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SOME MECHANISMS OF *LACTOBACILLUS REUTERI* ACTION AS A PROBIOTIC MICROORGANISM

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Some morphological, physiological and biochemical characteristics of the probiotic species of lactic acid bacteria *Lactobacillus reuteri* are presented in the work. This species, according to literature sources, were isolated from the normal human microbiota, other members of the class Mammalia, as well as broilers and sourdough. The study of literature and Internet sources identified the importance of using different strains of *L. reuteri* in modern dietary supplements.Some physiological features of this microorganism in terms of positive effects on the functioning of the human gastrointestinal tract and the formation of immunity were considered. According to processed datait is concluded that there is a necessity for further practical study of *L. reuteri* as a potential probiotic for the pharmaceutical and food industries.

Key words: probiotics; lactic acid bacteria; *Lactobacillus reuteri*; dysbacteriosis; drugs.

Introduction

The reality of our days is that the structure of nutrition in well-developed countries is very unbalanced with a huge lack of vitamins, micronutrients, dietary fibre and an increased amount of antibiotic substances, hormones. The irregular mealtime also has a considerable influence on our gastrointestinal tract (GIT) state.

Modern researches represent data that shows a negative effect of excessive antibiotic substances which are used for human health. The most common side effect of incorrect antibiotic using is dysbacteriosis, which causes diarrhoea, stomach pains, irritable bowel syndrome, and other disorders in the human body, such as yeast infections, lactose intolerance, infections of the urogenilal system. In complex therapy of side effects of antibiotic using prominent role belongs to probiotic drugs. Their efficiency depends on the biological properties of microorganisms used.

Probiotics – drugs or products, which contain living microbiota and can positively affect to health, improve GIT state and functioning. There are some researches, which are proving the positive effect of probiotic microorganism using and propose their introduction in pharmacy and food technologies [1–3].

In the last decades, there was a magnification of inflammatory diseases cases, which coincides with decreasing occurrence of *Lactobacillus reuteri* as a representative of normal microbiota in humans. Such decreasing is connected with high antibiotic using, improved hygiene and poor nutrition, such as lack of vitamins and dietary fibers [4]. That's why we decided to consider the role of these bacteria in the formation of normal human microbiota.

The aim of our work is to analyze the physiological properties of the lactic acid bacterium *Lactobacillus reuteri* as a potential probiotic microorganism, as well as to determine the priority areas of its practical application, in particular in the food and pharmaceutical industries.

Materials and methods

To obtain and further study *Lactobacillus reuteri*, we obtained an accumulating culture by resuspending an aliquot of baby food "Nestogen 4" in saline solution and inoculating the suspension on nutrient medium wort agar (6^0 B).

By performing a series of passages on wort agar (WA) with 2 % $CaCO_3$, a pure culture was obtained, which according to the morphological characteristics of the colonies, cell structure and their

tinctorial properties corresponded to the species *L. reuteri*.

Cells of the isolated strain, as well as other members of the family *Lactobacteriaceae* were stained with Gram staining according to standard methods and examined in a light field microscope "Biolam" at a magnification of 15×90.

Ultrapasteurized milk (3.2 % fat content) was used as culture medium.

Research results and discussion

General characteristics of Lactobacillus reuteri

The species *Lactobacillus reuteri* was isolated by german microbiologist Gerhard Reuter [21]. *L. reuteri* is a heterofermentative bacterium from *Lactobacillus* genus. Its strains were isolated from human faeces, breast milk, human vagina, human oral cavity, guinea pigs, rats, pigs, broilers and sourdough. *L. reuteri* species is known as true autochthonous for humans and animals.

Lactobacillus reuteri are grampositive rod-shaped bacteria that form beige skin colonies (Figs. 1, 2).

Interestingly, a significant number of these lactobacilli were found in the stomach and duodenum, because these departments have long been considered virtually sterile parts of the gastrointestinal tract. In addition to *L. reuteri*, the researcher identified other species of lactobacilli, including *L. gasseri*, which belonged to the indigenous gastrointestinal microbiota.

