

## Probiotics - Revisted

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**Abstract :** Probiotics are non-pathogenic micro-organisms which confer health benefits to the recipient. The derangement for normal body flora is responsible for various disorders which need treatment with probiotics because of their ability to supplement normal body flora. They are safe, cheap and widely prescribed. However, there may be chances of transferring resistance to other microbes including pathogens as well as occasional reports of systemic fungemia especially in immunocompromised and severely debilitated patients with the use of probiotics. Larger studies are required to document their definite role both therapeutically and prophylactically.

### INTRODUCTION

Probiotics are nonpathogenic micro-organisms or microbial mixtures used to improve the microbial balance and to confer health benefits to the recipient. The use of the term 'Probiotic' in greek means 'for life'. The derangement of normal body flora causes various disorders. Many revolutionary changes have taken place in the treatment of infectious diseases with the introduction of antibiotics. But there is risk of increased incidence of drug resistance and opportunistic infections with the use of antimicrobial agents<sup>1</sup>. This can be avoided if the use of antimicrobial therapy is minimized. The practice of probiotics via fermented milk products in animals was first documented by Weese et al<sup>2</sup>. Fuller (1991)<sup>3</sup> defined probiotics as a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance. A review of probiotics in health and diseases<sup>4-6</sup> has been carried out by various workers. In this article, an attempt has been made to further review the currently used probiotics in health and disease in great details.

### WHY ARE THEY IN DEMAND?

More than 500 different bacterial species (some of which having important health functions) are present in the gastrointestinal (GIT) tract. Normally a protective gut microflora develops and thus there is no need for bacterial supplement. But due to many factors like excessive use of antibacterial agents, change in life style and food habits, consumption of processed food, drug resistance and opportunistic infections, the probiotics always remain in great demand to the consumer for better therapies.

### PROBIOTICS IN USE

The probiotics are increasingly used in various clinical conditions<sup>3</sup>. Lactobacillus and bifidobacterium are the commonest bacteria used as probiotics as described under

- (a) **Bacteria :** i) *Lactobacillus* - examples are *acidophilus*, *plantarum*, *sporogenes*, *rhamnosum*, *lactis*, *casei*, *reuteri*, ii) *Bifidobacterium* - examples are *bifidum*, *infantis*, *longum*, *thermophilum*, iii) *Streptococcus* - examples are *lactis*, *intermedius*, *alivarius* sub sp *thermophilus*, iv) *Enterococcus* - e.g. *faecium*, v) *Prophionibacterium*, vi) *Leuconostoc*, vii) *Pediococcus*, viii) *Bacillus*,  
 (b) **Moulds and Yeast;** i) *A. niger*, ii) *A. cerevisiae*,  
 iii) *C. pintolopesii*, iv) *Saccharomyces boulardii*,

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### PRE-REQUISITES OF PROBIOTICS

These are : i) Non pathogenic and nontoxogenic, ii) Human origin iii) Stable in acid and bile, iv) Able to survive transit through GIT, v) Able to adhere to human intestinal mucosa, vi) Able to colonise in human GIT, vii) Should retain viability during storage and use<sup>4</sup>, viii) Production of antimicrobial substances towards pathogens, ix) Beneficial effects on human health,

*L.rhamnosum* strain GG is one of the most thoroughly explored lactic acid bacteria and meets most of these criteria. The plantarum species among lactobacillus has got many advantages like unique adhesiveness to the mucosa and tolerate low pH, whereby potential pathological micro-organisms fail to grow and thus microbial translocation is prevented.

### BENEFICIAL CLAIMS OF PROBIOTICS

The therapeutic and prophylactic claims of probiotics are depicted in Table - 2<sup>7</sup>

**Table - 2** Benefits of Probiotics

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|---|
| <b>A. Intestinal disorders :</b>  |
| <b>Diarrhoea:</b> a) Infectious diarrhoea, b) antibiotic associated diarrhoea (AAD), c) traveller's diarrhoea, d) lactose malabsorption associated diarrhoea (lactose intolerance), e) infantile diarrhoea, |
| <b>B. Other Uses :</b>  |
| a) Urogenital infections-vaginitis, b) hypercholesterolemia, c) cirrhosis liver, d) food allergy, e) hypertension, f) renal stones, g) cancer, h) vaccine adjuvant, i) growth and well being,               |
| <b>C. Miscellaneous Uses :</b>  |
| a) stabilization of flora, b) recolonization of bowel after antibiotic therapy,   |

### DIARRHOEA

a) **Infectable diarrhoea including infantile diarrhoea :** In developing countries, incidence of infectious diarrhoea is very high due to poor nutrition and sanitation. Diarrhoea is a major cause of death in infants in developing countries. Thus the use of probiotics has a definitive role in addition to improving sanitation and nutrition. Lactobacilli GG, bifidobacterium and streptococcus thermophilus have been shown to reduce the incidence of diarrhoea as compared

to control groups<sup>8,9</sup>. The supplementation of infant milk with *B. bifidum* and *S. thermophilus* reduced rotavirus shedding and episodes of diarrhoea in children<sup>9</sup>. The recovery (in terms of reduction in duration of diarrhoea) from acute diarrhoea has been reported to be good by lactobacilli GG, *L. reuteri*, *L. casei* and *S. boulardii*<sup>10</sup>. The infants are more susceptible to get diarrhoea because of immature intestinal mucosal barrier, low immunity and poor growth of intestinal bacterial flora, which takes 1 year to reach adult level and composition<sup>11</sup>. In AIDS associated diarrhoea, *S. boulardii* has been shown to resolve symptoms in 10 of 18 patients as opposed to 1 of 11 in placebo when given for 1 week<sup>12</sup>. *E. faecium* SF 68 has been shown to be effective in reducing diarrhoea in adults when given for 1 week<sup>13</sup>.

