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REVIEW

# Health benefits of probiotics: are mixtures more effective than single strains?

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**Abstract**
*Purpose* Most studies on probiotics utilise single strains, sometimes incorporated into yoghurts. There are fewer studies on efficacy of mixtures of probiotic strains. This review examines the evidence that (a) probiotic mixtures are beneficial for a range of health-related outcomes and (b) mixtures are more or less effective than their component strains administered separately.
*Results* Mixtures of probiotics had beneficial effects on the end points including irritable bowel syndrome and gut function, diarrhoea, atopic disease, immune function and respiratory tract infections, gut microbiota modulation, inflammatory bowel disease and treatment of *Helicobacter pylori* infection. However, only 16 studies compared the effect of a mixture with that of its component strains separately, although in 12 cases (75%), the mixture was more effective.
*Conclusion* Probiotic mixtures appear to be effective against a wide range of end points. Based on a limited number of studies, multi-strain probiotics appear to show greater efficacy than single strains, including strains that are components of the mixtures themselves. However, whether this is due to synergistic interactions between strains or a consequence of the higher probiotic dose used in some studies is at present unclear.

**Keywords** Multi-strain probiotics · Health benefits · Synergy · Probiotic efficacy

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## Introduction

Probiotics are defined as live organisms, which when administered in sufficient amounts, can have a beneficial effect on the host's health [1]. Probiotics have been demonstrated to be effective in a variety of conditions including travellers' diarrhoea, antibiotic-associated diarrhoea, upper respiratory tract infections, atopic eczema and some inflammatory conditions [2, 3]. Potential mechanisms of action include modulation of the intestinal immune system, and displacement of potential pathogens via competitive exclusion or production of antimicrobial agents [3].

Most studies on probiotics utilise single strains, sometimes incorporated into yoghurts containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii subspecies Bulgaricus.* Less is known about the efficacy of mixtures of probiotic strains, in particular whether mixing of strains results in additive or even synergistic effects in terms of bioactivity or in reduced efficacy due to mutual inhibition by the component strains. This review examines the evidence (a) that probiotic mixtures are beneficial for a range of health-related outcomes and (b) that mixtures are more or less effective than their component strains administered separately. It is important to note that although there are many studies comparing probiotics mixtures with single strains, the majority are not direct comparisons between a mixture and its component strains in an integrated study.

The studies in this review were arrived upon after a search of the PubMed database using the following search terms: multi-strain probiotic*, mixed-species probiotic*, probiotic mixture, probiotic interaction*, VSL#3, Ecologic 641, VIS-01, Yovis. From the studies located with this search, all appropriate subsequent references were scrutinised and reviewed where relevant.

## Animal studies (Table 1)

This section will review those animal studies that have made direct comparison between the effectiveness of probiotic mixtures and single strains, although usually not strains that are components of the mixture.

Shibolet et al. [4] compared the ability of VSL#3 mixture and *Lactobacillus GG* to prevent chemically induced colitis in rats. A reduction of inflammation in both probiotic groups was noted, with a significantly greater reduction observed in the group given the mixture. In this study, VSL#3 was used as both pre- and post-treatment in two chemically induced models of colitis, and only the colitis induced by iodacetamide and not DSS was improved by the probiotics. Although this study does suggest a beneficial role for probiotic mixtures in the treatment of some forms of colitis in animals, the lack of a direct comparison of the mixture with its component strains does not indicate that the former is more effective.

Lema et al. [5] investigated the effects of probiotics on *Escherichia coli 0157:H7* infections in lambs and cattle. The study demonstrated that the a 5-strain probiotic mixture led to a greater increase in shedding of *E. coli 0157:H7*, as well as increasing daily weight gain and gain-feed ratio, compared to either a 2-strain mixture or 2 of the component strains used individually. What is pertinent here is that all probiotic treatments, whether single- or multi-strain preparations, were given at the same total dose of

**Table 1** Animal studies

| Organisms/dose | *n* | End points | Outcome | Reference |
|---|---|---|---|---|
| Group 1: daily dose of VSL#3 mixture (*B. longum, B. infantis, B. breve; L. acidophilus, L. casei, L. delbrueckii ssp. Bulgaricus, L. plantarum; S. salivarius ssp. thermophilus* $3 \times 10^9$ CFU)<br>Group 2: daily dose of *Lactobacillus GG* $10^{10}$ CFU | 32 | Treatment of colitis (induced with iodoacetate or DSS) in rats with 7 days pre- and post-treatment with single- or multi-strain probiotic. End points assessed by histology, nitric oxide synthase (NOS) and prostaglandin E2 (PGE2) activity | Decreased lesion size in both colitis groups, greater decrease in VSL#3 group; similar decreases in MPO activity in both groups; decreased PGE2 and NOS in both groups, greater decrease in L. RHAMNOSUS GG group | [4] |
| Group 1: *L. acidophilus*<br>Group 2: *S. faecium*<br>Group 3: mixture of *L. acidophilus* and *S. faecium;*<br>Group 4*: mixture* of *L. acidophilus, S. faecium, L. casei, L. fermentum* and *L. plantarum.*<br>Each strain/combination given at $6 \times 10^6$ CFU/kg of feed | 30 | Faecal shedding of *E. coli 0157:H7* in lambs | Shedding, daily weight gain: feed ratio greatest in group given mixture of 5 strains. No difference between groups in duration of shedding | [5] |
| *Daily doses of:*<br>Group 1: *L. casei* $2.5 \times 10^8$<br>Group 2: *S. thermophilus* $8.3 \times 10^8$, *L. bulgaricus* $1.1 \times 10^8$<br>Group 3: *S. thermophilus* $1.1 \times 10^8$, *L. bulgaricus* $8.2 \times 10^8$, *L. casei* $0.8 \times 10^8$<br>Group 4: *L. casei* $4.9 \times 10^8$<br>Group 5: *L. casei* $1.0 \times 10^8$ | 24 | Survival after *S. Typhimurium* infection in mice after 7-day pre-treatment with various probiotic strains/mixtures | Higher survival in all treatment groups, most protection in group 3. Increased phagocytosis in group 2; no difference in phagocytosis between group 3 and control group. Increased serum IgA for single-strain groups | [6] |
| Group 1: *L. casei*<br>Group 2: *L. acidophilus*<br>Group 3: *L. casei* + *L. acidophilus*<br>All probiotics administered in fermented milks at total dose of $2.4 \times 10^9$ CFU/day | Not stated | Protection against *S. typhimurium* infection after 8-day pre-treatment with probiotic strains/mixtures | 100% 21-day survival in mix group (vs. 20% in all other groups); liver/spleen colonisation only prevented in multi-strain group. Higher circulating antibodies in mixture group ($5 \times$ control) vs. single-strain groups ($0.5 \times$ or $2 \times$ control).Higher intestinal antibodies in single-strain groups ($1–5 \times$ control) than multi-strain groups ($1–2 \times$ control). No protection offered by mixture administered post-*S. typhimurium* challenge | [7] |

$6 \times 10^6$ CFU/kg of feed. This suggests a synergistic effect of probiotics in the multi-strain preparation. Furthermore, since the results were greater for a 5-strain than a 2-strain mix, it also suggests greater efficacy with greater variety of strains at the same concentration. Another interesting factor is that there was no difference between groups in the duration of shedding, indicating that the effect of single- or multi-strains is temporary, and that no colonisation of the gut occurred.

