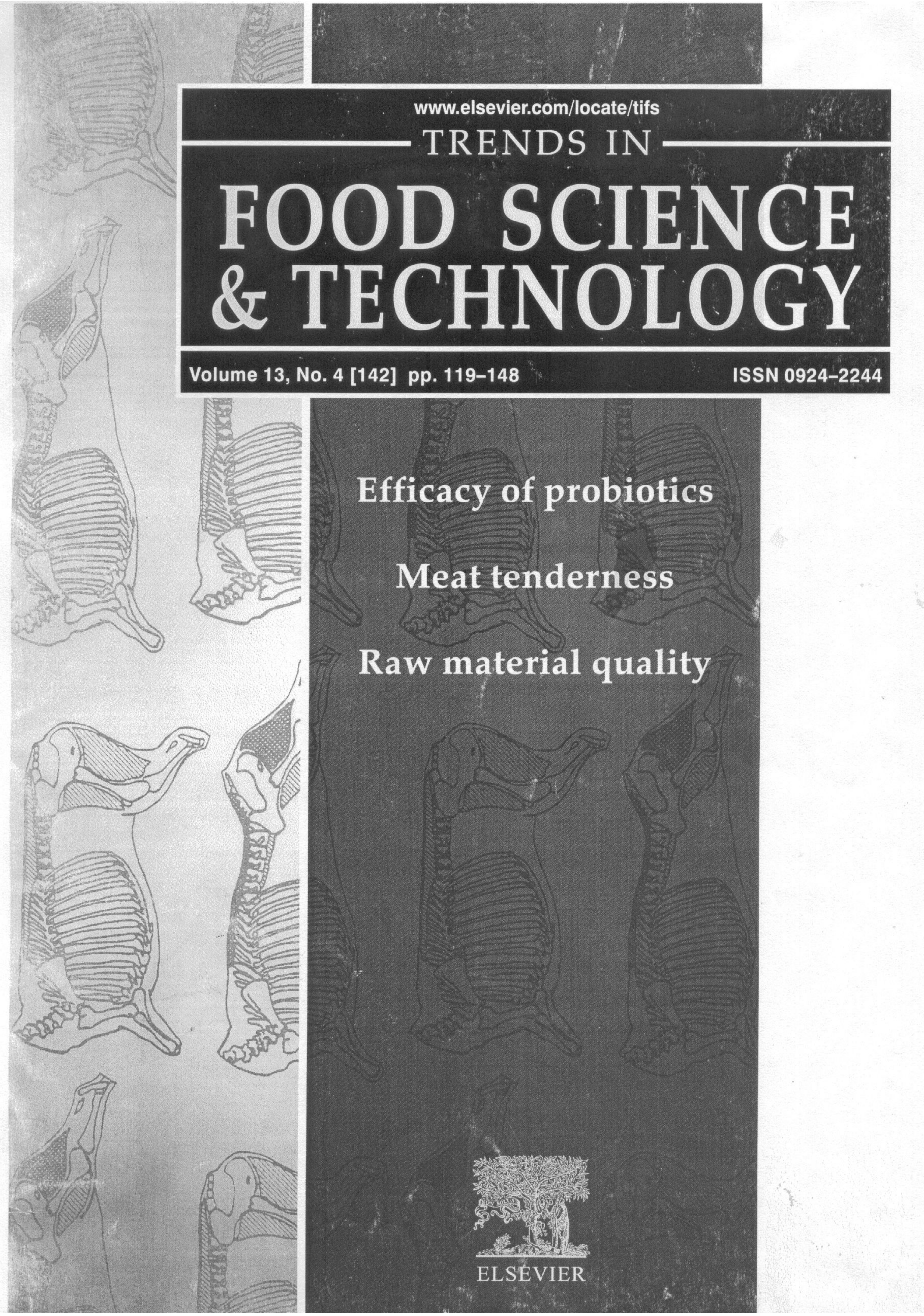

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Efficacy of probiotics

Meat tenderness

Raw material quality

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The possibilities of potentiating the efficacy of probiotics

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Probiotics could represent an effective alternative to the use of synthetic substances in nutrition and medicine. The data concerning the efficacy of probiotics are often contradictory and it will be important to search for ways to improve their efficacy. In order to enhance the efficacy of probiotics, it is necessary to obtain additional knowledge on their mode of action. The efficacy of probiotics may be potentiated by the several methods: the selection of more efficient strains; gene manipulation; the combination of several strains; and the combination of probiotics and synergistically acting components. This review focuses on the enhancement of the efficacy of probiotics by their combination with synergistically acting components of natural origin. This approach seems to be the best way of potentiating the efficacy of probiotics and is widely used in practise. By the above-mentioned method, more effective probiotic preparations will be developed.