**(b) Antibiotic associated diarrhoea (AAD) :** Diarrhoea is one of the most common adverse effect of antimicrobial agents. Its incidence in children ranges from 20% to 40%<sup>14</sup>. It is mostly related to disturbances in normal intestinal flora leading to many changes like (i) Loss of carbohydrates digesting gut bacteria resulting in osmotic diarrhoea<sup>15</sup>; (ii) Direct stimulation of gut motility. For example erythromycin acts as motilin agonist to cause diarrhoea<sup>16</sup>; (iii) Decrease in colonic fermentation to short chain fatty acids, which are required for the nutrition of the enterocyte as well as for absorption of water and electrolytes<sup>17</sup>; (iv) Overgrowth by pathogens, (v) Invasion and translocation of toxins by life threatening infections e.g. *C. difficile*. *C. difficile* leads to 20% of AAD<sup>18</sup> and has been reported to colonise 21% of hospitalized patients<sup>14</sup>. Longum in the form of fermented Yogurt has been reported to be useful if tetracyclines or Beta-lactam antibiotics<sup>19-20</sup>. *B. Longum* in combination with lactobacillus acidophilus has been shown to be more effective in clindamycin induced diarrhoea than when given alone<sup>21</sup>. *C. difficile* diarrhoea (CDD) is characterized by simple diarrhoea which may progress to colitis, pseudomembranous colitis and toxic megacolon<sup>14</sup>. Its prevention lies in consumption of probiotic containing both lactobacilli and bifidobacterium<sup>22</sup>. As far as treatment of CDD is concerned, a significant reduction in first episode of CDD or recurrences has been reported in various studies using *S. boulardii* given as 1 G/day for 4 weeks along with standard course of metronidazole or vancomycin<sup>23</sup> and another study using standard dose of *S. boulardii* for 4 weeks and vancomycin 2 G/day for 10 days<sup>24</sup>. Another probiotic *L. plantarum* is useful in recurrent CDD<sup>25</sup>. Although probiotics have shown beneficial effects in CDD, yet they are not recommended for routine prophylactic use a larger trials are needed.

**(c) Traveller's diarrhoea :** In traveller's diarrhoea, *L. rhamnosus* GG<sup>26</sup>, *S. Boulardii*<sup>27</sup>, lactobacilli GG<sup>28,29</sup> have been found to be useful. Lactic acid bacterial in the gut are helpful in replenishing the suppressed bacterial and inhibiting the growth of pathogenic bacteria<sup>30</sup>. Volatile fatty acids produced by some lactic acid bacteria control the colonization of gut by *S. sonnet* and *E. coli*<sup>31</sup>. Probiotics like *S. faecalis*, *C. butyricum* and *B. mesentericus* liberate butyrate and acetate, which by lowering intestinal pH, make a harmful environment for pathogenic *E. coli*, salmonella and methicillin resistant *S. aureus*<sup>32</sup>. The metabolites like butyrate and acetate help in intestinal epithelial cells proliferation<sup>33</sup> and glutamine produced by *B. mesentericus* plays a major role in maintaining intestinal mucosal barrier damaged by endotoxins<sup>34</sup>.

**(d) Lactose intolerance :** 90% of Africans, Asians and South Americans lack enzyme lactase, which utilizes lactose. Lactase disappears after weaning. Utilization of dairy products in such persons cause lactose intolerance characterised by flatulence and loose motions<sup>35</sup>. *S. thermophilus* and lactobacilli *delbrueckii* in live yogurt cultures has been found to be useful in lactose digestion<sup>36</sup>. *Lactobacillus acidophilus* as dietary adjunct for milk has been documented to assist lactose digestion in humans<sup>37</sup>. *L. rhamnosus* GG has been found to stimulate local IgA secretion in human intestine in rotavirus infection<sup>38</sup> while *S. boulardii* stimulate IgA secretion in rats intestine<sup>39</sup>. *E. acidophilus* has been shown to inhibit the attachment of *E. coli* to mucosal cells in pig ileum<sup>40</sup>. *L. rhamnosus* GG and *L. reuteri* have been shown to produce inhibitory substances like microcin<sup>41</sup> and reuterin<sup>42</sup> respectively, which are harmful to pathogens. *S. boulardii* destroys *C. difficile* toxin A receptor in rat ileum<sup>43</sup>. *Bifidobacterium* produces butyrate which stimulates the proliferation of normal intestinal epithelium and maintains mucosal barrier defences<sup>4</sup>.

**II) H. Pylori infections :** *Helicobacter pylori* (microaerophilic and gram negative bacilli) organisms lead to atrophic gastritis, gastric ulcers and gastric cancer. Lactobacilli *johnsonii* strain produce antibacterial substances which inhibit the growth, colonization and survival of *H. pylori*<sup>44</sup>. But lactobacilli GG<sup>45</sup> and *Bacillus clausii*<sup>46</sup> have been shown to be helpful in *H. pylori* infection but more studies are needed to establish their role in such infections.

