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BIOSAFETY ASSESSMENT OF LACTOBACILLUS PARACASEI – 010K STRAIN

Annotation: *The article evaluates the biosafety of microorganisms based on the study of the pathogenicity and nature of side effects of a new probiotic strain Lactobacillus paracasei – 010K isolated from koumiss. The paper presents data on the determination of potentially pathogenic signs in vitro, virulence, allergenic and irritant sensitizing effects and the study of toxicological effects. According to the existing classification of strains (Maximum permissible concentrations (MPC) of producing microorganisms, bacterial preparations and their components in the air of the working area Hygienic standards GN 2.2.6.709-98), the new strain of Bifidobacterium crudilactis 7-1C belongs to the 4th hazard class. It can be used to develop biological products in order to increase the vegetation of fish, prevent and treat the aura of fish, improve the microflora of the gastrointestinal tract of the stomach.*

Key words: *virulence, biosafety, sensitization, toxicity, allergenic risk, pathogenicity.*

Introduction

The use of modern biological knowledge, the development of Biotechnology opens up wide opportunities for solving the problems of the agricultural sector, veterinary medicine, food industry, healthcare, pharmacology, and Environmental Protection. Thanks to scientific achievements in the field of microbiology and the introduction of new developments in genetic engineering, it became possible to obtain high-tech strains of highly productive microorganisms [1-7].

In recent years, the biotechnology of probiotics, drugs used for the correction and prevention of microecological disorders in the gastrointestinal tract of humans and animals, has been intensively developing [8]. The effectiveness of probiotic drugs is determined by the combination of biological properties of the strains that make up the drug. Production bacteria must have a set of characteristics that allow them to compete with pathogenic and conditionally pathogenic microorganisms. These include: antagonistic activity, the ability to adhere and colonize the intestinal mucosa, acid formation activity, a certain level of resistance to hydrochloric acid and bile [9-11].

Another stage of introduction into the production of drugs based on cultures of microorganisms or their derivatives is the assessment of their biosafety – the study of pathogenicity, virulence, allergenicity [12-14]. Because of the growing nomenclature of strains used in biotechnology, the issue of the safety of microorganisms utilized in this business is relevant. The idea of biosafety aims to prevent biological agents-including microbes used in biotechnology-from becoming a threat.

The introduction of the requirements to create a contemporary regulatory framework for industrial strain certification and standardization, guarantee control over their use in production, and create a conceptually agreed-upon implementation mechanism with global experience in this area, the introduction of biosafety levels for industrial strains has become a more widespread issue.

The problem of the safety of microorganisms used in the biotechnology industry is relevant as a result of the expansion of the range of strains used. In a broad sense, biosafety includes the prevention of the potential danger of a biological agent, including microorganisms used in biotechnology [15].

A system of classifying microorganisms by Hazard Level has been developed by the National Institute of Health (USA), the European Federation of Biotechnology, and the Organization of Economic Cooperation & Development. This system is based on the taxonomic status of strains and

their potential pathogenic properties. This classification separates microorganisms employed in biotechnology into four classes: low-risk, medium-risk, high-risk, and harmless.

Based on the study of the nature of pathogenicity and adverse effects of probiotic strains, an assessment of the safety of the microorganism was carried out. 1 (Lactobacillus paracasei - 010K) strain, which showed activity in terms of probiotic properties, was identified as irritating in vitro in terms of potential–pathogenic symptoms, virulence, allergenic and sensitive effects. The aim of the work is to study the biosafety of the promising strain Lactobacillus paracasei - 010K, to study the harmful properties of the strain, the degree of toxicity (risk class) and sensitizing Acti ity in model experiments.

Research methods

A sample of koumiss was obtained on a farm in the Almaty region (Kazakhstan) from healthy horse meat by hand milking in sterile screw bottles and stored in cool boxes before being sent to a microbiological laboratory.