Paubert-Braquet et al. [6] and Perdigon et al. [7] studied infection by *Salmonella Typhimurium* in mice. The observations of Paubert-Braquet et al. [6] that higher levels of immunoglobulins and phagocytosis were achieved with a single-strain probiotic, while multi-strains gave rise to a greater survival rate after 7-day pre-treatment, suggest that the effect of immune enhancement is not enough to protect against a pathogen such as *S. Typhimurium*, implicating such mechanisms as the production of antimicrobial substances and competitive exclusion. Similarly, Perdigon et al. [7] observed immunostimulation by single strains of *L. casei* and *L. acidophilus*—greater than the combination in the case of localised intestinal immunity—but overall protection was only conferred by the mixture of strains. As with the Lema et al. [5] study, the same dose was used for each strain, suggesting an enhanced effect due to synergy between strains. The smaller localised effect of the multi-strain product may be due to inhibition between probiotic strains, preventing colonisation at a given location, but not preventing an overall protective effect, perhaps through antimicrobial production, or by outcompeting pathogenic strains.

These studies give support to the use of multi-strain probiotics in animals. They also lend support to the theory that a multi-strain product can be more effective than a single- or dual-strain probiotic in the treatment of certain clinical conditions, although it is worth stressing the paucity of studies involving direct comparison between single- and multi-strain probiotics in animal models. This is particularly relevant in the case of inflammatory bowel disease (IBD) since the efficacy of probiotic treatment in this condition is largely strain-specific and dependent upon the condition's pathology [8], and in the case of animal studies on IBD, a mixture does not seem to have been tested against any of its component strains, making this hypothesis difficult to support.

## Human studies

### Irritable bowel syndrome (IBS) and gut function (Table 2)

A study by Brigidi et al. [9], and two by Kim et al. [10, 11], examined the effects of the VSL#3 mixture on IBS symptoms. While Brigidi et al. [9] found an improvement in overall symptoms for 90% of patients, the Kim et al. [10, 11] studies reported only improvement in one different symptom for each study—decreased bloating [10] or flatulence [11]—despite using a similar dose of probiotic and a larger study size. It may be that the methods of assessment differed: Brigidi et al. [9] state only that "*symptoms assessment… and physical examination were performed*," while in the Kim et al. studies, patients completed a daily diary using a visual analogue scale to evaluate symptoms. With this in mind, it is difficult to compare the results of these studies, but given the open label nature of the Brigidi et al. [9] study and the large placebo effect in IBS of over 40%, [12] less emphasis should be placed on this study.

That Kim et al. [10, 11] found improvement in only one symptom in each study suggests either that the dose was insufficient, or that VSL#3 does not contain the correct probiotic strain/s to cause improvement in IBS symptoms. However, studies by Kajander et al. [13, 14] report improvements in IBS symptoms using doses of a similar size to those used by Kim et al. [10, 11]. This would suggest the latter explanation for the lack of symptomatic improvement in the Kim et al. [10, 11] studies. What remains is uncertainty as to the efficacy of multi-strain probiotics in the alleviation of symptoms at this level of dose.

Studies by Kajander et al. [13–15] of a four-strain mixture comprising *L. rhamnosus GG*, *L. rhamnosus Lc705*, *P. freudenreichii subsp. shermanii* and *B. lactis* build a consistent picture that this combination can reduce IBS symptoms, while altering gut microbiota in a transient fashion without changing faecal enzyme activity. That bloating/distension is a major symptom alleviated by such a probiotic indicates that the changes in gut flora brought about by this mixture can reduce the amount of gas produced in the gut, thereby reducing the distension. *L. rhamnosus GG* alone had little effect on IBS [16] in an eight-week study, suggesting either that only the other three strains have any effect on IBS, or that *L. rhamnosus GG* is only effective in a synergistic fashion in combination with other strains.

The study by Ouwehand et al. [17] found that a multi-strain probiotic composed of *L. reuteri, L. rhamnosus and P. freudenreichii* proved effective in both increasing number of bowel movements and decreasing mucin secretion in elderly subjects. The probiotic mixture was more effective than *L. reuteri* alone, although unfortunately it is difficult to draw conclusions about mixtures versus individual probiotics since only one component of the mixture was tested and its dose was over 10 times lower than the total bacterial dose in the mixture. Similarly, Olivares et al. [18] found an increase in bowel habit and stool volume in healthy subjects aged 23–43 given a

**Table 2** Human studies—Irritable Bowel Syndrome (IBS) and gut function

| Organisms | Study type | End points | Outcome | Reference |
|---|---|---|---|---|
| VSL#3 mixture: *B. longum, B. infantis, B. breve; L. acidophilus, L. casei, L. delbrueckii ssp. Bulgaricus, L. plantarum;* Total daily dose: $6 \times 10^8$ CFU | Open label study, $n = 10$ | Changes in symptoms, faecal flora and enzyme activity in IBS patients when treated with a probiotic mixture | Clinical improvement in 9/10 patients Transient increase in lactobacilli and bifidobacteria and decrease in *enterococci,* coliforms and clostridia*;* decreased urease and increased $\beta$-galactosidase activity; all levels returned to pre-treatment 10 days after treatment ended | [9] |
| Group 1: VSL#3 as above, $4.5 \times 10^9$ CFU per day<br>Group 2: starch powder | RDBPCT, $n = 25$ | Effect of supplementation with multi-strain probiotic on transit time and symptoms of patients with dIBS | Borderline improvement in bloating in probiotic group; no improvement in bowel function, abdominal pain, flatulence, and satisfactory relief | [10] |
| Group 1: VSL#3 as above, $4.5 \times 10^9$ CFU per day<br>Group 2: sterile powder | RDBPCT, $n = 48$ | Effects of a probiotic combination on symptoms and colonic transit in IBS patients | Reduced symptom score only in flatulence in probiotic group; decreased transit time in probiotic group | [11] |
| Group 1: mixture of *L. GG, B. breve, L. rhamnosus, P. freudenreichii subsp. Shermanii,* total count: 8–9 $\times 10^9$ CFU per day—equal proportions of each strain<br>Group 2: placebo capsule | RDBPCT, $n = 103$ | Reduction of IBS symptoms when treated with a probiotic mixture assessed by self-scoring and bowel habit | Reduction in scores for all 4 symptoms in 76% probiotic patients (vs. 43% placebo); increased frequency of bowel habit and improved sensation of completed evacuation in probiotic group | [13] |
| Group 1: *L. GG, L. rhamnosus Lc705, P. freudenreichii subsp. shermanii, B. lactis:* $1.2 \times 10^9$ CFU of each strain per day<br>Group 2: placebo capsule | RDBPCT, $n = 55$ | Changes in gut microbiota of IBS sufferers induced by multi-strain probiotic treatment | All supplemented strains detected in faecal samples; most analysed strains stable, decreased *bifidobacteria* in probiotic group ($\neq$ placebo group). Similar levels, and lack of change in, of short-chain fatty acids in both groups; decreased $\beta$-glucuronidase activity in probiotic group | [14] |
| Group 1: fermented milk drink containing a mixture of *L. rhamnosus GG, L. rhamnosus Lc705, P. freudenreichii subsp. shermanii, B. lactis:*$1.2 \times 10^9$ CFU of each strain per day<br>Group 2: placebo drink | RPCT, $n = 86$ | Effect of a multi-strain probiotic on IBS: abdominal symptoms, quality of life, gut microbiota, inflammatory markers | 34% decrease in symptom score in probiotic group ($\neq$ 9% in placebo group); abdominal pain and distension milder in probiotic group. No difference in stool consistency or diarrhoea. No difference in C-reactive protein; probiotic group had more stable microbiota | [15] |
| Fruit juice containing:<br>Group1: placebo<br>Group 2: *L. reuteri* $7.2 \times 10^8$ CFU/day<br>Group 3: *L. rhamnosus* 2–4 $\times 10^{10}$ CFU/day + *P. freudenreichii* 4–8 $\times 10^{10}$ CFU/day | Open study, $n = 28$ | Effects of single- or multi-strain probiotic on constipation and faecal composition in elderly | Increased defecation only in multi-strain group; small reduction in faecal azoreductase and mucin secretion in both probiotic groups, greater decrease in multi-strain group | [17] |
| Group 1: yoghurt containing *S. thermophilus* $4 \times 10^8$ CFU, *L. bulgaricus* $4 \times 10^9$ CFU<br>Group 2: mixture of *S. thermophilus* $4 \times 10^8$ CFU,<br>*L. gasserii* $4 \times 10^9$ CFU, *L. corniformis*-$4 \times 10^9$ CFU | RDBPCT, $n = 30$ | Bowel function in healthy adults (23–43) after probiotic supplementation | Increased frequency of bowel habit and stool volume, faecal lactic acid bacteria and short-chain fatty acids in probiotic group, reduced cytotoxic faecal supernatants in probiotic group | [18] |