**III) Inflammatory bowel disease (IBD) :** IBD is caused by luminal commensal bacterial flora (antigen) and immunological responses<sup>47</sup>. Because of immunodulatory and bowel flora manipulating properties, probiotics have been shown to be beneficial in IBD<sup>48</sup>. In Crohn's disease, commensal *E. coli* strain stimulate the release of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-8 (IL-8) by inflamed mucosa. *Lactobacillus casei* has been shown to downregulate the release of TNF- $\alpha$  by the inflamed mucosa and also inflammatory response by *E. coli*<sup>49</sup>. Kruis et al<sup>50</sup> reported relapse rates and 11.3% with mesalazine and 16% with *E. coli* when they were given for maintenance remission therapy for a period of 12 weeks in ulcerative colitis. Gioncetti P. et al<sup>51</sup> reported remission in 80% patients of ulcerative colitis with the use of probiotics given for 1 year. Another probiotic *S. boulardii* when given in patients with quiescent Crohn's disease as adjuvant therapy with mesalazine, produced lower clinical relapse rate as compared to mesalazine alone<sup>52</sup>. Gionchetti et al<sup>53</sup> has reported diminished occurrence of pouchitis, a common inflammatory condition which occurs in surgically created ileal reservoirs after total proctocolectomy for ulcerative colitis. More studies are required to evaluate the role of probiotics for the treatment of chronic IBD.

## OTHER USES

**Urogenital Infections :** Probiotics have been found to be useful in the management of nonsexually transmitted urogenital infections like urinary tract infections (UTI), bacterial vaginosis (BV) and vaginal candidiasis. Intravaginal insertion and perineal implantation of lactobacilli GR I strain twice weekly in patients suffering from recurrent UTI have been found to result in infection free period ranging from 4 weeks to 6 months<sup>54</sup>. The administration of

lactobacilli as vaginal suppositories significantly reduced recurrent UTI<sup>55</sup>. Lactobacilli rhamnosus GR-I and L-fermentum RC-14 significantly alter and improve vaginal flora as well as decrease yeast, bacteria and coliform count<sup>56,57</sup>. Lactobacilli acidophilus Yogurt two doses given for 7 days have been found to be useful in pregnancy bacterial vaginosis<sup>58</sup>. Vaginal flora prevents vaginal infections by creating unfavourable vaginal conditions for pathogens by producing H<sub>2</sub>O<sub>2</sub> and fermenting glycogen to produce lactic acid and an acidic pH. Since only few strains of lactobacilli have been shown to be beneficial in bacterial vaginosis and vaginal candidiasis, more studies are needed before probiotics can be recommended for routine prophylactic use in such conditions<sup>59</sup>.

**Hypercholesterolemia** : Consumption of Yogurt containing E.faecium is effective in lowering LDL and total cholesterol<sup>60</sup>. Maan GV et al<sup>61</sup> have also documented the beneficial effect of probiotics in lowering cholesterol. A study by Kiebling et al<sup>62</sup> has reported increase in HDL levels with the use of probiotics and fall in the ratio of LDL to HDL from 3.24 to 2.48. The proposed mechanisms include direct assimilation of cholesterol by bacterial cells, deconjugation of bile acids by bacterial acid hydrolases and inhibition of hepatic cholesterol synthesis<sup>63</sup>.

**Cirrhosis Liver** : Various studies<sup>64,65</sup> have reported that endotoxemia secondary to bacterial infection is responsible for hemodynamic alterations leading to cirrhosis liver, esophageal varices and portal hypertension. Administration of lactic acid bacteria in liver cirrhosis has been shown to increase mean blood velocity and flow in portal vein and reduction in splenic vein by way of fall in endotoxin levels<sup>66</sup>.

**Food allergy** : The colonic microbiota affects both the mucosal as well as systemic immunity in the host through modulation of both cellular and humoral mechanisms<sup>64</sup>. Administration of probiotics has been shown to be associated with disappearance of food allergy manifestations with decreased concentration of IgE in the serum<sup>67</sup>. Bifidobacteria and lactobacilli have been shown to increase IgA production in Peyer's patches in response to harmful agents<sup>68</sup>. This enhanced IgA response prevents some potentially allergenic dietary antigens from invading the intestinal mucosa and further alleviates intestinal inflammation and downregulates inflammatory responses<sup>69</sup> as well as induction of regulating T cells and counterregulation of Th 1 cells<sup>70</sup>. Lactobacilli also modify immunomodulatory properties of native food protein<sup>71</sup>.

Casein, a milk protein, upregulates the production of IL-4 and IFN- $\gamma$  in milk hypersensitive atopic infants but LGG degraded casein downregulates IL-4 production with no effect on TNF- $\alpha$  release. These facts highlight that probiotics potentially inhibit aberrant IgE secretion and eosinophil activation hypertension.

**Hypertension** : There are limited studies of the utility of probiotics in hypertension. A study by Hata Y et al<sup>72</sup> has reported that milk fermented with lactobacilli helveticus and saccharomyces cerevisiae, reduced systolic blood pressure by 9.4 mmHg and 14.1 mmHg after 4 and 8 weeks respectively and diastolic BP by 6.9 mm after 8 weeks of consumption in known hypertensives. The proposed mechanism may be due to bacterial cell wall component or ACE inhibiting peptides released by probiotics<sup>44</sup>.

