2, *P. acidophilus* LS, and *L. casei* L21	*S. enterica* subspecies *Enterica*	Chickens	[28]
L. acidophilus LA-5 and *B. bifidum* BB-12	*P. stomatis*, *P. multocida*, *P. canis*, *N. animaloris*, and *N. zoodegmatis*		[29]
P. acidilactici and *S. cerevisiae boulardii*	Enterotoxigenic *E. coli* (ETEC) F4	Pigs	[30]
L. acidophilus NCIMB 30184, *L. fermentum* NCIMB 30226, *L. plantarum* NCIMB 30187, and *L. rhamnosus* NCIMB 30188	Pathogenic *E. coli* and *E. faecalis*		[24]
S. cerevisiae, *E. faecium*, *L. acidophilus* and *Bacillus subtilis*	*E. coli*	Chickens (broilers)	[31]
L. acidophilus NCIMB 30184, *L. rhamnosus* NCIMB 30188, *L. plantarum* NCIMB 30187, *L. delbrueckii* ssp. bulgaricus NCIMB 30186, *L. casei* NCIMB 30185, *L. lactis* NCIMB 30222, *L. salivarius* NCIMB 30225, *L. fermentum* NCIMB 30226, *L. helveticus* NCIMB 30224, *B. bifidum* NCIMB 30179, *B. breve* NCIMB 30180, *B. infantis* NCIMB 30181, *B. longum* NCIMB 30182, *S.* thermophilus NCIMB 30189 *B. subtilis* NCIMB 30223	*S. typhimurium*, *C. difficile*	In-vitro distal colon model	[32]
L. acidophilus NCIMB 30184, *L. fermentum* NCIMB 30188, *L. plantarum* NCIMB 30187 and *L. rhamnosus* NCIMB 30226	*E. faecalis* NCTC 0075 and *E. coli* NCTC 9001	In-vitro agar diffusion test	[6]
L. rhamnosus and *L. reuteri*	Vaginal coliforms and yeast	Human (female)	[33]
L. crispatus, *L. salivarius*, *L. gallinarum*, *L. johnsonii*, *E. faecalis* and *B. amyloliquefaciens*	*Salmonella* Enteritidis A9	Chickens (broiler)	[34]
L. acidophilus, L. fermentum, *L. plantarum* and *E. faecium*	*Salmonella enterica*	Chickens (broiler)	[35]
B. amyloliquefaciens B-1895 and *B. subtilis* KATMIRA1933	Inhibits *Proteus mirabilis* biofilm formation	Invitro	[36]
E. faecalis (strains NM815, and NM915) and *E. faecium* NM1015	*C. difficile* infection	Mice	[37]
L. acidophilus (LA-5), and *B. animalis* subspecies *Lactis* (Bb12)	*E. coli* induced pyelonephritis	Sprague-Dawley rat	[38]
L. casei and *E. faecium*	*Entamoeba invadens*	Invitro	[39]
B. subtilis, L. acidophilus, *P. acidilactici*, *P. pentosus*, *Saccharomyces pastorianus*	Avian pathogenic *E. coli* and *Salmonella* Kentucky	White leg-horn chicks	[40]
L. gasseri and *L. rhamnosus*	Non-*Candida albicans* biofilm formation	In-vitro	[41]

3.3. Improvement of Human Health

Multi-strain probiotics had proved beneficial for the treatment of dysentery in addition to the standard regimen with a marked reduction in the extent of bloody stooling and a decreased average length of hospital stay [42]. These effects were a result of the alteration of the microbial and metabolic activities within the gut, and which are enough to modify the disease process and pathological conditions [43].

3.4. Multi-Strains Probiotics in Animal Husbandry

Probiotics may be a potential alternative for improving gastrointestinal health and growth promotion in different animal species [44]. Based on these, the roles of probiotics in the various livestock sub-sectors, including poultry, aquaculture, piggery, and ruminant nutrition, were discussed as follows.