mixture of *L. gasseri* and *L. corniformis* compared with standard yoghurt containing *S. thermophilus* and *L. bulgaricus.* While this study attests to the effect of a multi-strain probiotic, it gives no evidence as to the greater efficacy in using a greater number of strains, as the delivery vectors are different in either group.

Overall, with respect to IBS, the picture is unclear; multiple-strains are reported as both ineffective and

effective, but comparison of the respective studies is difficult due to differing methodologies and doses used. Since the mixtures tested contain different strains and the individual strains were not tested, it is difficult to determine whether the presence of a given strain in a mixture is crucial, or whether the effect of a mixture depends on the interaction of several strains. In the two studies in healthy, non-IBS populations [17, 18], it appears that gut function can be improved by supplementation with a multi-species probiotic.

## Inflammatory bowel disease (Table 3)

In animal models of active ulcerative colitis, a decrease in lactobacilli has been consistently observed [19], while in patients with Crohn's disease, decreased concentrations of bifidobacteria have been noted [20]. Although the aetiologies of these conditions have not been fully understood, these observations represent rationale for the hypothesis that a multi-strain probiotic, able to re-colonise the gut with these genera, may be of benefit to patients with IBD.

### *Ulcerative colitis (UC)*

Both Bibiloni et al. [21] and Venturi et al. [22], in small open label studies, observed high degrees of maintenance of remission with VSL#3 at different, high doses compared to placebo. Both reported increases in levels of faecal lactobacilli and bifidobacteria during treatment. A possible mechanism here is the alteration of gut microbiota leading to an improvement in the condition, although this is impossible to substantiate this as the authors did not perform analysis of the gut microbiota. Cui et al. [23] also noted a similar effect using a different multi-strain probiotic containing lactobacilli and bifidobacteria at a lower dose, reinforcing this hypothesis, although it must be noted that all patients were receiving the probiotics alongside antibiotics. These studies suggest that multi-strain probiotics may be effective at maintaining remission of UC.

### *Pouchitis*

Pouchitis is the most common complication after surgery for ulcerative colitis [24]. Treatment typically consists of antibiotic therapy [25], suggesting that one of the main aetiological factors is concentration of bacteria in the region. With this in mind, several studies have examined the influence of VSL#3 multi-strain probiotic mixture. All three studies [26–28] found that in patients with relapsing pouchitis, there was a very high incidence of relapse without probiotic intervention. However, intervention at varying doses with the VSL#3 mixture led to a drastic reduction in the incidence of relapse, as well as an increase in quality of life, measured by different questionnaires. An increase in lactic acid bacteria in the faeces up to 15 days after cessation of treatment was also seen.

This evidence suggests that a multi-strain probiotic may be an effective alternative to the long-term use of antibiotics in patients after surgery for UC, without the side-effects connected with use of antibiotics such as antibiotic-associated diarrhoea [29], although it should be noted that all the studies conducted so far have used small numbers of patients. From the data regarding faecal bacteria profiles, it appears that colonisation of lactobacilli and bifidobacteria is transient, suggesting that probiotic treatment would need to be ongoing, although this may be preferable to continued antibiotic treatment. Few data exist as to the comparative efficacy of single-strain and multi-strain probiotics, and as far as we can ascertain, no studies have been carried out comparing the effect of a probiotic mixture with that of one or more of its component strains. Thus, it is impossible to suggest whether a mixture is more or less effective. Similarly, there are few investigations of the optimum dose for use of multi-strain preparations, so these are areas needing further work.

## Treatment of *Helicobacter pylori* infection (Table 4)

Two studies that have used multi-strain probiotics alongside antibiotics to treat *H. pylori* infections indicate that such combinations are effective both at eradicating the infection, and reducing side-effects due to the disruption of gut microbiota caused by the triple therapy of antibiotics and protein pump inhibitors [30].

Cremonini et al. [31] compared treatment of patients with *Saccharomyces boulardii* or *L. rhamnosus GG*, or a combination of *L. acidophilus* and *B. lactis*. Reduced incidence of diarrhoea, along with reduced side-effects of the antibiotic treatments, was observed in both single-strain and mixture groups. However, there were no significant differences between the various probiotic regimens. Myllyluoma et al. [32] showed an increased eradication rate of *H. pylori* in a group taking a four-strain mixture (*L. rhamnosus GG*, *L. rhamnosus*, *B. breve, Propionibacterium shermanii*), although comparison was made with placebo rather than with single strains.

While these studies attest to the efficacy of multi-strain probiotics in the treatment of *H. pylori* infection, they provide no evidence for any greater effect of multi-strain over that of a single-strain treatment.

## Modulation of gut microbiota (Table 5)

One of the main side-effects of antibiotic therapy is the disruption of the intestinal flora [33]. Nord et al. [34], Orrhage et al. [35] and Zoppi et al. [36] all investigated the