Fig. 1. Colonies of L. reuteri on wort agar medium with the addition of 1 % $CaCO_3$ after 48 hours of cultivation [22]

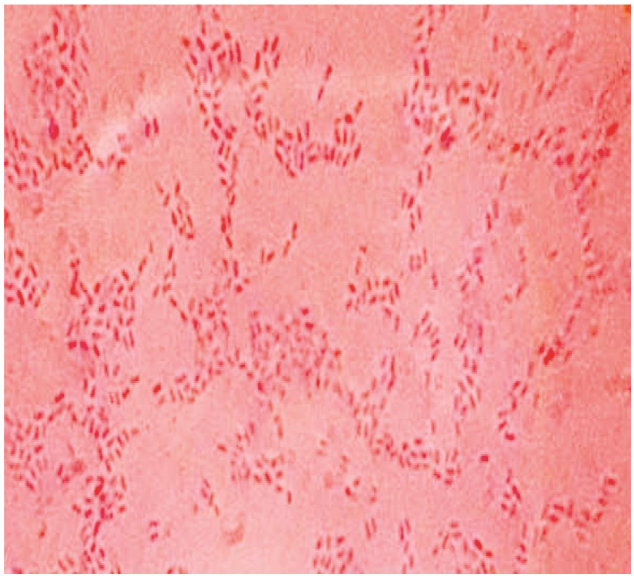

Fig. 2. Morphology of L. reuteri bacteria (magnification 15×90, fuccin staining) [22]

There is data that testified the occurrence of some *Lactobacillus* species in women breast milk in many countries with wide geographical range. *L. reuteri* occurs in 14 % (medium 1.3×10^3 CFU/ml) of women in the countryside and 15 % (medium 3.0×10^2 CFU/ml) of women in the urban area in all countries investigated. That means that the frequency of occurrence of *L. reuteri* in women breast milk does not depend on the living area of women or part of the world, but there is a significant difference in amounts of bacteria found [15].

Probiotic properties of Lactobacillus reuteri

Some literatury data prove the probiotic properties of this bacteria [1, 3]. For example, *L. reuteri* DSM 17938, *L. reuteri* NCIMB 30242, *L. reuteri* ATCC PTA 6475 were isolated from human GIT and thats why they are used in human food supplements and *L. reuteri* I5007, which was isolated from piglets colon is supposed to be used in piglets food supplements [1].

Many companies produce drugs ("Reuten", "Optilact") with different strains of *L. reuteri*. Swedish compsny "BioGaila" produces different drugs with the using of different *L. reuteri* strains, but the most common is using *L. reuteri* 55730 strain. There is data, that this strain has R-plasmids to lincomycin and tetracyclin. Based on its unique

biocharacteristics, this strain can be used in the courses of antibiotic therapy.

Like all representatives of genera *Lactobacillus*, *L. reuteri* produces a variety of antimicrobial substances such as lactic acid, hydrogen peroxide ets. The unique of this spesies is synthesis of reuterin and reutericyclin, which can inhibit the *in vitro* growth of many enteric pathogens, such as *E. coli, Salmonella typhimurium, Staphylococcus epidermidis, S. aureus, Helicobacter pylori,* and rotavirus [1, 3].

L. reuteri cells can convert glycerol into a reuterin. Its a broad-spectrum antimicrobial substance with a molecular mass of less than 200 g/mole. It is a neutral, water-soluble compound. Reuterin is an equilibrium mixture of monomeric, hydrated monomeric, and cyclic dimeric forms of β-hydroxypropionaldehyde in monomeric and dimeric forms NMR studies have shown that β-hydroxypropionaldehyde is the main component of reuterin. Based on MS data reuterin has a molecular weight of 148, but after the LC-MS it was found that 148 is the molecular weight of the cyclic dimeric structure, and the monomeric form has a molecular weight of 74 [20]. Reuterin can inhibit the growth of such bacteria genera: *Escherichia, Salmonella, Shigella, Proteus, Pseudomonas, Clostridium and Staphylococcus* and has less effect on bacteria genera: *Streptococcus, Pediococcus, Leuconostoc*, and *Lactobacillus* [16].