**Renal Stones** : Increased levels of oxalate in the urine is a risk

factor for the development of kidney stones. An intestinal microbe known as oxalobacter-formigenes can degrade oxalate through the enzyme oxalate - CoA decarboxylase and thus decreases its absorption. Patients with calcium oxalate stone have been shown to have lower rate of colonization with oxalobacter formigenes. Absence of intestinal oxalobacter among stone formers correlates with higher oxalate concentration and increased risk of hyperoxaluria<sup>73</sup>. Bifidobacterium has been found to be useful as a probiotic in oxalate stone diseases for prophylaxis against new stone formation<sup>74</sup>.

**Cancer** : Colorectal cancer is one of the leading causes of mortality in both men and women<sup>75</sup>. Probiotics have been found to be useful in the prevention and suppression of colonic cancer. In humans, administration of fermented dairy products containing lactobacilli or B. Longum has been found to be beneficial in colon cancer<sup>76,77</sup> and breast cancer<sup>78</sup>.

The proposed mechanisms include :

- detoxification of ingested carcinogens.
- Production of enzymes like Beta-glucuronidase, nitroreductase, Beta-glucosidase and urease by enteropathogens such as E.coli and C.perfringens.
- Lowering of intestinal pH creates bacteriocidal environment.
- Colonization at the expense of enteropathogens may bind the ultimate carcinogen by physically removing it via faeces.
- Stimulating immune system to defend better against cancer cell proliferation.
- Producing metabolic products (like butyrate) which improve programmed cell death (apoptosis).

One of the earliest genetic alterations associated with human cancer especially colonic cancer is Ras activation<sup>79</sup>. Increased levels of Ras p-21 have been correlated with increased cell proliferation, histologic grade, nuclear atypia and degree of undifferentiation<sup>80</sup>. Consumption of dietary B. longum cultures significantly suppressed the expression of total and mutated Ras p-21 in the colonic mucosa and tumours compared with control diet<sup>81</sup>. The proposed mechanism of inhibition of Ras activation by R. longum cultures includes modulation of induction of methyl guanine repair protein called O6 methylguanine DNA methyltransferase, which acts as a suicidal enzyme and accepts a methyl group on itself from guanine by demethylation and restores the original guanine in DNA. The administration of L.casie fermented milk in humans has been shown to significantly reduce the recurrence of bladder cancer<sup>82</sup>.

**Vaccine adjuvant** : Increase in rota virus specific IgM secreting cells was reported in a study<sup>83</sup> when children were given lactobacillus GG as an adjuvant to an oral vaccine to rotavirus compared to placebo on 8th vaccination day. Still more studies are required in this field.

**Growth and well being** : The administration of 50 ml of fermented curd for 6 months in malnourished children has shown gain in weight, height, lower incidence of diarrhoea and fever<sup>84</sup>. Administration of milk formulae supplemented with bifidobacterium lactis and streptococcus thermophilus reduced the occurrence of colic and irritability in infants as compared to control group<sup>85</sup>. A study by Robinson and Thompson<sup>86</sup> has reported that bottle fed infants during the first month of life gained an average

21.9 ozs weight when fed a standard formula and 26.5 ozs weight when *L.acidophilus* was added to the formula. The proposed mechanism of this enhanced growth includes infection control and increased bioavailability of nutrients.

**Various problems associated with probiotic use :** The major problem with probiotics is the maintenance of their viability to produce beneficial effects to the consumer. The probiotics need to survive in many environments like processing treatments, storage condition and human body condition (especially gastric acidity and action of bile salts). The ability to live through the stressful acidic conditions and bile solutions in human body varies among the strains of probiotic bacteria. Only those strains, which tolerate these adverse conditions, should be preferred. Storage at 4°C temperature is reported to be a most important factor in keeping probiotic bifidobacterium viable during 4 weeks storage time<sup>87</sup>. Viability of lactobacilli has been enhanced by encapsulation in artificial sesame oil for storage and in simulated high gastric or bile salt condition as compared to free cells<sup>88</sup>. The use of prebiotics like 150 maltoligosaccharides has been found to be associated with higher level of probiotics like lactobacillus and bifidobacterium after 1 month storage<sup>89</sup>. Still more studies are required in the field of viability.

#### Safety of probiotics

Fermented dairy products and cereals have been in use for centuries. Generally, probiotics are considered non-pathogenic but occasional isolated reports of their adverse effects have cropped up including liver abscess<sup>90</sup> and lactobacillemia<sup>91</sup>. *S.boulardii* has been used without complications in AIDS patients with chronic diarrhoea<sup>92</sup>. *L.plantarum* can be safely given in AIDS patients including immunosuppressed individuals. Salminen et al<sup>94</sup> reviewed the safety data and found no evidence of probiotics being involved in human infections. This is supported by epidemiological data on safety of dairy products<sup>95</sup>. But other limitations include risk of transfer of resistance via probiotics because these agents have to be resistant to antibiotics since they are to be used concurrently. Yet another limitation is depletion of nutrients, which are essential for the proliferation of pathogens. An example is the depletion of monosaccharides, which is essential for *C.difficile*<sup>96</sup>.