**Table 3** Human studies: inflammatory bowel disease (IBD)

| Organisms/dose | Study type | End points | Outcome | Reference |
|---|---|---|---|---|
| VSL#3 mixture:<br>*B. longum, B. infantis, B. breve;*<br>*L. acidophilus, L. casei, L. delbrueckii ssp. Bulgaricus, L. plantarum;*<br>*S. salivarius ssp. Thermophilus*<br>Total dose of $1.8 \times 10^{11}$ CFU, twice per day | Open study, $n = 32$ | Induction of remission in patients with mild-moderate active Ulcerative Colitis (UC) who were unresponsive to conventional therapy | Induction of remission in 53% of patients | [21] |
| VSL#3 mixture as above, $6 \times 10^{12}$ CFU per day | Open study, $n = 20$ | Maintenance of remission in patients with Ulcerative Colitis. Colonisation of intestine by probiotic bacteria, and | 75% of patients in remission after 12 months of treatment. Increased faecal lactic acid bacteria in all patients (15 days post-treatment levels returned to normal) | [22] |
| Group 1: Bifico mixture: "*Lactobacillus bifidus, acidophilic lactobacilli* and *Enterococcus*" [58]. Dose: $10^7$ CFU per day<br>Group 2: placebo (starch) capsule | RPCT, $n = 30$ | Prevention of relapse, and inflammatory markers, in patients with active Ulcerative Colitis | 20% relapse rate in probiotic group (vs. 93% in placebo group); increased faecal *lactobacilli* and *bifidobacteria* and anti-inflammatory cytokines in probiotic group | [23] |
| Group 1: VSL#3 mixture as above, $3 \times 10^{11}$ CFU per day<br>Group 2: placebo (starch) capsule | RDBPCT, $n = 40$ | Increase in remission in patients with chronic relapsing pouchitis | 15% relapse in probiotic group compared to 100% in placebo group. Probiotic group only had increased faecal concentration of all strains from mixture | [27] |
| Group 1: VSL#3 mixture as above, $9 \times 10^{11}$ CFU per day<br>Group 2: placebo (starch) capsule | DBPCT, $n = 40$ | Prevention of the onset of pouchitis after ileal pouch-anal anastomosis – assessed using histological and clinical data | 10% incidence of pouchitis in probiotic group (vs. 40% in placebo group). Lower Pouchitis Disease Activity Index (PDAI) score in probiotic patients with pouchitis than in placebo patients with incidence. Improvement in Inflammatory Bowel Disease Questionnaire (IBDQ) score and reduced stool frequency only in probiotic group | [26] |
| Group 1: VSL#3 mixture as above, $6 \times 10^{11}$ CFU per day<br>Group 2: placebo (starch) capsule | RPCT, $n = 36$ | Maintenance of remission and improved quality of life in patients with recurrent pouchitis | Remission maintained in 85% of probiotic group (vs. 6% placebo group). Deterioration of IBDQ scores seen over 12 months in placebo group; score remained the same for probiotic group | [28] |

effect of probiotic treatment on gut microbiota during such treatment. Orrhage et al. [35] using a fermented milk product (containing *L. delbrueckii and Strep. salivarius)* supplemented with *L. acidophilus and B. longum* plus oligofructose (OF) observed a reduction in the colonisation of *Clostridium difficile* compared with groups given the unsupplemented fermented milk with or without OF. In effect, this is a comparison of two varieties of probiotic/prebiotic mixture, one containing four probiotic strains and one with two; although the four-strain preparation appears to give more beneficial, this conclusion remains unsubstantiated since the comparison in this study is between two separate situations. Nord et al. [34] using a mixture of *L. acidophilus*, *B. bifidum*, *L. delbrueckii and S. thermophilus* reported similar results on *C difficile* carriage versus placebo. In this study, an accelerated re-colonisation of commensal strains alongside this reduction in *C. difficile* suggests that presence of commensal bacteria provides an environment that is not conducive to pathogen colonisation.

Zoppi et al. [36] compared eight different preparations in the treatment of children having antibiotic therapy for respiratory infections. In accordance with the studies above, the most successful preparations at returning gut microbiota to normal parameters were those with greater number both of strains and of cells. This again suggests a synergistic effect, but since the individual strains used were not among the constituents of the mixtures, it is difficult to

**Table 4** *Helicobacter pylori* treatment

| Organisms/dose | Study type | End points | Outcome | Reference |
|---|---|---|---|---|
| Group 1: *Lactobacillus GG:* $6 \times 10^9$ CFU<br>Group 2: *S. boulardii*: $5 \times 10^9$ CFU<br>Group 3: mixture of *L. acidophilus and B. lactis,* of $5 \times 10^9$ CFU<br>Group 4: placebo | RTBPC, $n = 85$ | Comparison of single-strain and multi-strain probiotics and placebo in preventing *H pylori*-therapy side-effects and *H. pylori* infection in asymptomatic patients | Reduced side-effects in all treatment groups, but little difference between probiotic groups. Reduced incidence of diarrhoea in probiotic groups; eradication of *H. Pylori* greater in multi-strain group but non-significant | [31] |
| Group 1: milk-based fruit drink containing mixture of *Lactobacillus GG, Lactobacillus rhamnosus, Bifidobacterium. breve, Propionibacterium shermanii,* total dose: $1.3 \times 10^{11}$ CFU<br>Group 2: milk-based fruit drink (placebo) | RDBPCT, $n = 47$ | Efficacy of eradication treatment of H Pylori infection alongside multi-strain probiotic or placebo | Better eradication in probiotic group. No difference in new/aggravated symptom scores, or bowel function, between groups; total symptom score reduced in probiotic group | [32] |

directly compare the effectiveness of a single strain with that of the single strain when used in conjunction with others.

A pattern emerges from these studies: a variety of multi-strain probiotics, with and without prebiotics, appear to be successful at altering gut microbiota to a more beneficial population, as well as at inhibiting pathogens that can take advantage of a compromised gut. What remains to be elucidated is whether this greater success is due to the sheer weight of numbers used, or due to synergistic effect between strains.

## Atopic dermatitis (Table 6)

Atopic dermatitis (AD) is traditionally treated with emollients and topical anti-inflammatory agents; however, several studies have shown that treatment with probiotics can ameliorate symptoms, as well as inhibit production of inflammatory cytokines. Many such studies have focussed on the use of a single-strain probiotic, while and only a few have focussed on the use of mixtures.

In a randomised, double-blind, placebo-controlled trial, Rosenfeldt et al. [37] treated a group of children suffering with AD with either a combination of $10^{10}$ CFU of each of *Lactobacillus rhamnosus 19070-2* and *Lactobacillus reuteri DSM 122460* or placebo. After treatment, 56% of the experimental group had a subjective improvement in symptoms as opposed to 15% of the placebo group, although the standardised scoring system for atopic dermatitis (SCORAD) scores did not reach statistical significance with regard to reduction of intensity. Also observed was a reduction in level of serum eosinophil cationic protein (sECP)—a cytotoxic protein used to monitor AD disease activity—in the treatment group, with no such decrease with placebo. When the subjects were subdivided into those with or without a food allergy (a potential contributor to the pathogenesis of AD), the probiotic mix was found to have a far more pronounced effect in those with allergy, suggesting an impact on inflammatory cytokine release, although these were not measured.

Viljanen et al. [38] measured levels of faecal inflammatory markers as well as faecal immunoglobulin A (IgA) in food-allergic AD infants. After a 4-week treatment with either *L. rhamnosus GG*, a mixture of four probiotic strains (including *L. rhamnosus GG*), or placebo, levels of IgA were seen to be higher in both treatment groups. There was no decrease in tumour necrosis factor-α (TNF-α), but a decrease in faecal anti-trypsin (AT) in both groups. The two treatment groups, however, did not differ significantly, despite differences in dosage: *L. rhamnosus GG* was given at $5 \times 10^9$ CFU, while the mixture contained *L. rhamnosus GG* $5 \times 10^9$ CFU, *L. rhamnosus LC705* $5 \times 10^9$ CFU, *Bifidobacterium breve Bbi99* $2 \times 10^8$ CFU and *Propionibacterium freudenrechii* ssp. *Shermanii JS* $2 \times 10^9$ CFU. This suggests two possible explanations; first, that *L. rhamnosus GG* was the only species to have any effect, since the presence of the other species had no additional effect; second, that there is a maximum effect on immune function achievable with probiotic treatment.

