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Some phenotypic properties of oral lactobacilli isolated from oral cavity in children

Lactobacillus spp., belonging to the lactic bacteria, is found to be as a part of normal microflora of the oral cavity, intestinal tract or vagina of the human beings. Normal microflora, including *Lactobacillus spp.*, prevents colonization of pathogenic microorganisms by competitive inhibition for microbial adhesion sites. However, normal microflora has not only beneficial but also harmful effects on the host; oral bacteria may be a source of endogenous infections, e.g. dental caries, which is one of the most common infectious diseases in humans (1, 5, 6).

The aim of this paper was to assess a salivary content of *Lactobacillus spp.* in oral cavity of school children, to identify isolated strains, to detect their ability to produce H_2O_2 and also to measure the relative cell surface hydrophobicity of the isolated lactobacilli.

MATERIAL AND METHODS

Microbiological material, growth media and identification of bacterial species. Specimens of unstimulated saliva were obtained from 43 children aged 9–10 years. A properly diluted specimens were immediately streaked onto Rogosa agar. Plates were incubated for 48 hrs at 37°C under anaerobic conditions, using Genbag (bioMerieux). Isolated colonies were identified by conventional macroscopic, microscopic or biochemical methods. Biochemical phenotypes of the obtained isolates were defined using identification system for *Lactobacillus spp.* – API 50 CHL (bioMerieux), containing 48 biochemical tests, based on fermentation of various substrates (sugars, glycosides, coumarins, alcohol polyhydric).

Assay of H_2O_2 production by isolated lactobacilli. The ability of oral lactobacilli to produce H_2O_2 was detected using Rogosa agar, containing MnO_2 (2). After inoculation with properly diluted specimens of unstimulated saliva, agar plates were incubated for 48 hrs at 37°C under anaerobic conditions. The zone-surrounded colonies of lactobacilli strains were suspected to produce H_2O_2.

Assay of cell surface hydrophobicity. The relative cell surface hydrophobicity of isolated lactobacilli was assessed using modified ammonium sulfate salt aggregation test (5). The autoaggregated strains were described as very strong hydrophobic, aggregated at 0.4–1.0 M $(NH_4)_2SO_4$ – as strong hydrophobic, at 1.2–1.6 M $(NH_4)_2SO_4$ – as hydrophobic, at 1.8–3.2 M $(NH_4)_2SO_4$ – as hydrophilic; strains not aggregated even at 3.2 M $(NH_4)_2SO_4$ were classified as strong hydrophilic.

RESULTS

Lactobacillus spp. were found in saliva of 39 (91%) assayed children. In 18 (42%) children, the number of these microorganisms was higher than 100,000 colony forming units (CFU)/ml saliva (Table 1). Strains of *Lactobacillus spp.*, possessing the ability to produce H_2O_2, were isolated only from 18 (42%) children and their number was lower compared to the total were of oral lactobacilli (Table 1). Among lactobacilli isolated from oral cavity of children, the most frequently identified species were *L. plantarum* (42%) and *L. rhamnosus* (26%) (Fig. 1).

Table 1. The salivary content of oral lactobacilli in the assayed school children

Number of oral lactobacilli (colony forming units CFU/ml of saliva)	Number (percent) of children possessing *Lactobacillus spp.*	Number (percent) of children possessing *Lactobacillus spp.* producing H_2O_2
1–1,000	2 (5)	3 (7)
1,001–10,000	7 (16)	5 (12)
10,001–100,000	12 (28)	8 (18)
> 100,000	18 (42)	2 (5)
Total	39 (91)	18 (42)

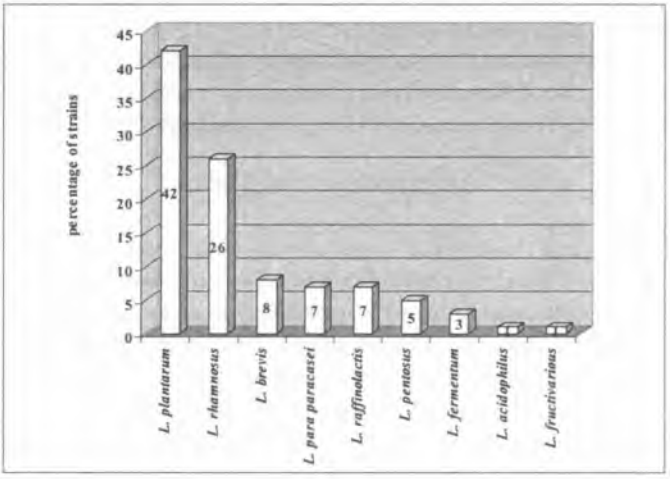

Fig. 1. *Lactobacillus spp.* species isolated from saliva in the assayed school children

As presented in Figure 2, among the isolated lactobacilli, strains possessing very strong hydrophobic properties of their cell surface dominated (81%). Besides, it was also found that 3% of lactobacilli were strong hydrophobic or hydrophobic. Only 4% of lactobacilli showed hydrophilic, while 12% – strong hydrophilic properties of their cell surface.

