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EFFECT OF PREBIOTICS ON LYZOSYMIC ACTIVITY OF THE *BACILLUS* GENUS BACTERIA

M.A. SAVEKA, O.A. VASYLCHENKO

National Aviation University, Kiev

Beneficial effects on a host health of probiotic strains of spore-forming bacteria of the Bacillus genus in combination with prebiotic substance lactulose were investigated. The influence of prebiotic lactulose on the Bacillus subtilis 39 *and Bacillus subtilis* 51 *lyzosymic activity were analyzed. The dependence of the growth inhibition zones extension on lactulose quantity introduced into cultural medium was displayed.*

Key words: *probiotics, prebiotics, synbiotics, lyzosymic activity, lactulose, Bacillus subtilis, Micrococcus luteus, growth inhibition zones*

Introduction

Probiotics are viable cell preparations that have beneficial effects on the health of a host by intestinal balance amendment via improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activities, growth-promoting factors and an increased immune response [1].

Probiotic preparations are becoming increasingly available to the public as beneficial functional foods that purport to promote health benefits of consumers. They are used in bacterioprophylaxis of gastrointestinal disorders in humans. Often these disorders are a direct result of antibiotic usage, which creates an imbalance in the normal intestinal microbiota composition. Bacteria most commonly used as probiotics include the lactic acid bacteria (LAB), e.g. lactobacilli, enterococci,

streptococci, and bifidobacteria. Probiotic preparations may be ineffective in term of fail to deliver the proper number of bacteria to the place of their main action, i.e. colon. But probiotic preparations that combine various probiotic bacteria with oligosaccharide substrates (prebiotics) get more popularity.

Nowadays the problem of finding effective, safe and economically beneficial probiotic strains of bacteria as well as in combination with prebiotics for creation of more potent drug is ongoing researches. Two strains of spore-forming aerobes as *B. subtilis* in combination with lactulose or lactitol can be used as effective probiotics for solving all these problems, because of its effective potential transition through the stomach to the colon, high antagonistic activity against pathogenic microorganisms and opportunistic pathogens of gastrointestinal tract [2], comparatively low cost of their cultivation and rapid action.

Experimental evidences suggest that the ingestions of substantial numbers of harmless bacteria indeed provide a beneficial effect on the enteric biota. Except for the lactic acid bacteria, *Bacillus* species are also used as probiotics [1-3]. Probiotic preparations contain bacterial spores, with the potential advantage that the spore can survive during transition through the stomach intact. *Bacillus* species are substantially different from other probiotic bacteria, being primarily aerobic saprophytes found in the soil. It's also known that some *Bacillus* species belong to transient normal microbiota of human gastrointestinal tract, thus, can be easily used as probiotics [2, 3].

Prebiotic effect of lactulose was proved in multiple investigations by increased bifidobacteria number in the colon of patients [4]. Lactulose stimulates the growth of normal bowel human microbiota, promotes infection resistance of the organism against a range of pathogenic microorganisms and opportunistic pathogens such as *Shigella*, *Salmonella, Rotavirus, Yersinia* etc [5]. The earlier scientific investigations showed the possibility of successful combinations of probiotic bacteria with prebiotic lactulose [6, 7].

It's essential to determine lyzosymic activity of *B. subtilis* 39 and *Bacillus subtilis* 51 during their co-cultivation with lactulose and prebiotic influence on the enzyme activity. Lyzosymic activity of the strain plays a main role in pathogenic

microrganisms and opportunistic pathogens growth inhibition, thus probiotic efficacy elevation.

Materials and methods

Lyzosymic activity of two *B. subtilis* strains at their cultivation with prebiotic lactulose was examined. For lyzosymic activity (LA) determination molten and cooled to temperature 45 °C meat-peptonic agar (MPA) nutrient medium was used. MPA was poured in monotonic layer in a Petri plates. After nutrient medium solidification and cooling one drop of broth with daily test cultures prepared according turbidity standard number 10 was inoculated. Inocula were incubated for 18 – 24 hours at temperature 37 °C. After incubation living cells of microorganisms were being treated with chloroform fumes for 30 min. After chloroform treatment, nutrient medium with 0.2 ml of 1 billion daily agar suspension of *Micrococcus luteus* culture were poured on macrocolonies. The results of the experiment were counted after the day of incubation at temperature 37 °C. Growth inhibition zones (GIZ) of *M. luteus* indicator culture were observed around lyzosyme-active cultures. Quantitative evaluation of LA was detected at maximum concentration of lyzosyme in the plates with inhibited indicator culture.

Results and discussions

Lyzosyme is an enzyme that belongs to a class of hydrolases. It selectively hydrolyzes glycosidic bonds in murein – complex biopolymer, which is the part of the bacterial cell wall [8].