3.5. Poultry Farming

In poultry, the addition of probiotics derived from *Lactobacillus, Bacillus* [45], and *Clostridium* species to feed has a positive impact on the growth yield, feed digestion [46], immunity [47], meat quality [48], and coliforms bacterial count [44][49]. The administration of multi-strain probiotics (comprising of *L. acidophilus* LAP5, *L. fermentum* P2, *P. acidophilus* LS, and *L. casei* L21) to specific-pathogen-free (SPF) chicks infected with *Salmonella enterica* subspecies *enterica* decreases the abundance of proteobacteria of which Salmonella is a member [28].

3.6. Aquaculture

The use of probiotics for health improvement has also found application in aquaculture. The addition of multi-strain probiotics in the feed of rohu (*Labeo rohita*) was revealed to stimulate cellulolytic and amylolytic enzymes secretions with improved growth output [50]. The multi-strain culture of *B. subtilis*, *B. licheniformis*, and *lactobacilli* probiotics significantly improves pacific white shrimps' growth (*Litopenaeus vannamei*) and enhances non-specific immunity and the abundance of *Bacillus* to influence the intestinal microbiota [51].

3.7. Swine Production/Piggery

The weaning period in piggery coupled with diets changes from simply digestible (milk) to solid feeds may result in intestinal perturbation, thereby causing diarrhea and a slow growth rate [52][53][54]. The ingestion of probiotic bacteria (like *P. acidilactici*) and yeast (*S. cerevisiae boulardii*) protect from microbial infection by enhancing intestinal defences and performance in different monogastric animals [55].

3.8. Ruminants Nutrition and Production

Some probiotics are suitable supplements in livestock feeds and may improve the rumen's microbial ecosystem, enhance feed digestion, and restores gut microflora in diarrhea in ruminants [56]. The administration of lactobacilli probiotics enhances calves' overall health status [57].

3.9. Synbiosis of Multi-Strain Probiotics with Other Biologically Active Molecules

Some probiotics are prepared as synbiotics (prebiotics) along with other active substances for maximum physiological effects. Ingestion of synbiotics made of multi-strain probiotics (containing *L. acidophilus* strain T16, *L. casei* strain T2 and *B. bifidum* strain T1) and 800 mg inulin (HPX) by gravid women with gestational diabetes mellitus decrease the rate of caesarean section and hyperbilirubinemia and hospitalization of newborns [58]. Administration of synbiotics (containing multi-strain probiotics and prebiotics) may alleviate some digestive system conditions, sepsis, and death in preterm babies [59] (Table 3).

Table 3. Use of multi-strain probiotics along with other substances.

Synbiotics	Actions	Host	References
L. acidophilus **strain T16,** ***L. casei*** **strain T2) and** ***B. bifidum*** **strain T1 plus 800mg inulin (HPX)**	**decreased the incidence of cesarean section rate and newborn's hyperbilirubinemia and hospitalization**	**Human (pregnant women)**	[58]
L. acidophilus**,** ***L. rhamnosus*****,** ***S. thermophilus*****, and** ***L. delbrueckii*** **subspecies** ***Bulgaricus*** **plus fluconazole**	**Enhance the treatment of Vaginal candidiasis caused** ***Candida albicans***	**humans**	[60]
L. plantarum, L. acidophilus, L. delbrueckii subspecies bulgaricus, B. bifidum, L. rhamnosus, E. faecium, S. salivarius subspecies thermophilus, Aspergillus oryza**, and Candida** ***pintolopesii*** **plus Zinc**	**Enhances growth performance, better feed utilization, increase in villus height in the duodenum and ileum**	**Chicken (broiler)**	[61]
Synbiotics A: ***Enterococcus*** **sp.,** ***Pediococcus*** **sp.,** ***Bifidobacterium*** **sp.,** ***Lactobacillus*** **sp. plus fructooligosaccharides Synbiotic B:** ***L. acidophilus, L. casei, L. salivarius, L. plantarum, L. rhamnosus, L. brevis, B. bifidum, B. lactis, S. thermophilus*****, prebiotic inulin (chicory root extract), protease, amylase, cellulase, hemicellulase, lipase, papain and bromelain**	**Modulate the caecal microbiota without any effects on Salmonella** ***Typhimurium*** **shedding**	**Chickens (layers)**	[62]