Reuterin also inhibits the growth of yeasts and fungi such as *Candida, Torulopsis, Saccharomyces, Saccharomycoides, Aspergillus, Fusarium* and protozoan *Trypanosoma cruzi*. Furthermore, it inhibits the replication of B2 and lambda phages in *Lactobacillus plantarum* and *E. coli*. The highest production of antibiotic was in the pH 6–8 range but also is possible in the pH 5–9 range. For maximum production, the temperature has to be in the range of 37–45 ^{o}C. The highest concentration is on the 6 hours, in some sources after 2 hours of fermentation. For stimulation of reuterin sinthesis the cells of *L. reuteri* need contact with other heterologous cells [17, 19].

Reutericylin isolated from *L. reuteri* LTH2584 is a negatively charged, hydrophobic molecule with a mass of 349 Da. Chemical analysis methods, such as RP-C8 chromatography, and anion-exchange chromatography, followed by rechromatography by reversed-phase high-pressure liquid chromatography have shown that this substance is a novel tetramic acid derivative structurally related to tenuazonic acid, but does not have such cytotoxic effect. There is data that it inhibits the growth of lipopolysaccharide mutant strains of *E. coli* and *Bacillus subtilis, L. sanfranciscensis*.

Content of fatty acids, such as wheat germ oil, the extracts from Tween 80 and oleic acid, in the growth media have shown a positive effect on inhibitory activities in cell extracts [11].

There are some evidence that *L. reuteri* can form biofilms on epitheliocytes of the intestine. Such biofilms have positive effects on human health:

1) synthesis of antibiotic substances such as reuterin;

2) modulation of cytokine production [8].

Also as many representatives of colon microbiota, *L. reuteri* can synthesize vitamin B12 and antioxidant enzymes. Some computer simulation models have shown that this species can synthesize L-lysine and folic acid [1]. Strain *L. reuteri* CRL1098 can convert glycerol in 1,3-propanediol through cobalamin-dependent reaction. It was found that cell extract of *L. reuteri* can support the necessary amount of the coenzyme B12 needed for the growth of *L. delbrueckii subsp. lactis* ATCC 7830 and *Salmonella enterica serovar Typhimurium* (metE cbiB), *E. coli* (metE) in minimal medium. Synthesis of cyanocobalamin in *L. reuteri* is due to the presence of cob genes which encode the CobA, CbiJ, and CbiK enzymes. Cobamid, isolated in cyanocobalamin form is identical to standard vitamin B12. This fact was approved by using reverse-phase high-pressure liquid chromatography [10].

Moreover, *L. reuteri* can produce histamine which is a very important mediator in immune system functioning. Usually, bacterial histamine activates H2 receptor that causes suppression of TNF, but *L. reuteri*-derived histamine increases levels of cAMP, which can suppress downstream of MEK/ERK MAPK signalling via protein kinase A (PKA). Such changes in cell-functioning cause transcriptional regulation changes lead to suppression of TNF (tumour necrosis factor) production [5].

All strains of *L. reuteri* can influence immune response via several mechanisms including modulation of pro-inflammatory cytokine. Depending on this property we can divide *L. reuteri* strains into two subsets, immunosupressive (ATCC PTA 6475 and ATCC PTA 5289) and immunostimulatory strains (ATCC 55730 and CF48-3A) [1].

Testings on newborn rats have shown, that *L. reuteri* strains differentially affect different parts of the immune system:

1) *L. reuteri* strains have shown a significant reduction of the levels of KC/GRO (IL-8) and IFN-γ in the intestine.

2) *L. reuteri* strains have shown the ability to reduce damage caused by the addition of LPS to the cow milk formula.

3) *L. reuteri* strain DSM 17938 can suppress the process of gut inflammation by concealing of synthesis of inflammatory cytokine IFN-γ and IL-13 [4].

4) *L. reuteri* has shown some effects on the regulation of NF-kB activation which caused suppression of TNF synthesis.

Also, *L. reuteri* increases levels of mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase and p38 MAPK that causes apoptosis. This is one of the mechanisms that promote apoptosis by activated immune cells, which regulate proliferation [6].

The ability to bind to asialo-GM1 and sulfatide with biotin-labelled bacterial cells (5×10^8 cells ml/1) differs within different strains, for example, strains JCM1081 and TM105 bonded with both labelled cells. Such bindings would inhibit some bacteria and it would help treat some diseases [9].