In a subsequent paper, the same authors [39] compared the effect of *L. rhamnosus GG* and the same probiotic mixture on severity of symptoms of AD in the children, half of whom suffered from cow's milk allergy. While each group's SCORAD rating decreased after the subjects were placed on a cow's milk elimination diet, there was no difference in improvement of symptoms between both

**Table 5** Human studies: modulation of gut microbiota

| Organisms/dose | Study type | End points | Outcome | Reference |
|---|---|---|---|---|
| Group 1: mixture of *L. acidophilus, B. bifidum, L. delbrueckii, S. thermophilus,* total daily dose: $2 \times 10^{10}$ CFU, equal proportions of all 4 strains<br>Group 2: placebo capsule | RPCT, $n = 23$ | Effect on faecal flora of adults 21–54 years taking a probiotic mixture alongside clindamycin | Probiotic group (group 1) showed increased total count and aerobes, delayed reduction of *bifidobacteria* and accelerated re-colonisation of *bifidobacteria,* reduced colonisation of *C. difficile* | [34] |
| Group 1: 250 ml fermented milk supplement containing:<br>*B. longum:* $5 \times 10^7$–$2 \times 10^8$ CFU/ml;<br>*L. acidophilus:* 2–3 $\times 10^8$ CFU/ml<br>15 g oligofructose (OF)<br>Group 2: 250 ml fermented milk supplement containing:<br>15 g oligofructise<br>Group 3: 250 ml fermented milk supplement placebo<br>N.B. Milk product also contained:<br>*L. delbrueckii*: $10^7$–$10^8$ CFU/ml<br>*S. salivarius:* $10^8$–$10^9$ CFU/ml | RDBPCT, $n = 30$ | Effect of OF with or without multi-strain probiotic on gut flora post-antibiotic therapy with cefpodoxime | Pro + OF group exhibited decrease in anaerobes and increase in lactobacilli; lower incidence of *C. difficile* colonisation (1/6) vs. 6/6) Yeasts increased in groups 1 and 2 | [35] |
| Group 1: Ceftriaxone (Cx) alone<br>Group 2: Cx + *S. boulardii* $1 \times 10^9$ CFU<br>Group 3: Cx + *Enterococcus SF68* $7.5 \times 10^7$ CFU<br>Group 4: Cx + lactulose<br>Group 5: Cx + *L. rhamnosus GG* $3 \times 10^9$ CFU<br>Group 6: Cx + *L. rhamnosus* $1.1 \times 10^9$ CFU, *L. bifidus* $6 \times 10^9$ CFU, *L. acidophilus* $6 \times 10^8$ CFU<br>Group 7: Cx + *B. bifidum* $10^9$ CFU, *L. acidophilus* $10^8$ CFU<br>Group 8: Cx + *S. salivarius* $2.04 \times 10^{11}$, *B. breve* $9.3 \times 10^{10}$, *B. infantis* $9.3 \times 10^{10}$, *B. longum* $9.3 \times 10^{10}$, *L. acidophilus* $2 \times 10^8$ CFU, *L. plantarum* $2.2 \times 10^8$ CFU, *L. casei* $2.2 \times 10^8$ CFU, *L. delbrueckii* $2 \times 10^8$ CFU, *S. faecium* $3 \times 10^7$ CFU | RPCT, $n = 51$ | Modulation of flora during treatment with ceftriaxone in children (mean age 5.1 years) | Multi-strain containing lactobacilli and bifidobacteria better at reducing dysbiosis; all preparations reduced clostridium spp; preparations 6, 7 and 8 had most significant effect on flora composition and numbers; no difference between groups in faecal enzyme activity | [36] |

probiotic groups and the placebo group. However, for the subgroup of IgE-sensitised children, the SCORAD ratings were significantly reduced in the *L. rhamnosus GG* group, with no reduction in the mixture group. One major confounder in this study is that a large proportion of subjects were treated with antibiotics during or shortly after the treatment periods. Removing these subjects from the analysis increased the effect of both the *L. rhamnosus GG* and mixture groups. No data are given as to whether there was a significant difference between these two groups.

Overall, the evidence for a probiotic mixture as a treatment for AD appears contradictory. The findings of Rosenfeldt et al. [37] point to probiotics reducing the levels of inflammatory cytokines, but not skin inflammation. The study by Viljanen et al. [38] again showed effects on inflammatory markers TNF-$\alpha$ and faecal anti-trypsin but no difference between single and mixed probiotic treatments, even with a higher overall dose with the mixture. In the second Viljanen et al. study [39], the data point to there only being an effect in IgE-sensitised children, and only with the single probiotic. In the latter two studies, evidence suggests *L. rhamnosus GG* as a useful addition to corticosteroid treatment for AD, but that the use of this species alongside others may reduce its efficacy. However, given

**Table 6** Human studies: atopic dermatitis

| Organisms | Study type | End points | Outcome | Reference |
|---|---|---|---|---|
| Group 1: mixture of *L. reuteri, L. rhamnosus*, $10^{10}$ CFU each strain<br>Group 2: placebo (skimmed milk powder) | RDBPCT, $n = 43$ | Amelioration of AD symptoms in children; plasma sECP levels | (Non-significant) improvement of symptoms, decreased in sECP | [37] |
| Group 1: *L. rhamnosus GG* $5 \times 10^9$ CFU;<br>Group 2: mixture of *L. rhamnosus GG* $5 \times 10^9$ CFU, *L. rhamnosus LC705* $5 \times 10^9$ CFU, *B. breve Bbi99* $2 \times 10^8$ CFU, P. *freudenrechii* ssp. *Shermanii JS* $2 \times 10^9$ CFU<br>Group 3: placebo (microcrystalline cellulose) | RDBPCT, $n = 230$ | Changes to levels of inflammatory markers | Reduction of IgA and AT, no change in TNF-α | [38] |
| Group 1: *L. rhamnosus GG* $5 \times 10^9$ CFU;<br>Group 2: mixture of *L. rhamnosus GG* $5 \times 10^9$ CFU, *L. rhamnosus LC705* $5 \times 10^9$ CFU, *B. breve Bbi99* $2 \times 10^8$ CFU, P. *freudenrechii* ssp. *Shermanii JS* $2 \times 10^9$ CFU<br>Group 3: placebo (microcrystalline cellulose) | RDBPCT, $n = 230$ | Amelioration of symptoms | Symptomatic improvement in both probiotic groups and placebo group | [39] |

the complex aetiology of the condition [40], improvement in the condition due to probiotics may be limited irrespective of dose and variety of strains.

## Diarrhoea including antibiotic-associated diarrhoea (AAD) (Table 7)

### *Acute diarrhoea in children*

Acute diarrhoea in infants often represents the first cause of hospitalisation and is usually caused by infection with rotavirus in both developed and developing countries [41]. Treatment of such diarrhoea generally consists of oral rehydration solutions to replace lost fluid and electrolytes [42] although these are generally ineffective at reducing the duration of the diarrhoea. It is thought that treatment with probiotics may reduce the time of rotavirus excretion and therefore the duration of the illness [43].

Barone et al. [44] found no significant differences in alleviation of symptoms between a single probiotic treatment (*Sacc. boulardii*) and 2 different multi-strain probiotic mixtures. However, the study used a small number of subjects, the probiotic doses were different and there was no placebo control, so firm conclusions are difficult to draw.

A study by Rosenfeldt et al. [45] indicates the efficacy of a mixture of *L. rhamnosus and L. reuteri* used at high doses ($10^{11}$ CFU 2×/day) in treatment of acute diarrhoea in children. By comparison with a control, the probiotic mixture reduced hospital stay and viral shedding. Although the study did not compare the mixture to individual strains, the authors noted that the combination seems to be no more effective than a single-strain probiotic, *Lactobacillus* GG. In a double-blind, randomised, placebo-controlled trial with a powdered formula containing *B. bifidum* and *S. thermophilus,* Saavedra et al. [46] observed a reduction in incidence, but not in severity or duration, of infant diarrhoea. The much lower dose used by Saavedra et al. [46] may account for the lack of effect on severity and duration, although with the use of different strains, the strain-specific nature of probiotic effect against pathogens would suggest that it is hard to compare the two studies.