Wilkins-Chalgren agar (Oxoid, UK) with the addition of soy peptone (5 g /l, Oxoid), L-cysteine-HCl (0.5 g/ l, Sigma-Aldrich), Twin 80 (1 ml / l, Sigma-Aldrich) was used to isolate lactic acid bacteria. The selective agents mupirocin (100 mg/l, Oxoid) and glacial acetic acid (1 ml/l; Sigma-Aldrich) were part of the medium. A freshly harvested sample of koumiss was serially diluted in an anaerobic Wilkins-Chalgren broth (Oxoid, UK) containing soy peptone, L-cysteine-HCl and Twin-80, and then incubated in a selective Wilkins-Chalgren agar under anaerobic conditions (CO_2 / H_2: 90). / 10) in anaerobic vessels (Oxoid) for 72 hours at 37°C. Bacterial colonies were collected and transferred to test tubes containing anaerobic Wilkins-Chalgren broth. The isolates were cultured for 24 hours at 37°C.

Genomic DNA was separated from daily bacterial cultures using the PureLink Genomic DNA Kit DNA isolation kit in accordance with the manufacturer's protocol (Invitrogen, Carlsbad, USA). The DNA concentration in the samples was determined using the qubit ® 2.0 fluorimeter qubittm dsdna HS Assay Kit (Life Technologies, Oregon, USA) kit.

With a single intravenous administration of large doses of microorganisms, the study of the potential pathogenic properties of a new biological strain was conducted in accordance with integral indicators characterizing the relationship between the macro and the microorganism: virulence, blood penetration rate ("threshold" dose) and distribution to internal organs (kidneys, liver, spleen), toxicity of EXO and endotoxins [1]. Using integral indicators that describe the relationship between macro and microorganisms with a single intravenous administration of large doses of microorganisms, such as virulence, blood penetration rate ("threshold" dose) and distribution to internal organs (kidneys, liver, spleen), EXO, and endotoxin toxicity, the study of potential pathogenic properties of new biotechnological strains was conducted [16].

Biosafety assessment of the Lactobacillus paracasei – 010K strain was studied in laboratory animals: nonlinear white mice, guinea pigs and rabbits.

The support of creatures was in agreement with the sterile rules for the development, gear and upkeep of exploratory and natural clinics (vivariums), the bolstering of creatures was carried out with common and briquetted bolster in understanding with the standards. The creatures experienced isolate and acclimatization in vivarium conditions for 14 days. Exploratory bunches of creatures were shaped by the strategy of irregular determination, taking into consideration body weight as a deciding marker. When conducting experiments, the general condition of animals, feed and water consumption were monitored daily; once a week, body weight was determined. Monitoring the condition of laboratory animals was carried out every morning. Decide the number of patients and the timing of ailment or passing of creatures, clinical signs (changes in behavior, appearance, nourishment and water utilization, responses to outside boosts, respiratory rate, color of the ears and appendages, the nearness of mirror withdrawals and tremors of the appendages, the improvement of drugs and comatose conditions, etc.).

Determination of the pathogenicity of strains was carried out in the laboratory of «scientific and production center of Microbiology and virology» LLP.

In vitro study of potential pathogenic traits of strains: in vitro tests using a dense nutrient medium supplemented with blood (blood agar) and egg yolk (yellow Agar) revealed that the strains lacked hemolytic and lecithinase activity.

Research results

S*tudying the virulence of the strain (L D 50):* Testing for acute toxicity of the Lactobacillus paracasei - 010K strain was carried out using the generally accepted method /Birger M.O.,1982/ on 8 groups of animals (5 white mice each, 3 females and 2 males, weighing 16-18 g) at concentrations from 10^3 to 10^{11} CFU/cm^3 (Table 1). The use of concentrations from 10^3 to 10^{11} CFU/ml did not lead to any noticeable physiological or behavioral changes.

Table 1 – results of the study of acute toxicity of the strain

№	Number of animals in practice	Input method	CFU/ ML amount	Sick animals	Animal yield	The surviving animals
1	5	Abdominal cavity	10^3	0	0	5
2	5	Abdominal cavity	10^5	0	0	5
3	5	Abdominal cavity	10^7	0	0	5
4	5	Abdominal cavity	10^9	0	0	5
Control	5	Abdominal cavity	Saline solution	0	0	5
5	5	Through the mouth	10^5	0	0	5
6	5	Through the mouth	10^7	0	0	5
7	5	Through the mouth	10^9	0	0	5
8	5	Through the mouth	10^{11}	0	0	5
Control	5	Through the mouth	Saline solution	0	0	5

The results of the experiment showed that neither intrauterine nor oral (by mouth) administration of the studied doses of the strain led to the death of the experimental participants. They were all healthy and active.

