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Probiotic lactic acid bacteria – the fledgling cuckoos of the gut?

Arnold Berstad[1]*, Jan Raa[1], Tore Midtvedt[2] and Jørgen Valeur[1]

[1]Unger-Vetlesen's Institute, Lovisenberg Diakonale Hospital, Oslo, Norway; [2]Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet, Stockholm, Sweden

It is tempting to look at bacteria from our human egocentric point of view and label them as either 'good' or 'bad'. However, a microbial society has its own system of government – 'microcracy' – and its own rules of play. Lactic acid bacteria are often referred to as representatives of the good ones, and there is little doubt that those belonging to the normal intestinal flora are beneficial for human health. But we should stop thinking of lactic acid bacteria as always being 'friendly' – they may instead behave like fledgling cuckoos.

Keywords: *bacteriocins*; *irritable bowel syndrome*; *lactic acid bacteria*; *obesity*; *probiotics*

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*Correspondence to: Arnold Berstad, Unger-Vetlesen Institute, Lovisenberg Diaconal Hospital, NO-0440 Oslo, Norway, Email: arnold.berstad@k2.uib.no

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Lactic acid bacteria, particularly the rod-shaped lactobacilli, are generally regarded as being of a friendly disposition. They are members of the normal microbial ecosystem in the intestinal tract and seem to play a key role in maintaining stability and diversity of the gut microbiome (1).

Lactic acid bacteria protect their own ecological niches by producing lactic acid, antibacterial peptides (bacteriocins), and H_2O_2, and this is how they counteract infections in the vagina (2). If the abundance of lactic acid bacteria for some reasons is declining, their protective function will inevitably be reduced, and epithelial tissues become more prone to staphylococcal infection and to overgrowth of *Candida albicans* (2). We envisage that something similar is taking place in the intestines of irritable bowel syndrome (IBS) patients (3).

In the presence of oxygen, lactic acid bacteria produce H_2O_2 that chemically and enzymatically will be converted into oxygen radicals with more potent antimicrobial activity than H_2O_2 itself. These reactive oxygen species may also act as signal molecules in the communication between gut microbiota and the intestinal mucosa (4). Like aerobic and facultative anaerobic bacteria, lactic acid bacteria are very tolerant to H_2O_2 and to reactive oxygen species. Anaerobic bacteria are, however, highly sensitive.

Lactic acid bacteria will efficiently capture remaining oxygen in the otherwise oxygen-starved environment distally in the intestine, thereby supporting the strictly anaerobic colonic bacteria in their production of short-chain fatty acids by anaerobic fermentation of unabsorbed food components – highly beneficial for human health (5). At the same time, they will, unlike facultative anaerobic bacteria that also consume oxygen, produce H_2O_2 and reactive oxygen species and thereby create a microenvironment around their aerobic niches that is more toxic for anaerobic bacteria than oxygen itself. Even in the strict anaerobic gut environment, oxygen may still be available in close vicinity of epithelial cells. Is so, that is, the most likely ecological niche for lactic acid bacteria in the anaerobic sections of the gut.

Imbalances of the gut microbial community (dysbiosis) are associated with various clinical conditions, including IBS (6). Earlier culture-based studies indicated low fecal levels of lactobacilli in patients with IBS (7), although newer studies using molecular techniques do not always agree (6). Failing anaerobicity within the colon may be a pathogenicity mechanism, since it may result in reduced diversity of the gut microbiota, increased aerobic glycolysis within human cells, toxin production (methylglyoxal), and oxidative stress (8, 9).

Fledgling cuckoos?

Lactic acid bacteria are present in high numbers in various fermented foods and drinks, and are widely used by young and old as dietary supplements or probiotics. It is widely

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Fig. 1. Fledgling cuckoo in the nest of a meadow pipit. Photo: Jan Berstad.

believed that such products are beneficial for human health. However, there are few well-documented clinical benefits (2) and hardly any benefits persisting after termination of probiotics intake (10).