Grandy et al. [47] observed a reduction in rotavirus diarrhoea in groups taking both single- and multi-strain products, albeit only a significant reduction in the single-strain group. The duration of fever was only reduced in the single-strain group despite the multi-strain product also containing that single strain (*Sacc. boulardii*). This suggests that the strain-specific effect was diminished either by the thousandfold reduction in the dose of that particular strain, or by the presence of the other probiotic microorganisms in the multi-strain product, possibly outcompeting *Sacc. boulardii* for nutrients or binding sites. Interestingly, duration of vomiting was only reduced in the multi-strain group despite a lower total dose, indicating either that this combination or one of its components was able to produce a beneficial effect that *Sacc. boulardii* alone was unable to provide.

### *Treatment of antibiotic-associated diarrhoea*

One of the major complications of treatment with antimicrobial agents is antibiotic-associated diarrhoea (AAD), occurring in 5–25% of patients [29]. One of the major causes of AAD is infection with *Clostridium difficile*, believed to be responsible for 15–25% of AAD cases [48].

Three randomised, double-blind, placebo-controlled trials have examined the role of probiotic combinations in

**Table 7** Human studies: diarrhoea, including antibiotic-associated diarrhoea (AAD)

| Organisms/dose | Study type | End points | Outcome | Reference |
|---|---|---|---|---|
| Group 1: yovis mix: *S. salivarius ssp. thermophilus, L. acidophilus, L. plantarum, L. casei, L. delbrueckii ssp. Bulgaricus, S. faecium:* $9.58 \times 10^{10}$ CFU in total<br>Group 2: lactogermine mix: *L. acidophilus, B. bifidum, S. thermophilus, L. bulgaricus:* $3.5 \times 10^{9}$ in total<br>Group 3: codex preparation: *S. boulardii:* 5 x $10^{9}$ | Prospective, $n = 33$ | Comparison of multi- and single-strain probiotics in treatment of diarrhoea in mentally retarded children assessed by number of defecations, time of belly normalisation and fever | No difference in any criteria between all 3 treatments | [44] |
| Group 1: mixture of *L. rhamnosus* and *L. reuteri*, $10 \times 10^{10}$ CFU each strain twice daily for 5 days<br>Group 2: placebo skimmed milk powder | RPCT, $n = 69$ | Effect of probiotic mixture in children 6–36 m hospitalised with acute diarrhoea | 20% decrease in duration, 48% reduction in hospital stay, reduced viral shedding in probiotic group. Fewer members of probiotic group had diarrhoea at end of study. More pronounced effect if treatment administered within first 60 h of illness | [45] |
| Group 1: powdered formula containing *B. bifidum:* $1.9 \times 10^{8}$ CFU/g and *S. thermophilus:* $0.14 \times 10^{8}$ CFU/g<br>Group 2: placebo (formula without probiotics) | RDBPCT, $n = 55$ | Incidence and severity of diarrhoea and rotavirus in hospitalised infants (5–24 m), assessed by frequency of defecation, clinical examination and faecal microbial evaluation | Reduced incidence of diarrhoea and rotavirus in probiotic group; similar severity and duration of diarrhoea between 2 groups | [46] |
| Group 1: placebo (sterile powder)<br>Group 2: *Sacc. Boulardii* $4 \times 10^{10}$ CFU/day<br>Group 3: *Sacc. Boulardii* $1.4 \times 10^{7}$, *L. acidophilus* $6.6 \times 10^{7}$, *L. rhamnosus* $3.\ 6 \times 10^{7}$, *B. longum* $8.8 \times 10^{6}$; total dose $1.25 \times 10^{8}$ CFU/day | RDBPCT, $n = 64$ | Treatment of acute rotavirus diarrhoea in children 1–23 months | Decreased duration in both probiotic groups, only significant in single-strain group; duration of fever only reduced in single-strain group; reduced duration of vomiting in multi-strain group | [47] |
| Group 1: lactinex mix: *L. acidophilus, L. bulgaricus.* Dose not stated, but each Lactinex packet contains $10^{8}$ CFU (www.bd.com)<br>Group 2: placebo (not stated) | RDBPCT, $n = 79$ | Prevention of antibiotic-associated diarrhoea (AAD) by *lactobacillus* blend or placebo | Reduced incidence of AAD in experimental group (8.3% vs. 21%) | [49] |
| Group 1: 97 ml yoghurt drink containing:<br>*L. casei:* $10^{8}$ CFU/ml, *L. bulgaricus:* $10^{8}$ CFU/ml, *S. thermophilus:* $10^{7}$CFU/ml, Total dose: $2.04 \times 10^{10}$ CFU twice daily<br>Group p2: placebo (sterile milkshake) | RDBPCT, $n = 135$ | Prevention of *C. difficile*-associated diarrhoea (CDCAD) and AAD | Reduced incidence of both forms of diarrhoea in experimental group (12% $\neq$ 34%); 75% reduction in relative risk for probiotic group | [50] |
| Group 1: mixture of *L. acidophilus* and *B .bifidum, t*otal dose: $2 \times 10^{10}$ per capsule (proportions not stated), one capsule/day for 20 days<br>Group 2: placebo (sterile capsule) | RDBPCT, $n = 150$ | Probiotic administration in the prevention of CDAD in the elderly | No difference in incidence between groups; more *C. difficile* carriers but, fewer toxins, recorded in probiotic group | [51] |

the treatment of AAD [49–51]. Gotz et al. [49] using *L. acidophilus* and *L. bulgaricus* found a 13% reduction in incidence of ampicillin-related diarrhoea, while Hickson et al. [50] using *L. casei, L. bulgaricus* and *S. thermophilus* found a 22% reduction in incidence in AAD and complete elimination of *C. difficile* toxin in faeces, albeit with a much larger dose of probiotics. In contrast, despite using a larger dose of probiotics, Plummer et al. [51] found no difference between treatment (*L. acidophilus* plus *B. bifidum*) and placebo groups, although the experimental group was found to have a reduced incidence of *C. difficile* toxin.

Overall, these studies support the use of multi-strain probiotics in the treatment of infant diarrhoea and AAD, although give no information on whether mixtures are more effective than single strains. What has yet to be defined is the most effective strains and doses for each particular type of diarrhoea, as well as which strains should be used for prevention, and which for treatment after the onset of diarrhoea, as the evidence above suggests some strain-specific effects in this regard.

Respiratory tract infections (RTI) (Table 8)

Two studies have examined the effect in adults of a combined multi-strain probiotic and multivitamin/mineral supplement containing *L. gasseri, B. longum* and *B. bifidum* on the incidence, duration and severity of common cold infections and aspects of immune function [52, 53]. Both studies found a reduction in severity and duration, as well as enhanced expression of immune cells, while only Winkler et al. [53] found a reduction in incidence. The major difference between studies is dose—the same probiotic strains were used for both, as well as the same assessment methods for the illness—suggesting that although the dose used by de Vrese et al. [52] ($5 \times 10^7$ CFU) was enough to attenuate symptoms and duration, a higher dose such as that used by Winkler et al. [53] ($5 \times 10^8$ CFU per day) was needed for prevention of infections. The lower dose may promote a systemic immune response sufficient to reduce severity and duration but not incidence, while the higher dose may stimulate systemic immunity via the mechanism of distribution of T and B lymphocytes, primed in the gut, which proliferate to the mucosal-associated lymphoid tissue (MALT), where the B cells differentiate into immunoglobulin-producing cells after specific antigenic exposure, leading to an inhibition of colonisation by pathogenic strains. Olivares et al. [54] also found an immunostimulatory effect in subjects given a multi-strain probiotic containing *L. gasseri* and *L. corniformis*, compared with a standard yoghurt containing *S. thermophilus* and *L. bulgaricus*, although this study provides no evidence for the efficacy of a greater number of strains, since two non-comparable treatments were used.