Morphological changes in internal organs: autopsy results of animals: in live form, they are dark red, with a smooth surface. The “drawings” of the brain and shells are clearly visible. The structure is light, simple in place, the surfaces are smooth, easy to separate from each other, and the materials to collect are invisible.

Ability to disseminate internal organs: Dissemination of internal organs takes place only during the first 24 hours after the introduction of culture.

Local irritant effect: When the studied growth was injected into the conjunctiva of the rabbit eye at a dose of 1*10^9 CFU/cm^3, a weak positive reaction was observed in the form of an injection of scleral and corneal vessels and mucosal secretions in thecornerof theeye.Onthe second day of observation,the above phenomeno ncompletely disappearedin all animals, and nodeviationfrom the physiological standard was observed for the next 5 days. Thus, the weak damage causedby the strainsinthisstudyhasa localirritant effect. Allergenic effect by sensitization: moderate allergen doses were established using guinea pigs,and 10^3, 10^5, 10^7 were in troduced per animal dose of CFU/ ML. Saline was used as control. Responses were recorded after 24 hours and with in 5 days. Results were scoredon the following scale:

0 – no visible reaction;

1 – pale pink erythema in the entire region or on its edge;

2 – bright pink erythema in the entire region or on its edge;

3 – whole erythema;

4 – infiltration and swelling of the skin in the presence or absence of erythema (thickening of the skin fold);

5 – erythema, pronounced infiltration, focal ulcers (necrosis), hemorrhages, crusts may appear.

In our experience – 0 points, no reaction. Thus, it was recognized that the studied strain practically does not have an allergic effect.

Allergenic effect on sensitizing effect: The establishment of an average allergenic dose was carried out on guinea pigs, which were injected with the studied culture at doses of 10^3,10^4,10^5,10^6 CFU/per animal. The control was a saline solution. The reaction was recorded after 10 days according to the diameter of the erythema. The average allergenic dose of the studied culture was 9.5*10^5 CFU per animal. Thus, this strain has practically no allergenic effect.

According to the classification of the strain (Maximum Permissible Concentration (MPC)GN2.2.6.709-98 Sanitary Standards for Microbial Producers, Bacterial Preparations and their Components in the Air in Work Areas), this active strain was classified as Hazard Class 4. To determine the tokicological indicators of the *Lactobacillus paracasei – 010K* strain, we studied the effect on the mass indicators of ten white mice. The results of the study obtained are presented in Table 2. According to the results of the research work, the toxicity of all used concentrations of the *Lactobacillus paracasei – 010K* strain was not established. At the end of the study, the mice were all alive and no physiological abnormalities were observed in their bodies. During the experiment, the difference in live weight of mice was on average 0.2 g.

Table 2 – Effect of Lactobacillus paracasei – 010K strain on weight indicators in mice

Groups	Average live weight, G		Absolute growth, G	Relative growth, %
	Primary	Last		
Control	2,2±0,6	28,3±0,8	6,1±0,2	0,43±0,01
1 Experimental group	22,1±0,7	28,4±0,7	6,3±0,1	0,45±0,01
2 Experimental group	22,3±0,6	29,2±0,5*	6,9±0,2*	0,49±0,01*
3 Experimental group	22,1±0,6	29,4±0,4*	7,3±0,2*	0,52±0,01*
Note: * – P≥0,05.				

According to the results, in the control group, the average live weight of animals increased from 22,2±0,6 g to 28,3±0,8 g, the absolute increase was 6.1±0.2 g. In 1 experimental group, the average live weight reached 28,4±0,7 g from 22,1±0,7 g, and the absolute increase was 6,3±0,1 g. In 2 experimental groups, the initial mean live weight was 22,3±0,6 g, the final indicator increased to 29,2±0,5 g, the absolute increase was 6,9±0,2 g, which is statistically significant (p≥0,05). In the 3 experimental groups, the average live weight increased from 22,1±0,6 g to 29,4±0,4 g, and the absolute increase was 7.3±0.2 g, which is also statistically significant (P≥0,05). The diagram of the indicators of relative growth of laboratory animals is shown in Figure 1.