Synbiotics	Actions	Host	References
Probiotics; (*L. rhamnosus, L. casei L. plantarum B. animalis*) prebiotics (383 mg of fructooligosaccharides and 100 mg of galactooligosaccharides)	**Improved gastrointestinal complications, sepsis, and mortality in premature infants**	**Preterm infants**	[59]

4. Future Consideration

To maximize all the benefits of probiotics consumption, research should determine the specific mechanisms of actions of probiotics microbes for more specific applications in respective disease conditions. There is also the need to study and understand each probiotics strain's best combination because some bacteria act synergistically, some additively, and some antagonistically. Additionally, the bioactive substances produced by some probiotics could be extracted to formulate supplements for use in specific conditions where individuals showed some reactions to the consumption of the whole-cells preparations. The harvesting and harnessing of the bioactive substances produced by individual constituents of mixed probiotics could also solve the challenges associated with the inconsistency of viable cells when live microbes are used. That will also enable large-scale production for commercialization. Finally, further studies in this direction could be an essential factor in the future research and development of multi-strain probiotics.

References

1. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol. 2014, 11, 506.
2. Alagawany, M.; Abd El-Hack, M.E.; Farag, M.R.; Sachan, S.; Karthik, K.; Dhama, K. The use of probiotics as eco-friendl y alternatives for antibiotics in poultry nutrition. Environ. Sci. Pollut. Res. 2018, 25, 10611–10618.
3. Aalaei, M.; Khatibjoo, A.; Zaghari, M.; Taherpour, K.; Akbari Gharaei, M.; Soltani, M. Comparison of single- and multi-str ain probiotics effects on broiler breeder performance, egg production, egg quality and hatchability. Br. Poult. Sci. 2018, 59, 531–538.
4. Timmerman, H.; Koning, C.; Mulder, L.; Rombouts, F.; Beynen, A. Monostrain, multistrain and multispecies probiotics—A comparison of functionality and efficacy. Int. J. Food Microbiol. 2004, 96, 219–233.
5. Wilkins, T.; Sequoia, J. Probiotics for gastrointestinal conditions: A summary of the evidence. Am. Fam. Physician 2017, 96, 170–178.
6. Chapman, C.; Gibson, G.; Todd, S.; Rowland, I. Comparative in vitro inhibition of urinary tract pathogens by single-and multi-strain probiotics. Eur. J. Nutr. 2013, 52, 1669–1677.
7. Adamberg, S.; Sumeri, I.; Uusna, R.; Ambalam, P.; Kondepudi, K.K.; Adamberg, K.; Wadström, T.; Ljungh, Å. Survival a nd synergistic growth of mixed cultures of bifidobacteria and lactobacilli combined with prebiotic oligosaccharides in a g astrointestinal tract simulator. Microb. Ecol. Health Dis. 2014, 25, 23062.
8. Yadav, R.; Shukla, P. An overview of advanced technologies for selection of probiotics and their expediency: A review. Crit. Rev. Food Sci. Nutr. 2017, 57, 3233–3242.
9. Bogucka, J.; Ribeiro, D.M.; Bogusławska-Tryk, M.; Dankowiakowska, A.; da Costa, R.P.R.; Bednarczyk, M. Microstruct ure of the small intestine in broiler chickens fed a diet with probiotic or synbiotic supplementation. J. Anim. Physiol. Ani m. Nutr. 2019, 103, 1785–1791.
10. Bajaj, B.K.; Razdan, K.; Claes, I.J.; Lebeer, S. Probiotic Attributes of the Newly Isolated Lactic Acid Bacteria from Infant s'gut. J. Microbiol. Biotechnol. Food Sci. 2020, 2020, 109–115.
11. Oak, S.J.; Jha, R. The effects of probiotics in lactose intolerance: A systematic review. Crit. Rev. Food Sci. Nutr. 2019, 5 9, 1675–1683.
12. Foligné, B.; Parayre, S.; Cheddani, R.; Famelart, M.-H.; Madec, M.-N.; Plé, C.; Breton, J.; Dewulf, J.; Jan, G.; Deutsch, S.-M. Immunomodulation properties of multi-species fermented milks. Food Microbiol. 2016, 53, 60–69.
13. Bernstein, C.N. Antibiotics, probiotics and prebiotics in IBD. In Nutrition, Gut Microbiota and Immunity: Therapeutic Targ ets for IBD; Karger Publishers: Basel, Switzerland, 2014; Volume 79, pp. 83–100.
14. Hajela, N.; Ramakrishna, B.; Nair, G.B.; Abraham, P.; Gopalan, S.; Ganguly, N.K. Gut microbiome, gut function, and pro biotics: Implications for health. Indian J. Gastroenterol. 2015, 34, 93–107.