According to Fuller (1992) probiotics are biopreparations containing living cells or metabolites of stabilized autochthonous microorganisms that optimize the colonization and composition of gut microflora in both animals and humans and have a stimulative effect on digestive processes and the immunity of the host. Probiotics are effectively being used in the food industry, agriculture, and human and veterinary medicine (O'Brien, Crittenden, Ouwehand, & Salminen, 1999; Shortt, 1999).

Despite a lot of knowledge obtained, the mode of action of probiotics has not been fully explained yet. The mode of inhibitive. action of probiotics against pathogens may be mediated by competition for receptors on the gut mucosa, competition for nutrients, the production of antibacterial substances, and the stimulation of immunity (Piard & Desmazeaud, 1991). Probiotics influence digestive processes by the improvement of the microbial population that is beneficial for the macroorganism, by enhancing its enzyme activity, by improving digestibility and feed utilization. The antitumour activity of probiotics may be realized in three ways: (a) the inhibition of tumour cells, (b) the suppression of bacteria producing β-glucosidase, β-glucuronidase, and azoreductase, which catalyze the conversion of procarcinogens to proximal carcinogens and (c) by the destruction of carcinogens such as nitrosamines, and by the decrease of nitroreductase activity which is involved in their synthesis. Probiotics influence blood cholesterol level by the inhibition of cholesterol synthesis, or decrease its level directly by assimilation (Zacconi *et al.*, 1992).

Bases for enhancing the efficacy of probiotics

The data concerning the efficacy of probiotics in practice are often contradictory (Simmering & Blaut, 2001). The preventive effect of lactobacilli and bifidobacteria against diarrhoea in pigs was confirmed by Depta *et al.* (1998) and Bomba *et al.* (1997). Some authors, however, have not confirmed the preventive effect of probiotics against diarrhoeic diseases of piglets (De Cupere, Deprez, Demeulenaere, & Muylle, 1992; Bekaert, Moermans, & Eeckhout, 1996). The variation in efficacy of probiotics under different conditions may be attributable to the probiotic preparation itself or may be caused by external conditions. Despite a lot of knowledge obtained, the mode of action of probiotics

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has not been explained yet. In order to enhance the efficacy of probiotics, it is necessary to obtain additional important knowledge on the mechanisms mediating their effect in the digestive tract. The anti-bacterial effect of each probiotic microorganism or its beneficial effect on the host may be mediated by one or a number of mechanisms that may be expressed to different degrees. This is the starting point for potentiating the efficacy of probiotics that may be realized by making one of the mechanisms more intensive or by extending the range of mechanisms of the probiotic organism.

The efficacy of probiotics may be enhanced by the following methods:

- the selection of more efficient strains of microorganism
- gene manipulations
- the combination of a number of strains of microorganism
- the combination of probiotics and synergistically acting components

The combination of probiotics with synergistically acting components of natural origin seems to be the best way of enhancing the efficacy of probiotic preparations from the practical point of view (Table 1). The next part of this study will deal with the above-mentioned questions.

Probiotics and prebiotics

Future challenges include the incorporation of one or more probiotics together or in combination with suitable prebiotic substrates to enhance the efficacy of the preparations for clinical use (Salminen *et al.*, 1998). A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. In order for a food ingredient to be classified as prebiotic, it must (1) be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract, (2) be a selective substrate for one or a limited number of beneficial bacteria commensal with the colon, which are stimulated to grow and/or are metabolically activated, (3) consequently, be able to alter the colonic flora in favour of a healthier composition, and (4) induce luminal or systemic effects that are beneficial to the hosts health. A way of potentiating the efficacy of probiotic preparations may be the combination of both probiotics and prebiotics as synbiotics which may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by activating the metabolism of one or a limited number of health-promoting bacteria and/or by selectively stimulating their growth improving the host's welfare (Gibson & Roberfroid, 1995).

Some oligosaccharides comply with all the criteria for prebiotics. Nemcová, Bomba, Gancarčíková, Herich, and Guba (1999) confirmed the synergistic effect of *Lactobacillus paracasei* and fructo-oligosaccharide combination on faecal microflora of weaned pigs. This effect was demonstrated by increased total anaerobes, aerobes, lactobacilli, and bifidobacteria counts, as well as by decreased clostridia, enterobacteriacea, and *Escherichia coli* counts.