**Areas for future research** include ;

- (a) Regulation of intestinal motility
- (b) Modulation of intestinal and systemic immune response
- (c) Reduction and protection of radiotherapy associated intestinal dysfunction
- (d) Prevention of intestinal cancers
- (e) Characterisation of the possible antimicrobial properties and their metabolic activities
- (f) Competitive exclusion
- (g) Cholesterol lowering effect

#### CONCLUSION

Various above mentioned studies have documented the beneficial effects of probiotics as preventive and therapeutic agents in various disorders including diarrhoeal diseases, urogenital infections, hypertension, hypercholesterolemia, oxalate renal stones, allergy, cancer and in children as growth promoters. However further larger

studies are needed to establish their role in the management of these conditions. On account of worldwide epidemic of resistant bacterial and increasing competition for health care resources, the use of cost effective microbial interference therapy should be a top priority. Some major limitations in establishing their role in the therapeutics may be lack of adequate knowledge about them among prescribers and probably the preparations available in the market contain non-viable organisms or strains which possess no antipathogenic activity. Probiotics are likely to emerge as alternative to conventional antimicrobial therapy since they are relatively cheap and may have lower risk of resistance.

#### REFERENCES

1. Henry, FC. Antimicrobial agents : General considerations. In : Hardman JG, Limbird, LE, Gilman AG; editors. Goodman and Gilman's. The Pharmacological basis of therapeutics. 10th ed. McGraw Hill. Medical Publishing Division, 2001;p.1143
2. Weese, JS; Anderson, EC. Preliminary evaluation of lactobacillus rhamnosus strain GG, a potential probiotic in dogs. Can. Vet. J. 2002;43:771-4
3. Fuller, R. Probiotics in human medicine. Gut. 1991;32:439-42
4. Anuradha, S; Rajeshwari, K. Probiotics in health and disease. JIACM 2995;6:67-72
5. Shweta, Bansal; S.K. Jain. Probiotics in health and diseases. JAPI 2001;49:735-741
6. Sharma, AK; Mohan, P; Nayak, BB. Probiotics: making a come back. Indian J. Pharmacol. 2995;37:358-365
7. Hitchins, AD; MaDonough, FE. Prophylactic and therapeutic aspects of fermented milk. Am. J. Clin. Nutrition. 1989;49:675-84
8. Szajewska, H; Kotowska, M; Mrukowicz, JZ; et al. Efficiency of lactobacillus GG in prevention of nosocomial diarrhoea in infants. J. Pediatr. 2001;138:361-65
9. Saavedra, JM; Bauman, NA; Oung, I; et al. Feeding of bifidobacterium bifidum and streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet. 1994;344:1046-49
10. Hhuang, JS; Bausvaros, A; Lee, JW; et al. Efficacy of probiotics use in acute diarrhoea in children : a meta analysis. Dig. Dis. Sci. 2002;47:2625-34
11. Hyman, PE; Clark, DD; Everett, SL; et al. Gastric acid secretory function in preterm infants. J. Pediatr. 1985;106:467-71
12. Saint-March, T; Rosello-Prats, L; Touraine, JL. Efficacite de saccharomyces boulardu dans let treatment des diaerehes du SIDA. Ann . Med. Interne (Paris)1991;142:64-5
13. Wunderlich, PF; Braun, L; Fumagali, I; et al. Double blind report on the efficacy of lactic acid producing enterococcus SF 68 in the prevention of antibiotic associated diarrhoea and in the treatment of acute diarrhoea. J. nt. Med. Res. 1989;17:333-8
14. McFarland, LV; Mulligan, ME; Kwok, PY; et al. Nosocomial acquisition of clostridium difficile infection. N. Eng. . Med. 1989;320:204-10
15. Firmansyah, A; Penn, D; Leenthal, E. Isolated colonocyte metabolism of glucose, glutamine, n-butrylate and beta-hydroxy-butyrate in malnutrition. Gastroenterol. 1991;101:1497-504
16. Peters, T; Mathijs, G; Depoortere, I; et al. Erythromycin is a motilin receptor agoist. Am. J. Physiol. 1989;257:470-4
17. Ruppin, H; Bar-Meir, S; Soergel, KH; et al. Absorption of short chain fatty acids by colon. Gastroenterol. 1980;78:1500-7
18. Kelly, CP; Pothoulakis, C; LaMont, JT. Clostridium difficile colitis. N. Eng. J. Med. 1994;330:257-62
19. Adam, J; Barret, C; Barret-Bellet, A. Essais cliniques controles en double insude I Ultra-Levure Lyophilisee. Etude multicentrique par 25 medecins de 388 Cas. Gaz. Med. Fr. 1977;84:2072-78
20. Colombel, JF; Cortot, A; Neut, C; et al. Yoghurt with bifidobacterium longum reduces erythromycin induced GI effects. Lancet 1987;2:43
21. Orrhage, K; Brismar, B; Nord, CE. Effects of supplement of bifidobacterium longum and lactobacilli acidophilus on the intestinal microbiota during administration of clindamycin. Microb. E. Col. Health Dis. 1994;17-25
22. Plummer, S; Weaver, MA; Harris, JC; et al. Clostridium difficile pilot study