15. Sabico, S.; Al-Mashharawi, A.; Al-Daghri, N.M.; Wani, K.; Amer, O.E.; Hussain, D.S.; Ansari, M.G.A.; Masoud, M.S.; Alokail, M.S.; McTernan, P.G. Effects of a 6-month multi-strain probiotics supplementation in endotoxemic, inflammatory and cardiometabolic status of T2DM patients: A randomized, double-blind, placebo-controlled trial. Clin. Nutr. 2019, 38, 1561–1569.

16. Akbari, E.; Asemi, Z.; Daneshvar Kakhaki, R.; Bahmani, F.; Kouchaki, E.; Tamtaji, O.R.; Hamidi, G.A.; Salami, M. Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: A randomized, double-blind and controlled trial. Front. Aging Neurosci. 2016, 8, 256.

17. Frech, T.M.; Khanna, D.; Maranian, P.; Frech, E.J.; Sawitzke, A.D.; Murtaugh, M.A. Probiotics for the treatment of systemic sclerosis-associated gastrointestinal bloating/distention. Clin. Exp. Rheumatol. 2011, 29, S22.

18. Jadhav, S.R.; Shandilya, U.K.; Kansal, V.K. Immunoprotective effect of probiotic dahi containing Lactobacillus acidophilus and Bifidobacterium bifidum on dextran sodium sulfate-induced ulcerative colitis in mice. Probiotics Antimicrob. Proteins 2012, 4, 21–26.

19. Wasilewska, E.; Zlotkowska, D.; Wroblewska, B. Yogurt starter cultures of Streptococcus thermophilus and Lactobacillus bulgaricus ameliorate symptoms and modulate the immune response in a mouse model of dextran sulfate sodium-induced colitis. J. Dairy Sci. 2019, 102, 37–53.

20. Yoon, J.S.; Sohn, W.; Lee, O.Y.; Lee, S.P.; Lee, K.N.; Jun, D.W.; Lee, H.L.; Yoon, B.C.; Choi, H.S.; Chung, W.S. Effect of multispecies probiotics on irritable bowel syndrome: A randomized, double-blind, placebo-controlled trial. J. Gastroenterol. Hepatol. 2014, 29, 52–59.

21. Karimi, G.; Jamaluddin, R.; Mohtarrudin, N.; Ahmad, Z.; Khazaai, H.; Parvaneh, M. Single-species versus dual-species probiotic supplementation as an emerging therapeutic strategy for obesity. Nutr. Metab. Cardiovasc. Dis. 2017, 27, 910–918.

22. Leventogiannis, K.; Gkolfakis, P.; Spithakis, G.; Tsatali, A.; Pistiki, A.; Sioulas, A.; Giamarellos-Bourboulis, E.J.; Triantafyllou, K. Effect of a preparation of four probiotics on symptoms of patients with irritable bowel syndrome: Association with intestinal bacterial overgrowth. Probiotics Antimicrob. Proteins 2019, 11, 627–634.