Species and strains of lactic acid bacteria used as probiotics and starter cultures for food preservation are selected on basis of their ability to produce antimicrobial substances (11–13). Is this the reason why probiotic lactic acid bacteria do not succeed to colonize the intestine? Do they behave like fledgling cuckoos that push their fellow nestlings out of the nest (Fig. 1) before they fly the nest themselves?

Weight gain and obesity

Excessive weight gain and obesity have become a global epidemic that may have a microbial cause (14, 15), albeit not infectious. The condition is associated with changes within the intestinal flora and can be transmitted through fecal transplantation in animals – and perhaps also in people (16). Commercial supplements of lactic acid bacteria stimulate weight gain in chicken and pigs, and some reports have suggested that the same may be the case also in humans (17). On the basis of a meta-analysis, Raoult and his team concluded that some lactic acid bacteria cause weight gain while others do not (18). That depends on the bacterial strain. It should also be kept in mind that species and strains causing weight gain in chicken and pigs not necessarily will do the same in humans, hence limiting the relevance of animal testing in order to screen for weight-gaining properties of specific probiotics for human use (19).

There are papers showing that probiotic lactic acid bacteria may prevent and treat obesity (20). However, the general limitations of all the referred trials have been small sample sizes and absence of follow-up studies demonstrating that the anti-obesity effects persist after termination of probiotics administration.

Antibiotics may cause weight gain in animals as well as in humans (21), and antibiotics have been used for many years as growth promoters for pigs and poultry (22). Following banning of such use of antibiotics in animal husbandry, growth-promoting probiotics have been taking their place. It may be that the effects of antibiotics and probiotics have a common ground, namely, affecting the ecological condition of the microbiota (23), favouring an 'obesogenic' microflora. Antibiotics administered in early life to animals may cause long-lasting changes in gut microbiota and obesity (24). It cannot be ruled out that this may also apply in humans (25), and accordingly, there are reasons to restrict using bacteriocin/antibiotics-producing lactic acid bacteria as probiotics – in particular to young children (11, 26).

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Killing of fellow nestlings

Lactic acid bacteria occurring naturally in milk are killed during the pasteurization process. As a consequence, pasteurized milk will not curdle in the same way as non-pasteurized milk. Today, starter cultures of specific lactic acid bacteria, grown in fermenters, are used in the manufacturing of soured milk products, such as yoghurts. If such starter culture bacteria are selected based on their ability to produce bacteriocins and antibiotics (12), it should not be disregarded that they behave like fledgling cuckoos.

Lactic acid bacteria produce bacteriocins that kill closely related gram-positive bacteria (1). Only a few days after last oral intake of lactic acid bacteria in probiotic preparations, it is normally not possible to detect them within the gut, and permanent colonization of added probiotics has never been documented (1).

Clinical experience by two of us (AB, JV) has convinced us about the validity of the fledgling cuckoos analogy. We have observed that the total number of lactic acid bacteria in feces of patients with IBS does not increase after probiotics use – quite the opposite. Our experience is in line with published data showing that the already sparse numbers of lactic acid bacteria in patients suffering from IBS are further reduced after treatment with probiotics (27). This is a logical consequence, as we see it, of adding bacteria that kill their closest relatives within the gut.

On the other hand, there are patients who have experienced positive effects of using probiotics, for instance, against IBS and other gastrointestinal discomforts. This may depend on species of probiotics and on subgroups of IBS tested (6). In general, however, effects are marginal and meta-analyses generally conclude that more and better studies are needed to confirm results from controlled clinical studies (28). It should also be taken into account that there may be a certain publication bias in this area, since negative results with commercial products not necessarily have been published. Farup and his team have, however, demonstrated that IBS patients had increased abdominal pain when treated with probiotic lactic acid bacteria (29).

It is a commonly held view that bacteriocins produced by lactic acid bacteria inhibit only closely related species and that bacteriocins therefore will not likely change the gross composition of the gut microbiota, at least not to the same extent as conventional antibiotics. This view is supported by studies showing that the microbial composition of feces is not largely altered after administration of probiotics (30). But this does not necessarily apply for all probiotic preparations. For example, *Lactobacillus rhamnosus* produces a 'bacteriocin' that inhibits bacterial species belonging to other families (13), and there are lactic acid bacteria producing antimicrobial substances that inhibit many different microbial species (11, 12). Probiotic lactic acid bacteria may therefore combat not only their closest relatives, but also other bacterial species in the healthy gut microbiota. We have not found any published papers on the effects of such bacteriocins on anaerobic intestinal bacteria, the most important players in maintaining ecological stability of gut microbiota.