Potentiated probiotics

Synbiotics seem to be preparations whose potentiated protective and stimulative effects occur only in the colon. Taking into account the pathogenesis of diarrhoeic diseases in young animals there is a need for protecting the digestive-tract mucosa throughout its length, i.e. also in the small intestine so that the adhesion of pathogenic microorganisms can be prevented. Potentiated probiotics are defined as biopreparations containing production strains of microorganisms and synergistically acting components of natural origin potentiate their probiotic effect on both small intestine and colon and their beneficial effect on the host by

Table 1. Possible potentiating factors of the effect of probiotic microorganisms

Component	*Reference*
Fructo-oligosaccharide	Nemcová *et al.* (1999)
Extract of *Coleus froskohlii Briq.*, roots	Yadava *et al.* (1995)
Origanum essential oil	Kyriakis *et al.* (1998)
Extracts of carrots	Babic *et al.* (1994)
Phytins	Nakashima (1997)
Whey	Edens *et al.* (1991)
Maltodextrin KMS X-70	Bomba *et al.* (1999)
Support culture in fermented milk	Saxelin *et al.* (1999)
Fermented milk	Hosoda *et al.* (1996)
Polyunsaturated fatty acids	Ringo and Gatesoupe (1998)
Salmonella vaccine	Methner *et al.* (1999)
S. typhimurium–specific antibodies	Promsopone *et al.* (1998)
Zinc	Holm and Poulsen (1996)

intensifying a mechanism or by extending the range of their probiotic action. Potentiated probiotics must comply with the criteria as follows: (a) they must be more effective than their components separately, (b) their potentiated protective and stimulative effects must be expressed in all parts of the digestive tract.

Based on the above-mentioned criteria, a synbiotic could be regarded as a potentiated probiotic, if a component potentiating its probiotic effect on the small intestine is added. From this it also follows that potentiated probiotics will probably be multi-component preparations.

Probiotics and phytocomponents

Lactic acid bacteria might prevent coliform diarrhoea by interaction with the enterotoxins. Such interaction might be indirect by influencing *E. coli* populations or metabolism or might be direct by neutralizing the enterotoxin itself. Plant extracts seem to have a similar ability, which might be used in potentiating the neutralization effect of lactobacilli against enterotoxin-producing *E. coli*. A crude alcohol extract of *Coleus froskohlii Briq*. roots showed a marked inhibitory action against an *E. coli* toxin-induced secretory response at 300 mg/loop dose in the ileal loops of rabbits and guinea-pigs. Coleonol A and B obtained by column chromatography of a benzene fraction of the alcohol extract of Coleus froskohlii roots exhibited anti-secretory (anti-diarrhoeal) action at a dose 1 mg/loop (Yadava, Gupta, Ahmad, Varma, & Tandon, 1995).

It is interesting to consider potentiating the efficacy of probiotics in the control of the post-weaning diarrhoea syndrome (PWDS) of piglets. The essential oils derived from the plant *Origanum* were proved to have *in-vitro* anti-microbial action against various bacteria, including *E. coli* (Sivropoulou *et al*., 1996). The medication with the *Origanum* essential oils seemed to be effective in the control of PWDS resulting in a mild atypical illness in the animals combined with very good growth performance (Kyriakis *et al*., 1998).

Babic, Nguyen-the, Amiot, and Aubert (1994) demonstrated that purified ethanolic extracts of carrots had an anti-microbial effect against a range of food-borne microorganisms. Free dodecanoic acid and methyl esters of dodecanoic and pentadecanoic acids were identified in the purified active extracts of carrots and could be responsible for the anti-microbial activity. A extract from the cortex of the African *Okoubaka* tree, has been successfully used for the treatment of enterotoxin-induced diarrhoea in horses.

It has been shown that some phytochemical components stimulate the production of lactic acid by lactobacilli. Phytins, including phytic acid (a naturally occurring compound formed during the maturation of seeds and cereal grains) stimulated the growth of lactic acid bacteria in a skim milk medium as measured by the number of live bacteria and the amount of acid produced. Nakashima (1997) reported that the stimulating effect of the phytin preparation on *Lactobacillus casei* is not attributable to phytin, but is exerted mainly by the Mn in the preparation, while the presence of other inorganic materials also augments the effect to some extent. Similar results were reported for green layer and sea lettuce.