Glűck and Gebbers [55] investigated colonisation by nasal pathogens and showed a 19% reduction in the group given probiotics (*L. rhamnosus GG*, *Bifidobacterium lactis*, *L. acidophilus*, *S. thermophilus*) compared to no reduction with placebo. Despite this reduction in colonisation, no data are given as to whether subjects became unwell during the study period, making conclusions as to actual health benefits difficult to draw. In a similar study, Hatakka et al. [56] found no effect of a probiotic mixture on incidence and duration of otitis and upper respiratory infections on children aged 6 months to 10 years; a lower dose than that used by Glűck and Gebbers [55] may explain the disparity between results. It may also be that ingested probiotics have less effect on the aural mucosa compared to that on the nasal mucosa, or that the effects are strain-specific.

In a 7-month study with over 1,000 subjects, Lin et al. [57] examined the protective effect of two single probiotics (*L. casei* and *L. rhamnosus*, given individually) and one multi-strain mixture containing the 2 lactobacilli and 10 other organisms. Reduced physician visits, as well as decreased incidence of bacterial, and viral respiratory disease were seen in all groups compared with placebo, but there was no significant difference in effectiveness between the preparations even though the multi-strain probiotic was given at a tenfold higher dose than the individual strains. However, in the case of prevention of gastro-intestinal tract infections, the probiotic mixture was significantly more effective than the single strains. This may be due to the exceptionally high dose given in the multi-strain treatment, resulting in larger numbers of probiotic bacteria competing with pathogens for binding sites and or nutrients in the gut. Another point of interest in this study is that despite large differences in dose, the two single strains did not have statistically different effects, suggesting strain-specificity in dose and effect for individual species.

These data support the theory that supplementation with certain multi-strain probiotics can reduce severity, duration, and possibly incidence of RTIs, and in the case of Lin et al. [57] that a multi-strain probiotic may be more effective than a single-strain. There is some evidence for immunostimulation, even in cases where illness still occurs. Further consistency could be added to this evidence with the establishment, by testing varied concentrations of probiotic bacteria, of an optimum dose that prevents pathogenic colonisation of the mucosa as well as the incidence and severity of illness. Testing this dose with and without vitamin and mineral supplementation may reveal a synergy between both types of supplement. Further work should be done to determine the relative efficacy of single- and multi-strain probiotics in this area.

**Table 8** Human studies: immune function, incidence and duration of respiratory tract infections (RTI)

| Organisms/dose | Study type | End points | Outcome | Reference |
|---|---|---|---|---|
| Group 1: multivitamin/mineral plus *L. gasseri*: $4 \times 10^7$ CFU, *B. longum:* $5 \times 10^6$ CFU, *B. bifidum*: $5 \times 10$ CFU, $5 \times 10^7$ CFU in total<br>Group 2: placebo (multivitamin/mineral only) | RDBPCT, $n = 479$ | Incidence and severity of common cold over a 3-month period in adults treated with probiotic and/or vitamin and mineral supplements, assessed by total symptom scoring, duration, cellular immune response and faecal probiotic bacteria counts | Reduced number of days with fever, duration of cold episodes (mean 2 days), total symptom score; increased T-suppressor cells in probiotic group compared to vitamin/mineral only group; increased numbers faecal *lactobacilli* and *bifidobacteria* in probiotic group from day 1–14 of study. No reduction in incidence of RTIs in either group | [52] |
| Group 1: multivitamin/mineral plus *L. gasseri*: $4 \times 10^8$ CFU, *B. longum*: $5 \times 10^7$ CFU, *B. bifidum*: $5 \times 10^7$ CFU, $5 \times 10^8$ CFU in total<br>Group 2: placebo (multivitamin/mineral only) | RDBPCT, $n = 477$ | Incidence, duration and severity of common cold in adults given probiotic multivitamin and mineral supplement or placebo over 3-month period | Incidence 13.6% lower, symptoms and number of days with fever reduced, in probiotic group; 9.3% shorter duration in probiotic group. Greater increase in immune function (CD4+ , CD8+ , T-lymphocytes, monocytes) in probiotic group | [53] |
| Group 1: fermented milk drink containing *Lactobacillus GG:* $7.1 \times 10^9$ CFU, *Bifidobacterium B420:* $8.4 \times 10^9$ CFU,<br>*L. acidophilus:* $3.2 \times 10^9$ CFU, *S. thermophilus:* $27 \times 10^9$ CFU. Total: $4.57 \times 10^{10}$<br>Group 2: placebo (standard yoghurt drink) | Open trial, $n = 209$ | Colonisation of nasal pathogens over a 28-day period in patients supplemented with a probiotic mixture or standard yoghurt | 19% reduction of colonisation in probiotic group; between days 1 and 21 pathogenic bacteria eliminated in 13/108 probiotic-fed subjects | [55] |
| Group 1: capsule containing *Lactobacillus GG,*<br>*Bifidobacterium breve, Propionibacterium freudenreichii;* $8–9 \times 10^9$ CFU of each strain per capsule, 1 capsule per day<br>Group 2: placebo (capsule containing microcrystalline cellulose) | RDBPCT, $n = 269$ | Occurrence and duration of otitis and upper respiratory infections in susceptible children (10 m–6y) treated with probiotic combination or placebo over a 24-week period; assessed by clinical examination and bacteriological screening of nasal swab | No effect on occurrence or recurrence; no reduction in carriage of potential pathogens | [56] |
| Group 1: *L. casei:* $6 \times 10^8$ CFU/day, 5 days/week<br>Group 2: *L. rhamnosus:* $3.42 \times 10^{10}$ CFU/day, 5 days/week<br>Group 3: mixture of *B. bifidum, B. infantis, B. longum, L. casei, L. acidophilus, L. salivarius, L. brevis, L. plantarum, L. helveticus, L. rhamnosus, S. thermophilus, E. faecium*: $10^{11}$ CFU/day, 5 days/week<br>Group 4: control group—no probiotic supplementation | RDBCT, $n = 1062$ | Prevention of paediatric bacterial, viral, gastrointestinal and respiratory diseases in children under 5 years old by supplementation with single- or multi-strain probiotic of placebo | Reduced incidence of respiratory infections (bacterial and viral) as well as physician visits in all groups; no significant differences between groups; reduction in GI infections only in multi-strain group | [57] |
| Group 1: yoghurt containing *S. thermophilus*: $4 \times 10^8$ CFU, *L. bulgaricus*: $4 \times 10^9$ CFU<br>Group 2: mixture of *S. thermophilus* $4 \times 10^8$ CFU, *L. gasserii* $4 \times 10^9$ CFU, *L. corniformis*: $4 \times 10^9$ CFU | RDBPCT, $n = 30$ | Multiple immune responses in adults (23–43) after probiotic supplementation | Increase in neutrophils in both groups, only maintained by probiotic group; increased natural killer (NK) cells and interleukin-4 and -10, decreased immunoglobulin E in probiotic group; greater increase in phagocyte activity in probiotic group | [54] |

