

## Progress in studies on application of probiotics in dentistry

### Postęp badań nad zastosowaniem probiotyków w stomatologii

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#### Abstract

The favourable effects of probiotics on human health have already been well documented. Their application in prophylaxis and therapy, especially of inflammatory diseases of alimentary tract (including antibiotic-associated diarrhoea) is not questioned any more. This study presents current data on the knowledge of the suitability of probiotic strains of the *Lactobacillus* genus for application in dentistry in adults with dental caries or chronic periodontitis. In the light of recent *in vitro* and *in vivo* studies, antagonistic effects of lactobacilli producing hydrogen peroxide toward cariopathogens and periopathogens were reviewed. The potential was analysed for using probiotic *Lactobacillus* strains in dentistry, pointing to their significance in prophylaxis and therapy of caries and chronic periodontitis.

**Keywords:** probiotics, *Lactobacillus*, dental caries, chronic periodontitis.

#### Streszczenie

Dobrze już udokumentowano korzystne oddziaływanie probiotyków na zdrowie człowieka. Ponadto, ich zastosowanie w profilaktyce i terapii, przede wszystkim chorób zapalnych przewodu pokarmowego (w tym biegunce związanej z antybiotykoterapią), nie budzi dziś wątpliwości. W tej pracy przedstawiono dane dotyczące aktualnego stanu wiedzy w zakresie przydatności stosowania szczepów probiotycznych z rodzaju *Lactobacillus* w stomatologii, u osób dorosłych z próchnicą zębów lub przewlekłym zapaleniem przyzębia. Omówiono, w świetle najnowszych badań *in vitro* i *in vivo*, antagonistyczne oddziaływanie pałeczek *Lactobacillus* wytwarzających nadtlenek wodoru wobec kariopatogenów oraz periopatogenów. Jednocześnie analizowano możliwości zastosowania probiotycznych szczepów *Lactobacillus* w stomatologii, wskazując na ich znaczenie w profilaktyce i terapii próchnicy oraz przewlekłego zapalenia przyzębia.

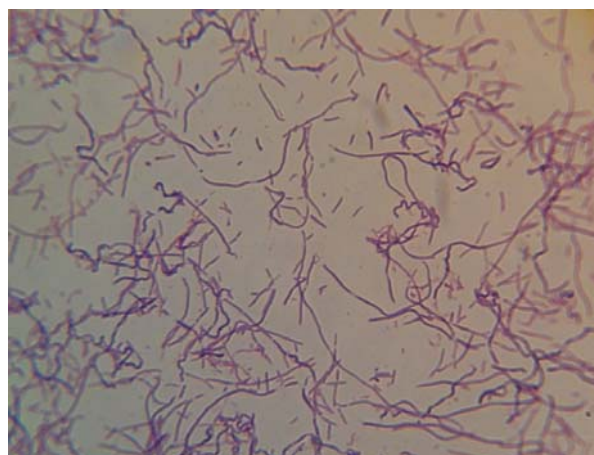
**Słowa kluczowe:** probiotyki, *Lactobacillus*, próchnica zębów, przewlekłe zapalenie przyzębia.

In line with the current WHO definition, probiotics involve living microbes which, administered per os to humans exert favourable health effects, independently of basic food [1]. This basic definition was supplemented with the so called criteria of Fuller: the probiotic agent should not be pathogenic nor be toxic for human body, manifesting no immune disturbances [2]. Thus, the probiotic strain should have GRAS (generally recognised as safe) status and safety of its application should be supported by *in vitro* and *in vivo* studies [3].

The probiotic strains include mainly lactic acid bacilli of *Lactobacillus* and *Bifidobacterium* genera and *Saccharomyces boulardii* yeasts. The probiotic strains used in dentistry include *Lactobacillus reuteri*, *L. brevis*, *L. rhamnosus* GG, *L. paracasei*, *L. acidophilus*, *L. plantarum* and *Bifidobacterium longum*. The genus of *Lactobacillus* covers a heterogeneous group of over 100 species of Gram(+) bacilli, widely present in the natural environment (**Figure 1**). At the same time the bacilli compose the microbiotas of humans and animals [3].

The favourable effect of probiotics on human health has already been well documented [4–8]. Moreover, their application in prophylaxis and

therapy, particularly of alimentary tract diseases (including antibiotic-associated diarrhoea) is not questioned any more [9–11]. The antineoplastic activity of probiotic strains is also indicated [12]. The European SYNCAN project is implemented



**Figure 1.** *Lactobacillus acidophilus* in Gram staining (1000x)

**Rycina 1.** *Lactobacillus acidophilus* w preparacie barwionym metodą Grama (pow. 1000x)

to analyse the antineoplastic action of probiotic agents [13].