- : effect of probiotic supplementation on the incidence of *C. difficile* diarrhoea. *Int. Microbiol.* 2004;7:59-62
23. *McFarland, LV; Surawicz, CM; Greenberg, RN; et al.* A randomized placebo-controlled trial of *saccharomyces boulardii* in combination with standard antibiotics for clostridium *difficile* disease. *JAMA.* 1994;27:1913-8. Erratum in *JAMA.* 1994;272:518
  24. *Surawicz, CM; McFarland, LV; Greenberg, RN; et al.* The search for a better treatment for recurrent clostridium *difficile* disease the use of high dose vancomycin combined with *saccharomyces boulardii*. *Clin. Infect. dis.* 2000, 31:1012-17
  25. *Wullt, M; Hagslatt, ML; Odenholt, I.* Lactobacilli planurum 299 v for treatment of recurrent clostridium *difficile* associated diarrhoea : a double-blind placebo-controlled trial. *Scand. J. Infect. Dis.* 2003;35:365-67
  26. *Hilton, E; Kolakowski, P; Singer, C; et al.* Efficacy of lactobacilli GG as a diarrhoeal preventive in travellers. *J. Travel Med.* 1997;4:41-43
  27. *Kollaritsch, H; Hoist, H; Grobara, P; et al.* Prevention of traveller's diarrhoea with *saccharomyces boulardii*. Results of a placebo controlled double blind study. *Fortschr. Med.* 1993;111:152-156
  28. *Oksanen, PJ; Salminen, S; Saxelin, M; et al.* Prevention of traveller's diarrhoea by lactobacilli GG. *Ann. Med.* 1990;22:53-6
  29. *Black, FT; Anderson, PL; Orsko, J; et al.* Prophylactic efficacy of lactobacilli in traveller's diarrhoea. In : Stiffen R. Ed. *Travel Medicine, Conference on international travel medicine.* Zurich Switzerland, Berlin : Springer. 1989;333-5
  30. *Salminen, S; Deighton, M.* Lactic acid bacteria in the gut in normal and disordered states. *Dig. Dis. Sci.* 1992;10:227-38
  31. *Pongpech, P; Hentges, DJ.* Incubation of *Shigella Sonnei* and enterotoxigenic *E. coli* by volatile fatty acids in mice. *Microbial Ecology in health and disease* 1989;2:153-61
  32. *Seo, G; Shimizu, K; Kono, M; et al.* Inhibition of growth of some enteropathogenic strains in mixed cultures of *Streptococcus faecalis* and clostridium *butyricum*. *Microbios letter.* 1989;40:151-60
  33. *Scheppach, W; Bartram, P; Richter, A; et al.* Effects of short chain fatty acids on human colonic mucosa in vitro. *JPEN.* 1992;16:43-6
  34. *Traveira, Da; Silva, AM; Kaulbach, HC; et al.* Shock and multiple organ dysfunction after self administration of salmonella endotoxin. *N. Eng. J. Med.* 1993;328:1457
  35. *Palmer, KR; Penman, ID.* Disease of alimentary tract and pancreas. In Halett C. Edwin C, Edwin RC, John AA Hunter, Nicholas AB, editors. *Davidson's Principles and Practice of Medicine* 18th edition. London. UK. Churchill. Livingstone, 1999, p.651
  36. *Kolars, JC; Levit, MD; Aouji, M; et al.* Yogurt and autodigesting source of lactose. *N. Eng. J. Med.* 1984;310:1-3
  37. *Kims, HS; Gilliland, SE.* Lactobacillus *acidophilus* as dietary adjunct for milk to aid lactose digestion in humans. *J. Dairy Science.* 1983;66:959-66
  38. *Kaila, M; Isolauri, E; Saxelin, M; et al.* Viable versus inactivated lactobacilli strain GG in acute rotavirus diarrhoea. *Arch. Dis. Child.* 1995;72:51-3
  39. *Buts, JP; Bernasconi, P; Vaeman, JP; et al.* Stimulation of IgA secretory component of immunoglobulins in small intestine of rats treated with *saccharomyces boulardii*. *Dig. Dis. Sci.* 1990;35:251-6
  40. *Blomberg, L; Henriksson, A; Conway, PL.* Inhibition of adhesion of *Escherichia coli* K 88 to piglet ileal mucus by lactobacilli spp. *Appl. Environ. Microbiol.* 1993;59:34-9
  41. *Silva, M; Jacobus, NV; Deneke, C; et al.* Antimicrobial substance from human lactobacillus strain. *Antimicrob. Agents. Chemother* 1987;31:1231-3
  42. *Kabuki, T; Saito, T; Kawai, Y; et al.* Production, purification and characterization of reuterin 6, a bacteriocin with lytic activity produced by lactobacilli reuters LA6. *Int. J. Food Microbiol.* 1997;34:145-56
  43. *Pothoulakis, C; Kelly, CP; Joshi, MA; et al.* *Saccharomyces boulardii* inhibits clostridium *difficile* toxin A binding and enterotoxicity in rat ileum. *Gastroenterol.* 1993;104:1108-15
  44. *Usprobiotics (home page in the internet).* California : Usprobiotics org. C. 2004 (updated 2005 Jan, 05; cited 2005 Jan. 25). Probiotics basics. Available from <http://www.usprobiotics.org/index.htm>