23. Sun, Q.; Zhang, S.; Liu, X.; Huo, Y.; Su, B.; Li, X. Effects of a probiotic intervention on Escherichia coli and high-fat diet-induced intestinal microbiota imbalance. Appl. Microbiol. Biotechnol. 2020, 104, 1243–1257.

24. Chapman, C.; Gibson, G.; Rowland, I. Effects of single-and multi-strain probiotics on biofilm formation and in vitro adhesion to bladder cells by urinary tract pathogens. Anaerobe 2014, 27, 71–76.

25. VidyaLaxme, B.; Rovetto, A.; Grau, R.; Agrawal, R. Synergistic effects of probiotic Leuconostoc mesenteroides and Bacillus subtilis in malted ragi (Eleucine corocana) food for antagonistic activity against V. cholerae and other beneficial properties. J. Food Sci. Technol. 2014, 51, 3072–3082.

26. Kondepudi, K.K.; Ambalam, P.; Karagin, P.H.; Nilsson, I.; Wadström, T.; Ljungh, Å. A novel multi-strain probiotic and synbiotic supplement for prevention of Clostridium difficile infection in a murine model. Microbiol. Immunol. 2014, 58, 552–558.

27. Bidossi, A.; De Grandi, R.; Toscano, M.; Bottagisio, M.; De Vecchi, E.; Gelardi, M.; Drago, L. Probiotics Streptococcus salivarius 24SMB and Streptococcus oralis 89a interfere with biofilm formation of pathogens of the upper respiratory tract. BMC Infect. Dis. 2018, 18, 653.

28. Chang, C.H.; Teng, P.Y.; Lee, T.T.; Yu, B. The effects of the supplementation of multi-strain probiotics on intestinal microbiota, metabolites and inflammation of young SPF chickens challenged with Salmonella enterica subsp. enterica. Anim. Sci. J. 2019, 90, 737–746.

29. Zambori, C.; Morvay, A.A.; Sala, C.; Licker, M.; Gurban, C.; Tanasie, G.; Tîrziu, E. Antimicrobial effect of probiotics on bacterial species from dental plaque. J. Infect. Dev. Ctries. 2016, 10, 214–221.

30. Daudelin, J.-F.; Lessard, M.; Beaudoin, F.; Nadeau, É.; Bissonnette, N.; Boutin, Y.; Brousseau, J.-P.; Lauzon, K.; Fairbrother, J.M. Administration of probiotics influences F4 (K88)-positive enterotoxigenic Escherichia coli attachment and intestinal cytokine expression in weaned pigs. Vet. Res. 2011, 42, 69.

31. Ahmed, S.T.; Hoon, J.; Mun, H.-S.; Yang, C.-J. Evaluation of Lactobacillus and Bacillus-based probiotics as alternatives to antibiotics in enteric microbial challenged weaned piglets. Afr. J. Microbiol. Res. 2014, 8, 96–104.

32. Tejero-Sariñena, S.; Barlow, J.; Costabile, A.; Gibson, G.R.; Rowland, I. Antipathogenic activity of probiotics against Salmonella Typhimurium and Clostridium difficile in anaerobic batch culture systems: Is it due to synergies in probiotic mixtures or the specificity of single strains? Anaerobe 2013, 24, 60–65.

33. Reid, G.; Bruce, A.W. Selection of Lactobacillus strains for urogenital probiotic applications. J. Infect. Dis. 2001, 183, S77–S80.

34. Neveling, D.P.; van Emmenes, L.; Ahire, J.J.; Pieterse, E.; Smith, C.; Dicks, L. Effect of a Multi-Species Probiotic on the Colonisation of Salmonella in Broilers. Probiotics Antimicrob. Proteins 2019, 1–10.

35. Chen, C.-Y.; Tsen, H.-Y.; Lin, C.-L.; Yu, B.; Chen, C.-S. Oral administration of a combination of select lactic acid bacteri a strains to reduce the Salmonella invasion and inflammation of broiler chicks. Poult. Sci. 2012, 91, 2139–2147.