Tilapia, the world's second largest fish species in aquaculture, has its biological origin in eutrophic lakes in East Africa where it feeds on planktonic algae and detritus. It is a very hardy species in its natural environment, but in aquaculture it is susceptible to opportunistic pathogens like other cultured fish species. Tilapia has been used as a very interesting organism for model studies on dysbiotic gut conditions of relevance for humans (31) and has elegantly confirmed our 'fledgling cuckoos' hypothesis. The fish stayed healthy on the addition of a probiotic containing *Lactobacillus* strains, but upon termination of the treatment the fish developed gut microbiota dysbiosis similar to that caused by antibiotics, resulting in reduced resistance to infection. Moreover, the probiotic bacteria disappeared from the gut after termination of probiotics use.

It is established knowledge that interfering with the intestinal flora may have harmful consequences for human health, but there are not yet reliable biomarkers for a healthy gut microbiota, with the possible exception of indole. Indole is produced by microbial tryptophan catabolism in the colon, and a normal indole concentration seems to indicate a healthy intestinal flora (32). Indole is metabolized and excreted within urine as indoxyl sulfate. In cases of chronic kidney failure, there are increased concentrations of indoxyl sulfate in the blood, and since this metabolite works as a uremic toxin, interventions seek to reduce its urinary excretion, for instance, by administering probiotics (33). It is noteworthy that there is a clear correlation between reduced urinary excretion of indoxyl sulfate and scarcity of lactic acid bacteria in the feces (34). And more importantly, low perioperative levels of indoxyl sulfate in the urine constitute the principal predictor of a fatal outcome for bone-marrow transplants (35).

Rare side effects

In special circumstances, probiotic bacteria may translocate into the blood and cause bacteraemia/sepsis, liver abscess, endocarditis, and death (2). In one study, mortality increased in patients with acute pancreatitis who were tube fed with probiotics (36). The use of probiotics should therefore be considered with care, at least in such cases, hence in line with the advice from The Norwegian Scientific Committee for Food Safety (VKM) against the use of probiotics for critically ill patients (www.vkm.no/dav/009488e0b8.pdf).

Concluding comments

When they are members of a stable gut microbiota, lactic acid bacteria undoubtedly play an important role in maintaining good health. The mechanisms involved are apparently quite complex, involving immune modulation, production of peroxides, acid and bacteriocins, and also proteins that alter epithelial permeability and bind to intestinal receptors for pathogen (37). Their immunomodulating properties may, for example, be of fundamental importance in the development of mucosal and systemic immune tolerance (38). Therefore, if they for some reasons are outnumbered in the gut microbial ecosystem, resulting in disease conditions, it is a tempting strategy to replace them by oral administration of lactic acid bacteria produced in fermentation culture. The idea has proved to be good in the prevention of allergy and asthma (39), and it seems to be a very good idea to select strains of probiotic bacteria that will survive in the intestine and produce GABA (γ-aminobutyric acid) (40), an inhibitory neurotransmitter deficient in children suffering from ADHD (41). Lactic acid bacteria with strong bacteriocidal effects have been exploited in the eradication of methicillin-resistant *Staphylococcus aureus* infections (42). But use of probiotics may have its downsides. Strains of lactobacilli producing bacteriocins and other antimicrobial substances may have an 'antibiotic-like' effect for short duration, but at the same time they may eradicate their closest relatives and pave the way for a dysbiotic gut microbiota, resulting in other health problems. Furthermore, we do not know what might be the implications of overwhelming the complex gut microbiota by introducing very high numbers of one or a few alien bacterial species.

The warning of the author Edith Wharton (1862–1937) is apparently relevant also in our case: 'Beware of monotony; it's the mother of all the deadly sins'.

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