As a result of conducted experiments, it’s found that the probiotic strain *B. subtilis* 39 has a quite expressed lyzosymic activity (GIZ = 15 ± 1 mm) in contrast to *B. subtilis* 51 (GIZ = 1 ± 0,3 mm) (Fig. 1). With prebiotic lactulose addition into the cultural medium lyzosymic activity significantly increases, at the same time enzymatic activity depends on the concentration of lactulose in the medium.

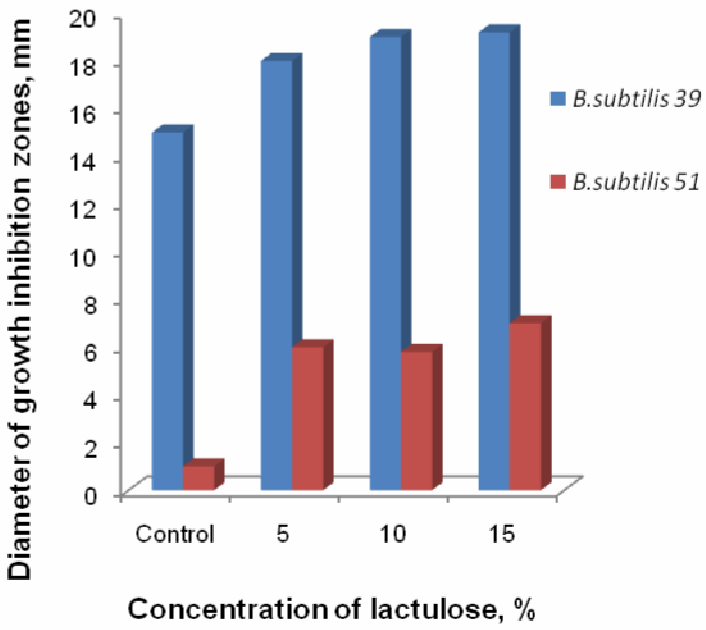

Fig.1. Lactulose effect on bacilli probiotic strains lyzosymic activity

Lyzosymic activity increasing was also observed with *P. aeruginosa* 4141. The initial GIZ diameter of test-culture *M. luteus* 5 ± 1,2 mm, lactulose addition into the cultural medium changes the enzymatic activity that depends on prebiotic concentration in the medium (GIZ = 8 ± 1,5 mm).

CONCLUSIONS

Provided investigations clearly show that lactulose adding into the nutrient medium results in a increased lyzosyme biosynthesis by strains *B. subtilis* 39 and *B. subtilis* 51. Prebiotic addition significantly elevates antagonistic activity of bacilli against pathogens, amplifying the useful qualities of probiotic preparation. Thus, lactulose can be successfully applied together with both *B. subtilis* strains in a new combined synbiotic product. Also this study gives essential impulse for the further investigations of several probiotic bacteria strains combination with different nature prebiotics for finding the most efficient and economically profitable one that may significantly amplify the useful qualities of chosen probiotic bacteria strains.

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ВПЛИВ ПРЕБІОТИКІВ НА ЛІЗОЦИМНУ АКТИВНІСТЬ БАКТЕРІЙ РОДУ BACILLUS

М. А. Савека, О. А. Васильченко

Національний авіаційний університет, м. Київ

Досліджено позитивний вплив на здоров'я організму-господаря пробіотичних штамів спороутворюючих бактерій роду Bacillus *у поєднанні з пребіотиком лактулозою. Проаналізовано вплив лактулози на лізоцимну активність пробіотичних штамів* Bacillus subtilis 39 *та* Bacillus subtilis 51. *Показано залежність збільшення діаметру зон пригнічення росту патогенних та умовно-патогенних мікроорганізмів від кількості лактулози, внесеної у поживне середовище.*

Ключові слова: *пробіотики, пребіотики, синбіотики, лізоцимна активність, лактулоза,* Bacillus subtilis, Micrococcus luteus, *зони пригнічення росту.*

ВЛИЯНИЕ ПРЕБИОТИКОВ НА ЛИЗОЦИМНУЮ АКТИВНОСТЬ БАКТЕРИЙ РОДА BACILLUS

М.А. Савека, О.А. Васильченко

Национальный авиационный университет, г. Киев

Исследовано положительное влияние на здоровье организма-хозяина пробиотических штаммов спорообразующих бактерий рода Bacillus *в комбинации с пребиотиком лактулозой. Проанализировано влияние лактулозы на лизоцимную активность пробиотических штаммов* Bacillus subtilis 39 *и* Bacillus subtilis 51. *Показана зависимость увеличения диаметра зон*

угнетенного роста патогенных и условно-патогенных микроорганизмов от количества лактулозы, внесённой в питательную среду.

Ключевые слова: *пробиотики, пребиотики, синбиотики, лизоцимная активность, лактулоза,* Bacillus subtilis, Micrococcus luteus, *зоны угнетенного роста.*