  45. *Armuzzi, A; Cremonini, F; Ojetti, V; et al.* Effect of lactobacilli GG supplementation on Antibiotic-Associated GI side effects during *H. pylori* eradication therapy. A pilot study. *Digestion.* 2001;63:1-7
  46. *Nista, EC; Candelli, M; Cremonini, F; et al.* Bacillus clausii therapy to reduce side effects of anti-*H. pylori* treatment : randomized, double-blind, placebo-controlled trial. *Ther.* 2004;20:1181-8
  47. *Macpherson, A; Khoo, UY; Forgacs, I; et al.* Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacterial. *Gut.* 1996;38:365-75
  48. *Schultz, M.* Probiotics and inflammatory bowel disease. *Am. J. Gastroenterol.* 2000(1Suppl.) S19-21
  49. *Borrue, N; Carol, M; Casellas, S; et al.* Increased mucosal TNF- $\alpha$  production in Crohn's disease be down regulated ex-vivo by probiotic bacteria. *Gut.* 2002;51:659-64
  50. *Kruis, W; Schultz, E; Fric, P; et al.* Double blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. *Ailment Pharmacol. Ther.* 1997;111:853-8
  51. *Gionchetti, P; Rizzello, F; Malteuzzi, D; et al.* Microflora in the IBD pathogenesis. Possible therapeutic use of probiotics. *Gastroenterol. Intranat.* 1998;11:108-10
  52. *Gulsandi, M; Mezzi, G; Sorghi, M; et al.* *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. *Dig. Dis. Sci.* 2000;45:1462-4
  53. *Gionchetti, P; Rizzello, F; Hentges, DJ; et al.* Prophylaxis of pouchitis onset with possible therapy, a double blind placebo controlled trial. *Gastroenterology.* 2003;124:1202-09
  54. *Bruce, AN; Reid, G.* Intravaginal instillation of lactobacilli for prevention of recurrent urinary tract infection. *Can. J. Microbiol.* 1988;34:339-43
  55. *Reid, G; Bruce, AW; Taylor, M.* Influence of three day antimicrobial therapy and lactobacillus vaginal suppositories on recurrence of urinary tract infections. *Clin. Ther.* 1992;14:11-6
  56. *Reid, G; Charbonneau, D; Erd, J; et al.* Oral use of lactobacilli GR-1 and L-fermentin RC-14 significantly alters vaginal flora : randomized placebo controlled trial in 64 health women FEMS. *Immunol Med. Microbiol.* 2003;35:131-4
  57. *Sandler, B.* Lactobacillus for vulvovaginitis. *Lancet.* 1979;2:791-2
  58. *Neri, A; Sabah, G; Samra, Z.* Bacterial vaginosis in pregnancy treated with yogurt. *Acta. Obstet. Gynecol. Scand.* 1993;72:17-9
  59. *Vanderhoof, JA; Probiotics :* Future directions. *Am. J. Clin. Nutr.* 2001;73:1152-5
  60. *Hivak, P; Odraska, J; Ferencik, M; et al.* One year application of probiotic strain enterococcus *faecium* M-74 decreases serum cholesterol levels. *Bratisl Lek. Listy.* 2005;106:67-72
  61. *Maan, GV; Sperry, A.* Studies of a surfactant and cholesterolemia in the Massai. *Am. J. Clin. Nutrition.* 1974;27:464-9
  62. *Kiebling, G; Schneider, J; Jahreis, G.* Long term consumption of fermented dairy product over 6 months increases HDL cholesterol. *Eur. J. Clin. Nutr.* 2002;56:843-9
  63. *Lovegrove, J; Jackson, K.* Coronary heart disease. In : Mattila-Sandholm T, Saarela M, editors. *Functional dairy products.* Cambridge UK; Woodhill Publishing, 2003
  64. *Genesca, J; Gonzalez, A; Segura, R; et al.* Interleukin-6, nitric oxide and the clinical and hemodynamic alterations of patients with liver cirrhosis. *Am. J. Gastroenterol.* 199;94:169-77
  65. *Goulis, J; Patch, D; Bourroughs, AK.* Bacterial injection in the pathogenesis of variceal bleeding. *Lancet.* 199;353:139-42
  66. *Santis, AD; Famularo, G; Simone, CD.* Probiotics for hemody alterations of patients with liver cirrhosis. *Am. J. Gastro.* 2000;95:323-4
  67. *Trapp, CL; Chang, CC; Halpern, GM; et al.* The influence of chronic Yoghurt consumption in population of young and elderly adults. *Int. J. Immunother* 1993;9:53-64
  68. *Yasui, H; Nagaoka, N; Mike, a; et al.* Detection of bifidobacterium strains that induce large quantities of IgA. *Microb. Ecol. Health Dis.* 1992;5:155-62
  69. *Kirjavainen, PV; Probiotics and the management of food allergy.* In : Mattila-Sandholm T, Saarela M, editors. *Functional dairy products.* Cambridge, UK Woodhill Publishing. 2003
  70. *Vonder Weid, T; Billiard, C; Schiffrin, E.* Induction by a lactic acid bacterium of a population of CD4T cells with low proliferative capacity that produce transforming growth factor and IL10. *Clin Diagnost. Lab Immunol.* 2001;8:695-701
  71. *Sutas, Y; Horne, M; Isolauri, E.* Down regulation of anti CD3antibody induced IL-4 production by bovine caseins hydrolysed with lactobacillus G6 derived enzymes. *Scand. J. Immunol.* 1996;43:687-89