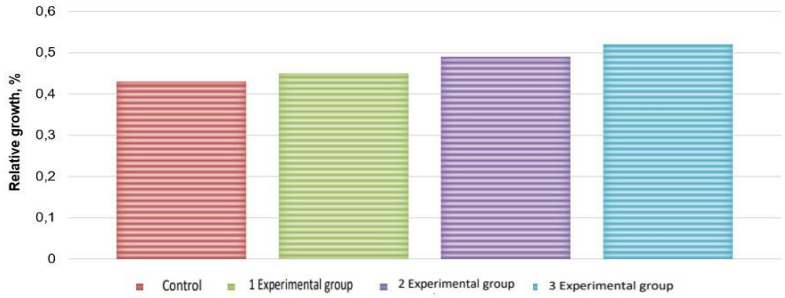

Figure 1 – relative growth indicators of mice, %

The relative growth indicators shown in the figure were 0,43±0,01% in the control group, 0,45±0,01% in 1 experimental group, 0,49±0,01% in 2 experimental groups and 0,52±0,01% in 3 experimental groups. The increase in experimental groups is higher than in the control group, which indicates the effectiveness of experimental conditions. Similar results were obtained in the studies of N.A. Tabakov. In his work, when feeding laboratory mice with a feed additive with the addition of mineral elements, the weight of the experimental groups of mice increased significantly compared to the control group, and the feed additive did not have a negative effect on the physiological state of the mice [17].

Discussion

The problem of microbial safety in the biotechnology industry is relevant due to the expansion of the range of strains used.Biosafety includes the prevention of the potential danger of a biological agent, including microorganisms used in biotechnology.At the present stage of development and improvement of biosafety criteria for biotechnological strains, it is clearly shown that a mandatory and necessary condition for the admission of producer strains to industrial use is their avirulence, low invasiveness, the absence of toxigenic properties and the ability to disseminate in internal organs and the ability to disseminate inIf the strain qualifies as non-pathogenic, it can be used in the biotechnology industry. Strains of all types of microorganisms proposed for the production of probiotics should be non-virulent, non-toxic, safe for humans, including, if necessary, immunological safety.

Conclusion

The objective of this study was to evaluate the safety of oral administration of Lactobacillus paracasei – 010K. Ingestion of this bacteria did not produce significant toxic effects in both male and female mice.The animals appeared healthy and behaved normally throughout th estudy period. An indicator of the overall health of the animals in the toxicity study was the changein body weight. A significant loss of body weight in the animals could be due to some adverse effect, such asanorexia, diarrhea, or dehydration. Thus, one of the reasons why the bacterium was not toxic to mice was the normal change in body weight of the experimental animals during the study period.

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ОЦЕНКА БИОБЕЗОПАСНОСТИ ШТАММА LACTOBACILLUS PARACASEI 010K

В статье дана оценка биобезопасности микроорганизмов на основе изучения патогенности и характера побочных эффектов нового пробиотического штамма Lactobacillus paracasei – 010К, выделенного из кумыса. В статье представлены данные по определению потенциально патогенных признаков in vitro, вирулентности, аллергенному и раздражающему сенсибилизирующему воздействию, а также изучению токсикологических эффектов. Согласно существующей классификации штаммов (Предельно допустимые концентрации (ПДК) микроорганизмов-продуцентов, бактериальных препаратов и их компонентов в воздухе рабочей зоны по гигиеническим нормативам ГН 2.2.6.709-98), новый штамм Bifidobacterium crudilactis 7-1С относится к 4-му классу опасности. Он может быть использован для разработки биопрепаратов с целью увеличения вегетативной активности рыбы, профилактики и лечения ауры рыбы, улучшения микрофлоры желудочно-кишечного тракта желудка.