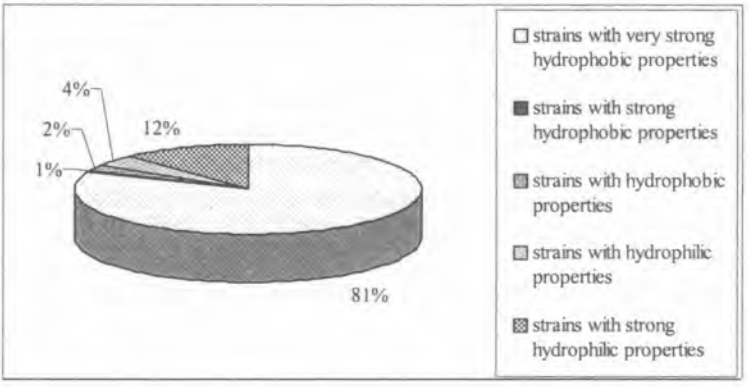

Fig. 2. Cell surface properties of *Lactobacillus spp.* isolated from saliva in the assayed school children

DISCUSSION

It is well-known that lactobacilli are present predominantly in the oral cavity, intestinal tract or vagina. The most frequently isolated species of lactobacilli from human microflora are: *L. acidophilus, L. salivarius, L. casei, L. plantarum, L. rhamnosus, L. cellobiosus, L. fernentum, L. buchneri* and *L. brevis* (1, 3, 6). However, *L. plantarum* and *L. rhamnosus*, identified most frequently in the assayed children, are typical of oral cavity microflora (1).

According to the literature data (1, 6), lactobacilli exert an inhibitory effect on a variety of microorganisms in the oral cavity, intestinal tract or vagina by producing organic acids, free fatty acids, ammonia, H_2O_2 and/or bacteriocins. Our data and those reported by other authors indicate that due to the low prevalence and content of oral lactobacilli producing H_2O_2, this phenomenon can not be regarded as a factor controlling growth of oral microflora (2).

Adhesion of bacterial cell, i.e. their attachment to host tissues is the first step of colonization or/ and infection. This is due to non-specific or specific cell-cell interactions. The cell surface hydrophobicity of bacteria is an important non-specific adhesion factor, which allows them to adhere to smooth surfaces, e.g. teeth surface (4, 7). The very strong or strong hydrophobic properties of cell surface of isolated oral lactobacilli in the assayed children, favouring formation of dental plaque, may be due to the presence of teichoic and lipoteichoic acids in cell wall of *Lactobacillus spp.* (4).

The number of oral lactobacilli in saliva reflects their content in dental plaque and allows to assess a potential risk of dental caries development. The high salivary content of lactobacilli, exceeding 100,000 CFU/ml saliva, found in the 42% of the assayed children, can be regarded as high potential risk of carious lesion propagation. However, development of dental caries depends not only on the high salivary content of cariogenic bacteria, e.g. lactobacilli, but also on other factors such as the susceptible "host" (teeth) and the environment (content of dietary carbohydrates, mainly sucrose) (4).

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SUMMARY

The aim of this paper was to assess a salivary content of *Lactobacillus spp.* in school children, to identify the isolated strains, to detect production of H_2O_2 by oral lactobacilli and also to measure the relative cell surface hydrophobicity of the isolates. Strains of *Lactobacillus spp.* were isolated from saliva of 91% of the assayed children, while strains possessing the ability to produce H_2O_2, were isolated from 42% of these children. In 42% of children the total number of lactobacilli was higher than 100,000 colony forming units (CFU)/ml saliva. The most frequently isolated species were *L. plantarum* and *L. rhamnosus*. 81% strains of isolated lactobacilli showed very strong hydrophobic properties of cell surface, favouring formation of dental plaque. *Lactobacillus spp.*, was found with high prevalence in the assayed school children. The high salivary content of *Lactobacillus spp.*, belonging to the cariogenic bacteria, together with very strong or strong hydrophobic properties of cell surface of isolated lactobacilli, indicate that this group of children is predisposed to dental caries development, provided existence of other risk factors.

Niektóre cechy fenotypowe pałeczek kwasu mlekowego występujących w jamie ustnej u dzieci

Celem pracy była ocena częstości występowania pałeczek *Lactobacillus spp.* w ślinie dzieci szkolnych, identyfikacja izolowanych szczepów, ocena produkcji H_2O_2 przez pałeczki kwasu mlekowego, a także określenie właściwości hydrofobowych powierzchni komórki uzyskanych izolatów. Szczepy *Lactobacillus spp.* były izolowane ze śliny od 91% badanych dzieci, podczas gdy szczepy posiadające zdolność produkcji H_2O_2 izolowano od 42% dzieci. U 42% badanych dzieci liczba szczepów *Lactobacillus spp.* była wyższa niż 100 000 jednostek tworzących kolonie CFU/ml śliny. Najczęściej izolowanymi gatunkami były *L. plantarum* i *L. rhamnosus*. 81% izolowanych szczepów *Lactobacillus spp.* wykazywało bardzo silne właściwości hydrofobowe powierzchni komórki, sprzyjające tworzeniu się płytki nazębnej. Pałeczki te występowały z dużą częstością u badanych dzieci szkolnych. Wysoka zawartość bakterii próchnicogennych z rodzaju *Lactobacillus* w ślinie oraz silnie hydrofobowa powierzchnia komórek izolowanych pałeczek kwasu mlekowego świadczą o potencjalnym narażeniu tej grupy dzieci na próchnicę zębów, przy istnieniu dodatkowych czynników predysponujących.