Probiotics and non-specific substrates

Prebiotics are specific substrates selectively fermented in the colon. It has been demonstrated that to enhance the efficacy of probiotics, non-specific substrates can be used as well. The effect of caecal flora, cultured in lactose-based broths, against *Salmonella* was enhanced by adding lactose to chick diets. The reduction in caecal colonization was accompanied by an increase in the concentration of volatile fatty acids and a decrease in the caecal pH (Corrier, Hinton, Ziprin, Beier, & De Loach, 1990).

The combination of peptides and lactobacillus reduced mortality following diarrhoea, halved the incidence of digestive disorders and improved animal growth significantly. While peptides or lactic bacteria alone improved animal productivity, their combination resulted in a synergy of action. Supplementation of whey in a diet for turkey poults enhanced the effect of *Lactobacillus reuteri* by increasing body weight gain and resistance to salmonellae (Edens, Parkhurst, & Casas, 1991). The addition of whey significantly increased both the cell numbers and lactic acid production by lactobacilli and streptococci (Bury, Jelen, & Kimura, 1998). The whey proteins, α-lactalbumin and β-lactoglobulin, were found to be excellent growth promoters of bifidobacteria. More recently, it has been recognized that several of the whey proteins confer anti-bacterial and immuno-associated protection to the neonate against disease and that these and other whey proteins also have putative biological effects when ingested, including an anti-cancer action (McIntosh *et al*., 1998).

Bomba, Nemcová, Gancarčíková, Herich, and Kaštel' (1999) investigated the influence of the preventive administration of *L. casei* subsp. casei and maltodextrin KMS X-70 on *E. coli* O8: K88 adhesion in the gastrointestinal tract of conventional and gnotobiotic piglets. *L. casei* administered in combination with maltodextrin significantly decreased the number of *E. coli* colonizing the jejunum of conventional piglets in comparison with the control.

Probiotics and metabolites of microorganisms

Lactic acid bacteria—among them many probiotics—have been found to produce anti-microbial substances (Ouwehand, Isolauri, Kirjavainen, & Salminen, 1999). They include toxic metabolites of oxygen, the lactoperoxidase-thiocyanate system, organic acids, and bacteriocins.

Organic acids together with probiotics and specific carbohydrates (yeast-cell walls) are often suggested as alternatives to the use of antibiotic growth promoters (Jensen, 1998).

Boldnan, Jung, Schneider, Block, and Klenke (1998) found that formic acid decreased the population of coliform bacteria in the gastrointestinal tract of weaned piglets. An alternative to the use of organic acids in combination with probiotics in diets is the use of fermented feed. Under certain conditions probiotic strains may be used as the sole fermenting agent in milk. However, in many cases use of a support culture is preferable. The combination of the probiotic culture and the support culture enhanced the acidification rate (Saxelin *et al.*, 1999). Several investigations have shown fermented liquid feed to improve growth performance in pigs and to establish a prophylactic barrier against gastrointestinal disorders. The administration of the milk fermented with *Lactobacillus acidophilus* LA-2 caused a remarkable decrease (71.9% on average) in faecal mutagenicity and increased *Lactobacillus* spp. and *Bifidobacterium* spp. populations in the human intestine (Hosoda, Hashimoto, He, Morita, & Hosono, 1996).

The *in-vitro* bactericidal activity of certain fatty acids has been known for a long time and may be of importance *in vivo* in preventing, eliminating, and in some cases, treating infections. The number of lactic-acid bacteria associated with the epithelial mucosa and from faeces was highest in the digestive tract of fish what were fed diets with added 7% linolic acid (18:3 a-3) or 4% of polyunsaturated fatty acids. It is suggested that dietary fatty acids affect the attachment sites for the intestinal microbiota, possibly by modifying the fatty acid composition of the intestinal wall (Ringo & Gatesoupe, 1998).

Several methods for the control of diarrhoeagenic *E. coli* by bacteriocins have been described. There is potential for using nisin and colicins in foods and agriculture to inhibit sensitive diarrhoeagenic *E. coli* strains. It is suggested that combining probiotic microorganisms with bacteriocins could improve their positive effect on the host.

Probiotics and antibiotics

Probiotics are often considered as 'natural' substitutes for feed antibiotics. Based on the results of some studies, it may be feasible to combine the probiotic and antibiotic treatments to obtain an additive advantage. The probiotic bacteria may be established more easily in the digestive tract of animals if the natural flora is weakened by the use of an antibacterial feed additive (Nousiainen & Setälä, 1993).