**Table 9** Summary of studies with probiotic mixtures vs. component strains

| End point | Outcomes | Conclusions | Organisms | Reference |
|---|---|---|---|---|
| In vivo studies | | | | |
| Atopic dermatitis in children | No difference in treatment groups except for IgE-sensitised patients | Mix = SS | *L. rhamnosus GG* vs. Mixture of *L. rhamnosus GG, B. breve, L. rhamnosus* and *P. freudenrechii* | [39] |
| Atopic dermatitis in children | Reduction of IgA and AT, no change in TNF-α | Mix = SS | *L. rhamnosus. GG* vs. Mixture of *L. rhamnosus GG, B. breve, L. rhamnosus* and *P. freudenrechii* | [39] |
| Diarrhoea in children (rotavirus-associated) | Decreased duration<br>Reduced duration of fever;<br>Reduced duration of vomiting; | Mix < SS<br>Mix < SS<br>Mix > SS | *Sacc. Boulardii* vs. Mixture of *Sacc. Boulardii, L. acidophilus, L. rhamnosus, B. longum* | [47] |
| Pathogen infection in mice | 21-day survival;<br>Prevention of liver/spleen colonisation;<br>Levels of circulating antibodies;<br>Levels of intestinal antibodies in single-strain groups;<br>No protection offered by mixture administered post- *S. typhimurium* challenge | Mix > SS<br>Mix > SS<br>Mix > SS<br>Mix > SS<br>Mix = SS | *L. casei* vs., *L. acidophilus* vs. Mixture of both strains | [7] |
| Pathogen infection in lambs | Shedding, daily weight gain and gain: feed ratio;<br>Duration of shedding | Mix > SS<br>Mix = SS | *L. acidophilus, S. faecium* individually<br>Mixture of *L. acidophilus* and *S. faecium*<br>Mixture of *L. acidophilus, S. faecium, L. casei, L. fermentum* and *L. plantarum* | [5] |
| Pathogen infection in mice | Survival rate;<br>Rate of phagocytosis;<br>Levels of serum IgA | Mix > SS<br>Mix = SS<br>Mix < SS | *L. casei* vs.<br>Mixture of *S. thermophilus, L. bulgaricus* vs.<br>Mixture of *S. thermophilus, L. bulgaricus L. casei* | [6] |
| Prevention of paediatric bacterial, viral, gastrointestinal and respiratory diseases in healthy children | Reduced physician visits;<br>Incidence of bacterial, respiratory and viral infections;<br>Reduction in GI infections | Mix = SS<br>Mix = SS<br>Mix > SS | *L. casei* vs.<br>*L. rhamnosus* vs.<br>Mixture of *B. bifidum, B. infantis, B. longum, L. casei, L. acidophilus, L. salivarius, L. brevis, L. plantarum, L. helveticus, L. rhamnosus, S. thermophilus, E. faecium* | [57] |
| In vitro studies | | | | |
| Adhesion of pathogen (*E. Sakazakii*) | Prevention of pathogen adhesion | Mix > SS | *Lactobacillus GG, L rhamnosus, P. freudenreichii, B. breve* vs. Mixtures of 2, 3 and 4 strains | [59] |
| Adhesion of pathogens | Inhibition of individual stains;<br>Inhibition of all pathogenic strains | Mix > SS<br>Mix > SS | *Lactobacillus GG, L rhamnosus, P. freudenreichii, B. breve* vs. Mixtures of 2, 3 and 4 strains | [60] |

**Table 9** continued

| End point | Outcomes | Conclusions | Organisms | Reference |
|---|---|---|---|---|
| Adhesion, barrier function, cell death and inflammatory response | Protection against barrier function decline;<br>anti-inflammatory effect;<br>Increase in IL-8 secretion | Mix = SS<br>Mix < SS<br>Mix = SS | *L. rhamnosus GG, L. rhamnosus, P. freudenreichii, B. breve* vs.<br>Mixture of all 4 | [61] |
| Inflammatory response to Enterotoxigenic *E. coli* | Reduced expression of IL-8, GRO-α and ENA | Mix = SS | *L. GG, B. animalis*, mixture of both strains | [62] |
| Mucus-binding properties of probiotics | Enhanced adhesion of *L. GG, L rhamnosus, P. freudenreichii* | Mix > SS | *Lactobacillus GG, L. rhamnosus, P. freudenreichii, B. breve* vs.<br>Mixtures of 2, 3 and 4 strains | [63] |
| Mucus-binding of probiotics | Increase in binding when incubated with single-or multi-strain product | Mix > SS | *B. lactis, L. rhamnosus GG L. delbrueckii subsp bulgaricus, L. acidophilus, L. johnsonii* vs.<br>Mixtures of 2 of each of these strains | [64] |
| Pathogen growth inhibition (*Shigella sonnei)* | Increased inhibition of *S. sonnei* growth in coculture | Mix > SS | Probiotics: *L. casei, L. acidophilus* vs. Mixture of the two strains | [65] |
| Pathogen growth inhibition | Inhibition of *E. coli* and *S. enteritidis* growth;<br>Inhibition of *V. Cholerae* | Mix > SS<br>Mix = SS | *L. paracasei B21060 and B21070, L. acidophilus B21190* vs.<br>Mixture of all 3 strains | [66] |
| Pathogen growth inhibition (13 strains isolated from pancreatic necrosis) | Inhibition of all pathogens | Mix > SS | *Lactobacillus acidophilus W70, Lactobacillus casei, Lactobacillus salivarius, Lactococcus lactis, Bifidobacterium Bifidum, Bifidobacterium infantis* vs.<br>Mixture of all 6 strains (Ecologic 641) | [67] |

## Conclusions

From the studies reviewed, there is good evidence that probiotic mixtures have beneficial effects against a wide range of disorders. The evidence that mixtures are more effective than their component strains is more limited, especially in certain areas of use e.g. IBD. This is summarised in Table 9, which lists 16 studies in which a direct comparison was made between a probiotic mixture and one or more of its component strains. Of these 16 studies, 12 showed the mixture to be more effective than the single strains, against a variety of end points such as inhibition of pathogen growth and atopic dermatitis. Furthermore, there were very few studies that reported a greater effect by a single-strain probiotic than that of a mixture. However, in many of the in vivo studies, the comparison is not strictly valid due to the fact that in each study, the dose of multi-strain probiotic differed from that of the single strain. In order to demonstrate more definitively the greater effectiveness of a multi-strain probiotic, more studies are needed using identical doses in matched populations, testing against a variety of conditions, such as inflammatory bowel disease.

It is of interest that of the 12 studies showing greater effectiveness of probiotic mixtures, eight used a *Lactobacillus* sp. as well as another non-bifidobacterial strain, while only six used a *Bifidobacterium* sp. along with a *Lactobacillus*. This suggests greater efficacy of lactobacilli within a mixture. In all four studies showing no greater effect from the mixture, *Bifidobacterium* spp were used, suggesting that bifidobacteria may be inhibited by the presence of other species within a multi-strain probiotic mixture. Of these four studies in which the mixture was not more effective, all involved one or more lactobacilli species along with bifidobacteria and another genus. This suggests that a greater variety of genera reduce the effectiveness of a multi-strain probiotic. It may be that these different species inhibit each other, possibly by production of antagonistic agents, or by competition for either nutrients or binding sites within the gastrointestinal tract.

A further potential advantage of multi-strain probiotics in addition to exerting additive or synergistic effects on a single health end point is that strain-specific effects of individual probiotic components could together influence a wider range of end points. Currently, the evidence for this is lacking. More research is needed with a variety of multi-strain preparations, to clarify which species within a mixture have a synergistic relationship that might enhance the preparation's effectiveness and allow the development of probiotic products with broader spectrum of activity.

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