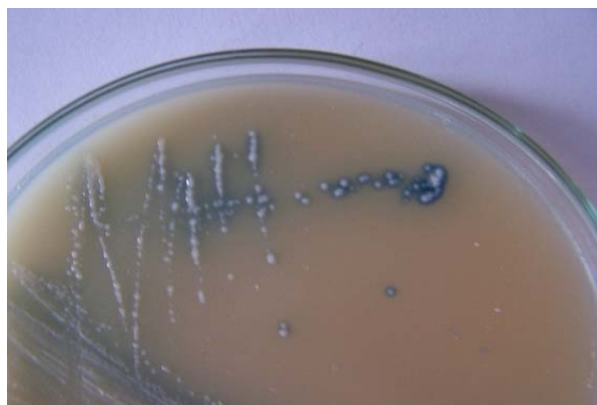
The first studies on the application of probiotic agents in dentistry involved investigations on dental caries, conducted in 2001 on children with active caries. Supplementation of the diet with milk containing *L. rhamnosus* strain was found to reduce the course of the disease [14]. The results were followed by introduction of probiotics in to prophylaxis and therapy of dental caries as supplementary preparations.

Dental caries represents a restricted pathological process, induced by oral cavity bacteria, resulting in demineralization and destruction of solid dental tissues with development of a carious defect [15]. Global population studies confirmed universal worldwide manifestation of dental caries, representing the most frequent disease of civilization-linked nature [16, 17]. Much higher levels of caries occurred in adults than in children in all 26 countries all over the world. In Europe the highest indices of DMFT were detected in its north-western part, particularly in Poland, Finland, Denmark and Austria [18]. Etiopathogenesis of early carious lesion is determined mainly by activity of caries-inducing streptococci of *Streptococcus* genus, including *S. mutans* (the Mutans group). The species is recognised to represent a classical cariopathogen. Bacteria of the species manifest the ability to produce soluble and insoluble extracellular polysaccharides (dextran, fructan), forming stroma of developing dental plaque [19]. Other cariopathogens include *Streptococcus salivarius* (the Salivarius group) and *Streptococcus sanguinis* (the Mitis group). In such bacteria the sugars supplied with food are rapidly metabolized to organic acids. The arising organic acids become accumulated in dental plaque, reducing its pH to less than 5.5, which initiates the development of an early lesion focus.

The published papers pointing to the favourable clinical effect of probiotics administration in dental caries justify further studies. Therefore, in my studies I analysed the *in vitro* potential for an antagonistic effect of *Lactobacillus spp.* bacilli against *Streptococcus mutans* [20]. The studies were conducted on 120 patients aging 20 to 49 years, in two groups. Group 1 comprised 45 persons with active caries and group 2 included 75 caries-free persons. The activity of caries was assessed using WHO criteria, including DMFT, GI (Gingival Index) and PLI (Plaque Index) [17]. The investigated material involved samples of saliva at rest, in which manifestation of *Lactobacillus* bacteria was examined and their ability to produce hydrogen peroxide. Isolation of *Lactobacillus* strains and estimation of their ability to produce hydrogen peroxide were conducted in the Department of Medical Microbiology, Poznan University of Medical Sciences. Strains of *Lactobacillus spp.* were identified using API 50 CHL test (bioMerieux)

while the ability to produce hydrogen peroxide was determined by culturing the isolates in a differentiating medium of TMB-Plus agar, prepared as described by Rabe and Hillier [21], as illustrated in **figures 2** and **3**. The tests permitted to find out that in all patients with active caries the isolated bacilli of *Lactobacillus* did not produce hydrogen peroxide. In the group of caries-free persons strains of *Lactobacillus* isolated from 35% of patients did not produce hydrogen peroxide while in 27.5% of patients the presence of *Lactobacillus spp.* could not be demonstrated.

The obtained results may indicate that activity of caries is linked to presence of *Lactobacillus spp.* strains producing no hydrogen peroxide. On the other hand, it is known oral lactobacilli are that they may play a significant role in the progression of dental caries, which is mainly linked to production by the bacteria of lactic acid [22, 23]. Results of my studies are consistent with the data and for the first time they document the pathogenetic relationship between active caries and presence of *Lactobacillus spp.* strains producing no hydrogen



**Figure 2.** *Lactobacillus acidophilus* strain producing hydrogen peroxide on TMB-Plus medium

**Rycina 2.** Szczep *Lactobacillus acidophilus* wytwarzający  $H_2O_2$  na podłożu różnicującym TMB-Plus



**Figure 3.** *Lactobacillus acidophilus* strain non-producing hydrogen peroxide on TMB-Plus medium

**Rycina 3.** Szczep *Lactobacillus acidophilus* nie wytwarzający  $H_2O_2$  na podłożu różnicującym TMB-Plus

peroxide. Salivary manifestation of *Lactobacillus* spp. bacteria producing hydrogen peroxide was demonstrated already earlier [24–25]. However, the obtained results were not analysed in comparison to activity of the carious process.