72. Hata, Y; Yamamoto, M; Ohni, M; *et al.* A placebo controlled study of the effect of sour milk on blood pressure in hypertensive subjects. *Am. J. Clin. Nutr.* 1996;64:767-71
73. Troxel, SA; Sidhu, H; Kaul, P; *et al.* Intestinal oxalobacter formigenes colonization in calcium oxalate stone formers and its relation to urinary oxalate. *J. Endourol.* 2003;17:173-6
74. Kodama, T; Mikami, K; Akakura, K; *et al.* Detection of oxalobacter formigenes in human faeces and study of related genes in a new oxalate-degrading bacterium. *Hinyokika Kyo.* 2003;49:371-6
75. Parkar, SL; Tong, T; Bolden, S; *et al.* Cancer statistics. *CA-Cancer J. Clin.* 1997;47:5-27
76. Kampman, E; Goldholm, RA; Van-den Brandt, PA; *et al.* Fermented dairy products, calcium and colorectal cancer in the Netherland Cohort Study. *Cancer. Res.* 1994;54:363-5
77. Singh, J; Rivenson, A; Tomita, M; *et al.* Bifidobacterium longum, a lactic acid producing intestinal microflora inhibit the colon cancer and modulate the intermediate biomarkers of colon carcinogenesis. *Carcinogenesis.* 1997;18:1371-7
78. Von't Veer, P; Von Leer, EM; Rietdijk, A; *et al.* Combination of dietary factors in relation to breast cancer occurrence. *nt. J. Cancer.* 1991;47:649-53
79. Barbaad, M. Ras oncogenes : their role in neoplasia. *Eur. J. Clin. Investig.* 1990;20:225-35
80. Kotsinas, A; Spandidos, DA; Romanowski, P; *et al.* Relative expression of wild type and activated ki-ras 2 oncogene in colorectal carcinomas. *Int. J. Oncol.* 1993;3:841-5
81. Reddy, BS; Rivenson, A. Inhibitory effect of bifidobacterium longum on colon, mammary and liver carcinogenesis induced by 2-amino-3 methylimidazole (4,5-f) quinoline, a food mutagen. *Cancer Res.* 1993;53:3914-8
82. Asoy, Akaza H; Kotakae, T; *et al.* Preventive effects of a lactobacilli casei preparation on the recurrence of superficial bladder cancer in a double-blind trial *Eur. Urol.* 1995;27:104-9
83. Isolauri, E; Josensuu, J; Suomalainen, H; *et al.* Improved immunogenicity of oral DX RRV reassortant rotavirus vaccine by L. Casei GG. *Vaccine.* 1995;13:310-2
84. Saran, S; Gopalan S; Krishna, TP. Use of fermented foods to combat stunting and failure to thrive. *Nutrition.* 2002;18:393-6
85. Jose, M; Saavedra, Adel Abi Hanna; Nancy Moore; *et al.* Long term consumption of infant formula containing live probiotic bacterial tolerance and safety. *Am. J. Clin. Nutr.* 2004;79:261-7
86. Robinson, EL; Thompson, WL. Effect on weight gain and addition of lacidophilus to the formula of newborn infants. *J. Pediatrics.* 1952;41:395-8
87. Jurgen Schrezenmeir Michaelde Vrese. Probiotics, prebiotics and synbiotics - approaching a definition. *Am. J. Clin. Nutr.* 2001;73:361-4
88. Hou, RC; MyLin, MMC Wang; *et al.* Increase of viability of entrapped cells of lactobacilli debrueckii ssp bulgaricus in artificial sesame oil emulsions. *J. Dairy Sci.* 2003;86:424-8
89. Ming-Ju, Chen; Kun-Nanchen, Chin-Wen, Lin. Optimization of the viability of probiotics in a fermented milk drink by the Response Surface Method. *Asian Aust. J. An. Im. Sci.* 2004;17:705-11
90. Rautio, M; Jousimies Somer, H; Kauma, H; *et al.* Liver abscess due to lactobacilli rhamnosus strain indistinguishable from L.rhamnosus strain GG *Clin. Infect. Dis.* 1999;28:1159-60
91. Saxelin, M; Chuang, NH; Chassy, B; *et al.* Lactobacilli and bacteremia in Southern Finland. 1989-92. *Clin. Infect. Dis.* 1996;232:564-6
92. Elmer, GW; Moyer, KA; Surawicz, CM; *et al.* Evaluation of saccharomyces boulardii for patients with HIV-related chronic diarrhoea and in healthy volunteers receiving antifungals. *Microecol. Ther.* 1995;25:23-31
93. Cunningham-Rundles, S; Ahrne, S; Bengmark, S; *et al.* Probiotics and immune response. *Am. J. Gastroenterol.* 2000;95(Suppl) :S22-25
94. Salminen, SJ; Donahue, DC. Safety assessment of lactobacillus strain GG (AICC 53103). *Nutrition Today.* 1996;31:325-45
95. Saxelin, M. *Lactobacillus GG* A human probiotics strains with thorough clinical documentation. *Food Reviews Int.* 1997;13:293
96. Wilson, KH; Penni, F. Role of competition for nutrients in suppression of clostridium difficile by the colonic microflora. *Infect. Immun.* 1988;56:2610-14

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