36. Algburi, A.; Alazzawi, S.A.; Al-Ezzy, A.I.A.; Weeks, R.; Chistyakov, V.; Chikindas, M.L. Potential Probiotics Bacillus subti lis KATMIRA1933 and Bacillus amyloliquefaciens B-1895 Co-Aggregate with Clinical Isolates of Proteus mirabilis and P revent Biofilm Formation. Probiotics Antimicrob. Proteins 2020, 12, 1471–1483.

37. Mansour, N.M.; Elkhatib, W.F.; Aboshanab, K.M.; Bahr, M.M. Inhibition of Clostridium difficile in mice using a mixture of potential probiotic strains Enterococcus faecalis NM815, E. faecalis NM915, and E. faecium NM1015: Novel candidates to control C. difficile infection (CDI). Probiotics Antimicrob. Proteins 2018, 10, 511–522.

38. Sabetkish, N.; Sabetkish, S.; Mohseni, M.J.; Kajbafzadeh, A.-M. Prevention of renal scarring in acute pyelonephritis by probiotic therapy: An experimental study. Probiotics Antimicrob. Proteins 2019, 11, 158–164.

39. Sarjapuram, N.; Mekala, N.; Singh, M.; Tatu, U. The potential of Lactobacillus casei and Entercoccus faecium combinati on as a preventive probiotic against Entamoeba. Probiotics Antimicrob. Proteins 2017, 9, 142–149.

40. Redweik, G.A.; Stromberg, Z.R.; Van Goor, A.; Mellata, M. Protection against avian pathogenic Escherichia coli and Sal monella Kentucky exhibited in chickens given both probiotics and live Salmonella vaccine. Poult. Sci. 2020, 99, 752–76 2.

41. Tan, Y.; Leonhard, M.; Moser, D.; Ma, S.; Schneider-Stickler, B. Inhibitory effect of probiotic lactobacilli supernatants on single and mixed non-albicans Candida species biofilm. Arch. Oral Biol. 2018, 85, 40–45.

42. Sharif, A.; Kashani, H.H.; Nasri, E.; Soleimani, Z.; Sharif, M.R. The role of probiotics in the treatment of dysentery: A ra ndomized double-blind clinical trial. Probiotics Antimicrob. Proteins 2017, 9, 380–385.

43. Guarner, F.; Malagelada, J.-R. Gut flora in health and disease. Lancet 2003, 361, 512–519.

44. Zhang, Z.; Kim, I. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood char acteristics, cecal microbial shedding, and excreta odor contents in broilers. Poult. Sci. 2014, 93, 364–370.

45. Deniz, G.; Orman, A.; Cetinkaya, F.; Gencoglu, H.; Meral, Y.; Turkmen, I. Effects of probiotic (Bacillus subtilis DSM 172 99) supplementation on the caecal microflora and performance in broiler chickens. Rev. Méd. Vét. 2011, 162, 538–545.

46. Mountzouris, K.; Tsirtsikos, P.; Kalamara, E.; Nitsch, S.; Schatzmayr, G.; Fegeros, K. Evaluation of the efficacy of a pro biotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performan ce and modulating cecal microflora composition and metabolic activities. Poult. Sci. 2007, 86, 309–317.

47. Yang, C.; Cao, G.; Ferket, P.; Liu, T.; Zhou, L.; Zhang, L.; Xiao, Y.; Chen, A. Effects of probiotic, Clostridium butyricum, on growth performance, immune function, and cecal microflora in broiler chickens. Poult. Sci. 2012, 91, 2121–2129.

48. Mahajan, P.; Sahoo, J.; Panda, P. Effect of probiotic (Lacto-Sacc) feeding, packaging methods and seasons on the micr obial and organoleptic qualities of chicken meat balls during refrigerated storage. J. Food Sci. Technol. (Mysore) 2000, 37, 67–71.