Continuing the studies on the role of probiotics in diseases of oral cavity I addressed the problem of antagonistic interactions between clinically important periopathogens and strains of *Lactobacillus* spp. in microflora of oral cavity in chronic periodontitis in adults [26–29].

Chronic periodontitis is an inflammatory disease, leading to destruction which involves gingivae, radical cementum, periodontium and osseous alveolus. Chronic periodontitis has a moderate or severe clinical course. Incidence of the disease and its severity increase with age [30]. Epidemiological investigations confirm that chronic periodontitis belongs to the most frequent chronic diseases in humans. In western Europe in around 36% of persons aged 35 to 44 years a moderate and in around 10% a severe form of the disease is revealed; in eastern Europe the proportions are higher, 45% and 30–40% respectively. In Poland a moderate form of chronic periodontitis was diagnosed in around 32% of persons and a severe form in around 16% of individuals [31].

The principal etiological factor of chronic periodontitis involves plaque-forming bacteria, defined as periopathogens. Bacteria of the plaque exert harmful effects on fibroblasts, epithelial cells and endothelial cells and on components of extracellular substance. Also, they may affect cells of the immune system, stimulating them to produce mediators of inflammatory reactions [32–34]. Currently, on the basis of studies of Socransky's team specific groups of bacterial species are distinguished (termed complexes) with particular significance for etiopathogenesis of chronic periodontitis [35]. *Porphyromonas gingivalis*, together with the species of *Tannerella forsythia* and *Treponema denticola*, form the so-called red complex, which seems to be linked to pathogenic signs of periodontitis in adults [36, 37]. Another significant group of bacteria forms the so-called orange complex, encompassing 13 species, including *Fusobacterium nucleatum* and *Prevotella intermedia*, manifesting a particular pathogenetic significance. Microbiological investigations indicate that bacteria of the orange complex form an indispensable link, promoting colonization on periodontal tissues by the red complex. Another group of bacteria forms the so-called green complex, comprising three species: *Capnocytophaga sputigena*, *C. gingivalis* and *Eikenella corrodens* [37]. The species are also associated with morbid signs of chronic periodontitis in adults but induce a more benign clinical course, as compared to that induced by red complex bacteria. Moreover, separate periopathogens are distinguished, in particular *Aggregatibacter acti-*

*nomycetemcomitans*, which may accompany the red complex. Currently, a particular significance of *A. actinomycetemcomitans* is recognised in etiopathogenesis of periodontitis [38, 39].

The data have inspired me to undertake studies on the potential for antagonistic effects of probiotics against periopathogens [29]. The studies were conducted on 30 patients, 35 to 49 years of age, in two separate groups. Group 1 involved 16 patients with moderate chronic periodontitis while group 2 consisted of 14 patients with severe chronic periodontitis. The control group included healthy patients, 20 to 42 years of age. Chronic periodontitis was diagnosed in line with WHO criteria [17, 40]. In the studies of the saliva of all patients with moderate chronic periodontitis hydrogen peroxide-producing bacilli of *Lactobacillus* spp. were identified. The hydrogen peroxide-producing bacilli of *Lactobacillus* spp. were demonstrated only in patients with the moderate form of chronic periodontitis.

The present data indicate that in pathogenesis of periodontitis a significant role is played by pro-inflammatory cytokine response of Th 17, expressed by high levels of IL-17 and TNF- $\alpha$  in gingival fluid of patients with chronic periodontitis [41, 42]. IL-17 induces activation of neutrophils with inflammatory reaction, which additionally accentuates synergic interaction of TNF- $\alpha$ , IL-22 and IL-26. Moreover, IL-17 releases neutrophils while IL-22 induces antibacterial peptides, involving a nonspecific immune response against bacterial and mycotic pathogens [43, 44]. Thus, the cytokine response of Th17 fulfils an important role in immune protection of the host and it may provide the principal mediator in pathogenesis of several inflammatory diseases, as indicated by results of recent studies [45–48].