Ключевые слова: *вирулентность, биобезопасность, сенсибилизация, токсичность, аллергенный риск, патогенность.*

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LACTOBACILLUS PARACASEI – 010К ШТАМЫНЫҢ БИОҚАУІПСІЗДІГІН ЗЕРТТЕУ

Мақалада қымыздан оқшауланған Lactobacillus paracasei – 010К жаңа пробиотикалық штаммының патогенділігі мен жанама әсерлерінің сипатын зерттеу негізінде микроорганизмдердің биоқауіпсіздігіне баға берілген. Мақалада ықтимал патогендік белгілерді in vitro, вируленттілікті, аллергенді және тітіркендіргіш сенсибилизациялық әсерді анықтау, сондай-ақ токсикологиялық әсерлерді зерттеу туралы мәліметтер келтірілген. Штаммдардың қолданыстағы жіктемесіне сәйкес (Гн 2.2.6.709-98 гигиеналық нормативтері бойынша жұмыс аймағының ауасындағы микроорганизмдер-өндірушілердің, бактериялық препараттардың және олардың компоненттерінің шекті рұқсат етілген концентрациясы (ШРК), Bifidobacterium crudilactis 7-1С жаңа штаммы қауіптіліктің 4-ші класына жатады. Оны балықтың вегетативті белсенділігін арттыру, балық аурасының алдын алу және емдеу, асқазан-ішек жолдарының микрофлорасын жақсарту мақсатында биологиялық препараттарды әзірлеу үшін пайдалануға болады.

Түйін сөздер: *сүт қышқылды бактериялар, вируленттілік, биоқауіпсіздік, уыттылық, аллергендік қауіп, патогенділік.*

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АСТЫҚ ӨҢДЕУ ЖӘНЕ МАЙ ДАҚЫЛДАРЫН ҚАЙТА ӨҢДЕУДІҢ ЕКІНШІЛІК ШИКІЗАТТАРЫН ПАЙДАЛАНЫП МАҚСАРЫ МАЙЫН ТАЗАРТУ ТИІМДІЛІГІН АРТТЫРУ

Аңдатпа: *Мақалада астық өңдеу және май дақылдарын қайта өңдеудің екіншілік шикізаттарын пайдаланып мақсары майын тазарту тиімділігін арттыру мақсаттын іске асыру үшін орындалған міндеттер негізінде әдеби көздерге сараптамалық сыни шолу жүргізіп, мақсары майын тиімді тазарту технологиясының нысанын мен мақсаты айқындалды. Екіншілік шикізаттарын сүзу материалдары ретінде қолдану жолдары талданып, оңтайлы сүзу материалы ретінде зығыр талшығы ұсынылады. Қайта өңдеудің екіншілік шикізатынан тұратын сүзгі материалын өндірістік тұрғыда жабдықта қолдану үшін көп факторлы эксперименттік зерттеулер жүргізілді. Қайта өңдеудің екіншілік шикізатынан тұратын сүзу материалдарымен тазарту кезінде мақсары майының шығымының шамасы сорғы қысымына байланысты өскендігін көрдік. Сорғы қысымының* $180 \cdot 10^{-3}$ *Па әсерінде майдың шығымы төмендеп, сүзу материалдарының өткізу қабілетінің төмендегенімен байланыстыра аламыз және сүзу жылдамдығының артуына байланысты мақсары майының шығымының да артқанын көре аламыз. Зығыр талшығы арқылы мақсары майын рамалы сүзгіде 0,1 л/с жылдамдықта сүзу нәтижесінде мақсары майының шығымы 93%-ға дейін артты. Мақсары майының май қышқылды, бейорганикалық құрамы мен дәрумендер мөлшерін зерттеу нәтижесінде, құрамындағы қаныққан май қышқылдарының мөлшері төмендеп, моно және полиқанықпаған қышқылдардың (олеин, беген, цис-10-гептадецен мен цис-линол қышқылдары) мөлшері артқанын көреміз, сонымен қатар бейорганикалық құрамы барлық нормаларға сай, ал Е дәрумендерінің мөлшерінің жоғарлағанын байқаймыз.*

Зерттеу жұмысында өсімдік майына тазартуға арналған ФПР (фильтр пресс рамный) маркалы рамалы қатарлы сүзгі-престі пайдаланылады. Нәтижесінде қайта өңдеудің екіншілік шикізатынан тұратын сүзгі материалдарын рамалы қатарлы сүзгі-