Prophylactic use of lactobacilli-containing preparations may protect against the side effects of antibiotics. The principal risk of the therapeutic use of antibiotics consists in bacterial translocation with potential subsequent septicaemia caused by an increase in the number of resistant species and a decrease in the number of more sensitive bacteria. The application of *L. casei, L. acidophilus* and *L. bulgaricus* in conjunction with antibiotics prevented both the increase in the number of ampicillin-resistant bacteria and their translocation into the liver. Wasson, Criley, Glabaugh, Koch, and Peper (2000) suggested that oral administration of a *Lactobacillus*-containing product is ineffective in preventing clinical disease in guinea pigs administered clindamycin phosphate. Lactobacilli, when applied with antibiotics, may eliminate the impairment of the gut micro-ecosystem by maintaining the equilibrium between microbial types in the gut, thus preventing the impairment of the mucosa's immune response.

Probiotics and vaccines

Methner, Barrow, Berndt, and Steinbach (1999) tested the combination of competitive exclusion (CE) and vaccination against *Salmonella* infection in chickens of different ages. The results of their model study demonstrated principally that a combination of CE and immunization against *Salmonella* infection result in a degree of protection considerably beyond that afforded by the use of one of the two methods. The administration of the live *Salmonella* vaccine strain prior to or simultaneously with the CE culture revealed the best protective effect, because these combinations ensure an adequate persistence of the vaccine strain as a prerequisite for the expression of inhibitive effects in very young chicks and the development of strong immune protection in older birds. The combination of *L. acidophilus, Streptococcus faecium,* and *S. typhimurium*—specific antibodies when administered via spray to broiler chicks at 1 day of age and administered orally for the first 3 days after placement significantly reduced *S. typhimurium* colonization of the caecum and colon in market-aged broilers (Promsopone *et al.*, 1998). The therapeutic activity of recombinant strains of *Streptococcus gordonii* in experimental vaginitis was confirmed by Beninati *et al.* (2000).

Probiotic bacteria and trace elements

Some microbes have the ability to bind metal ions present in the external environment at the cell surface or to accumulate them in the cell. These properties have been exploited also in probiotic preparations.

Yeasts and lactobacilli are able to concentrate selenium from their growth media into their cells in high concentrations and both produce an organic form of selenium from inorganic Se. Recently, Se-enriched yeasts and lactobacilli have been commercialized as a Se supplement, which is much more bioavailable.

Trivalent chromium is the active constituent of the glucose tolerance factor (GTF), which is a cofactor needed to potentiate insulin. The yeast is able to produce GTF and thus to serve as a feed supplement of the bioactive and non-toxic form of chromium (Ingledew, 1999).

Iron is an essential nutrient for all microbes, except the genus *Lactobacillus*, which uses manganese and cobalt instead. The binding of iron by bifidobacteria and lactobacilli, used extensively as probiotics, can serve to reduce the availability of iron to pathogenic microorganisms.

Certain species of lactobacilli are able to concentrate high levels of manganese, which supports their pseudocatalase and superoxide dismutase activity. The addition of Mn to the growth medium increases the viable count of *L. casei* spp. casei and *L. plantarum* compared to unsupplemented cultures (Calomme, Van Den Branden, & Van Den Berghe, 1995).

Zinc is important for both the stability and action of alcohol dehydrogenase as well as for many other microbial Zn-metallo-enzymes. However zinc's antimicrobial effects are well known. The inhibiting effect of zinc has been used successfully in the treatment of *E. coli* diarrhoea in postweaning piglets (Holm & Poulsen, 1996).

Future developments

If probiotics are to represent a real and effective alternative to antibiotics and chemotherapeutics it is absolutely necessary to ensure their consistently high efficacy. To ensure the high efficacy of probiotic preparations requires a complex solution aimed at the following: (a) the product and (b) how it is applied.

As concerns the product itself, future research should be aimed at the selection of strains with strong probiotic effects, what will comply with the main criteria of selection. It will be important to search for ways to potentiate the efficacy of probiotic microorganisms in all parts of the digestive tract. In addition to prebiotics, which potentiate the effect of probiotics in the colon, there should be components that, in combination with probiotic preparations, will ensure their high efficacy in the small intestine also.

As concerns the form of application, research and development should be aimed at methods that will ensure the maximum efficacy of probiotics at the time of their consumption. Current knowledge confirms that probiotic preparations as well as fermented products are most effective in a fresh state when given together with a medium. Based on current knowledge it may be expected that the developments in the field of biotechnological research will result in a very simple 'fermentor' which will make it possible for the customers to prepare probiotic preparations and fermented products directly.

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