49. Lee, K.; Lillehoj, H.S.; Siragusa, G.R. Direct-fed microbials and their impact on the intestinal microflora and immune sys tem of chickens. J. Poult. Sci. 2010.

50. Bairagi, A.; Sarkar Ghosh, K.; Sen, S.; Ray, A. Evaluation of the nutritive value of Leucaena leucocephala leaf meal, ino culated with fish intestinal bacteria Bacillus subtilis and Bacillus circulans in formulated diets for rohu, Labeo rohita (Ha milton) fingerlings. Aquac. Res. 2004, 35, 436–446.

51. Xie, J.-J.; Liu, Q.-Q.; Liao, S.; Fang, H.-H.; Yin, P.; Xie, S.-W.; Tian, L.-X.; Liu, Y.-J.; Niu, J. Effects of dietary mixed prob iotics on growth, non-specific immunity, intestinal morphology and microbiota of juvenile pacific white shrimp, Litopenae us vannamei. Fish Shellfish Immunol. 2019, 90, 456–465.

52. Dlamini, Z.; Langa, R.; Aiyegoro, O.; Okoh, A. Effects of probiotics on growth performance, blood parameters, and antib ody stimulation in piglets. S. Afr. J. Anim. Sci. 2017, 47, 765–776.

53. Castillo, M.; Martín-Orúe, S.M.; Nofrarías, M.; Manzanilla, E.G.; Gasa, J. Changes in caecal microbiota and mucosal m orphology of weaned pigs. Vet. Microbiol. 2007, 124, 239–247.

54. Rhouma, M.; Fairbrother, J.M.; Beaudry, F.; Letellier, A. Post weaning diarrhea in pigs: Risk factors and non-colistin-bas ed control strategies. Acta Vet. Scand. 2017, 59, 31.

55. Lee, S.; Lillehoj, H.; Dalloul, R.; Park, D.; Hong, Y.; Lin, J. Influence of Pediococcus-based probiotic on coccidiosis in br oiler chickens. Poult. Sci. 2007, 86, 63–66.

56. El-Tawab, M.A.; Youssef, I.; Bakr, H.; Fthenakis, G.; Giadinis, N. Role of probiotics in nutrition and health of small rumin ants. Pol. J. Vet. Sci. 2016, 19, 893–906.

57. Adetoye, A.; Pinloche, E.; Adeniyi, B.A.; Ayeni, F.A. Characterization and anti-salmonella activities of lactic acid bacteria isolated from cattle faeces. BMC Microbiol. 2018, 18, 96.

58. Karamali, M.; Nasiri, N.; Shavazi, N.T.; Jamilian, M.; Bahmani, F.; Tajabadi-Ebrahimi, M.; Asemi, Z. The effects of synbiotic supplementation on pregnancy outcomes in gestational diabetes. Probiotics Antimicrob. Proteins 2018, 10, 496–503.

59. Güney-Varal, İ.; Köksal, N.; Özkan, H.; Bağcı, O.; Doğan, P. The effect of early administration of combined multi-strain and multi-species probiotics on gastrointestinal morbidities and mortality in preterm infants: A randomized controlled trial in a tertiary care unit. Turk. J. Pediatr. 2017, 59, 13–19.

60. Kovachev, S.M.; Vatcheva-Dobrevska, R.S. Local probiotic therapy for vaginal Candida albicans infections. Probiotics Antimicrob. Proteins 2015, 7, 38–44.

61. Shah, M.; Zaneb, H.; Masood, S.; Khan, R.U.; Ashraf, S.; Sikandar, A.; Rehman, H.F.U.; Rehman, H.U. Effect of dietary supplementation of zinc and multi-microbe probiotic on growth traits and alteration of intestinal architecture in broiler. Probiotics Antimicrob. Proteins 2019, 11, 931–937.

62. Khan, S.; Chousalkar, K.K. Short-term feeding of probiotics and synbiotics modulates caecal microbiota during Salmonella Typhimurium infection but does not reduce shedding and invasion in chickens. Appl. Microbiol. Biotechnol. 2020, 104, 319–334.