There are experimental data that has shown a significant effect of *L. reuteri* on an allergic airway response in OVA-sensitized mice. Oral treatment with viable *L. reuteri* leads to a decrease in levels of inflammatory cytokines (MCP-1, TNF, IL-5, IL-13) in the BAL fluid, but it does not affect increasing of amounts Th1-type cytokines IL-12 and IFN-γ or the antiinflammatory cytokine IL-10. Such results show that there is a possibility of using *L. reuteri* living cells for treating an asthmatic response in a mouse model of allergic airway inflammation [13].

To prove the probiotic properties of *L. reuteri* some open clinical investigations were made. Samples for stomach, duodenum, or ileum biopsy were taken from 10 healthy people and 9 volunteers with ileostomy underwent. Before the experiment, endogenous *L. reuteri* ATCC 55730 was found in the stomach of 1 subject and the duodenum of 3 subjects (out of 10 subjects), 3 ileostomy subjects (out of 6 tested). All volunteers were supplemented with 4×10^8 CFU of live culture *L. reuteri* ATCC 55730 per day during 28 days. After this period primary biopsy was held, and it has shown the presence of *L. reuteri* ATCC 55730 in the stomachs of 8 and the duodenums of all 10 subjects and the ileostomy of 6 subjects. Moreover, secondary biopsy has shown decreasing in gastric mucosal histiocyte number. On the other hand, increasing duodenal B-lymphocyte number and CD4-positive T-lymphocytes in the ileal epithelium has been detected [12].

A study of 114 hypercholesterolaemic adults that got 6-week treatment with microencapsulated *L. reuteri* NCIMB 30242 has shown significant reductions in LDL-cholesterol, total cholesterol and non-HDL-cholesterol (HDL-C) over placebo [14].

In a placebo-control experiment, *L. reuteri* Prodentis has shown significant improvement in the state of the oral cavity (reducing of plaque index, bleeding on probing and pocket probing depths) only in 30 days of use probiotic gum that contains 2×10^8 CFU/piece. The control group that used placebo did not show any significant improvement in oral cavity state [18].

There are some contradictory results about using *L. reuteri* as a supplement in baby food to treat infant colic. For example, *L. reuteri* DSM 17938 did not affect treating infant colic in both breastfed and formula-fed children [2]. But on the other side, there are some of evidence that *L. reuteri* DSM 17938 has shown an effect on colic treatment in breast-fed infants [7].

Strain *L. reuteri* I5007 was added to piglets food during the weaning and post-weaning periods, with the aim to stimulate the development of normal GIT microbiota and to stimulate the intestinal immune system. Well developed gastrointestinal microflora can prevent post-weaning diarrhoea and stimulate the growth of piglets [1].

Conclusions

After processing literature data we can conclude, that *L. reuteri* is a very interesting and perspective species with a variety of strains that have important biological and technological properties. There are many ways of using *L. reuteri* in pharmacy, food technology and veterinary science, so it is very important to investigate and involve this species in corresponding industries in Ukraine to improve and develop the technologies and assortment of probiotic products on the European level.

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ДЕЯКІ МЕХАНІЗМИ ДІЇ *LACTOBACILLUS REUTERI* ЯК ПРОБІОТИЧНОГО МІКРООРГАНІЗМУ

У роботі розглянуто окремі морфологічні, фізіологічні та біохімічні характеристики пробіотичного виду молочнокислих бактерій *Lactobacillus reuteri.* Цей вид, відповідно до літературних джерел, виділено з нормальної мікробіоти людини, інших представників класу Mammalia, а також бройлерів та хлібної закваски. Опрацювання літературних та інтернет-джерел дало змогу визначити важливість застосування різних штамів *L. reuteri* у сучасних біологічно активних харчових добавках. У роботі розглянуто деякі фізіологічні особливості цього мікроорганізму з погляду позитивного впливу на функціонування шлунково-кишкового тракту людини та формування імунітету. На основі розглянутих теоретичних даних зроблено висновок про необхідність подальшого практичного вивчення *L. reuteri* як потенційного пробіотика для фармацевтичної та харчової промисловості.

Ключові слова: пробіотики; молочнокислі бактерії; *Lactobacillus reuteri*; дисбактеріоз; лікарські препарати.