In the conducted investigations elevated levels were demonstrated of TNF- $\alpha$ , IL-1 and IL-17, and the levels of cytokines were significantly higher than in moderate form of the disease [26]. The elevated levels of IL-17, and also of TNF- $\alpha$  and IL-1 $\beta$  in chronic periodontitis were described earlier by other authors [41, 42, 49]. Nevertheless, in this study the overproduction of IL-17 and TNF- $\alpha$  and of IL-1 was for the first time found to be typical of the two clinical forms of chronic periodontitis. The obtained results permit to distinguish patients with a high cytokine pro-inflammatory response from those with a moderate response. Intensity of pro-inflammatory cytokine response, high or moderate, corresponds to severe and moderate clinical form of chronic periodontitis respectively. Therefore, it can be concluded that chronic periodontitis is determined by the persisting pro-inflammatory response, the intensity of which may be of significance for clinical course of the disease. The conclusion is supported by the data documenting the relationship between progression of articular damage in patients with rheumatoid arthritis and high production of IL-17 and TNF- $\alpha$  [50].

In order to make the conclusion more credible, I have conducted studies on adult patients with moderate chronic periodontitis treated with an oral probiotic preparation [28]. The investigated group included 35 patients, 31 to 46 years of age, in whom clinical examination allowed to diagnose moderate chronic periodontitis with mean duration of the disease ranging from 18 to 27 months. Two weeks following hygienization procedures in 19 of the patients tablets were administered, containing hydrogen peroxide-producing probiotic strain of *Lactobacillus reuteri*. The diet supplement of probiotic tablets for chewing, containing *Lactobacillus reuteri* strain ( $10^8$  CFU *Lactobacillus reuteri* ATCC PTA 5289, Prodentis), was applied by the patients twice daily, following tooth brushing. After completing the treatment, in the subgroup of participants a significant clinical improvement was found in 14 patients (73.7%), expressed by reduced values of clinical indices: SBI (Sulcus Bleeding Index), PPD (Periodontal Pocket Depth), CAL (Clinical Attachment Level). Moreover, a reduction was disclosed in levels of estimated pro-inflammatory cytokines (TNF- $\alpha$ , IL-1f and IL-17). The results remain in line with those of Vivekananda et al. [51] and of Vicario et al. [52], documenting reductions in values of the indices in patients with moderate chronic periodontitis using probiotic tablets and hygienization procedures. Also Twetman et al. using a chewing gum containing strains of *Lactobacillus reuteri* demonstrated lowered values of GI and SBI indices and reduced levels of TNF- $\alpha$  and IL-8 cytokines [53]. Other authors pointed to improved clinical indices in patients with gingivitis [54, 55]. In addition, Lin et al. demonstrated that *L. reuteri* inhibits production of pro-inflammatory cytokines [56].

In my studies no clinical improvement was noted in 25% of patients with moderate chronic periodontitis and levels of estimated pro-inflammatory cytokines (TNF- $\alpha$ , IL-1f and IL-17) showed no significant alterations [28]. This may indicate an insufficient colonization of gingival pockets by the applied probiotic strain of *Lactobacillus reuteri*. It remains possible that the process of periodontal colonization in some patients requires a more prolonged period of administering probiotic tablets, containing *Lactobacillus reuteri* strain. In the conducted studies the hydrogen peroxide-producing strains of *Lactobacillus* were shown to be present significantly more frequently in patients with moderate chronic periodontitis than in those with severe chronic periodontitis. This indicates that the strains may prevent development of the disease. *Lactobacillus reuteri* represents one of the well recognised strains of probiotic bacteria with a documented activity in several bacterial infections [4, 6–8, 57, 58]. Reuterin, produced by the strain, involves a 3-hydroxypropionaldehyde with a broad spectrum of antibacterial activity [59]. It is active within a broad spectrum of pH and remains resi-

stant to action of lipo- and proteolytic enzymes and it exerts a suppressive effect on synthesis of pro-inflammatory cytokines. Reuterin blocks adherence and prevents against colonization by pathogens [60].

Thus, the presented data indicate that administration of probiotics in dentistry is advisable, particularly in dental caries and in chronic periodontitis in adults. Probiotic agents, containing hydrogen peroxide-producing *Lactobacillus* bacteria may exert antagonistic effects against cariopathogens and periopathogens, with the resulting reduced cariogenic process and reduced progression of chronic periodontitis. The chronic periodontitis is associated with significant concentrations of pro-inflammatory cytokines, TNF- $\alpha$  and IL-17 in gingival fluid of gingival pockets. Treatment with probiotic agents containing *Lactobacillus reuteri* strain leads to significantly reduced levels of the cytokines. The observations show for the need of further studies in order to implement novel strategies in prophylaxis and therapy of caries and periodontal diseases.

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### Conflict of interest statement

The author declare that there is no conflict of interest in the authorship or publication of contribution.

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