Antoni MIECZNIKOWSKI, Katarzyna PIASECKA-JÓŹWIAK, Beata CHABŁOWSKA
Institute of Agricultural and Food Biotechnology
Instytut Biotechnologii Przemysłu Rolno-Spożywczego
ul. Rakowiecka 36, 02-532 Warszawa, Poland
e-mail: antoni.miecznikowski@ibprs.pl; katarzyna.piasecka@ibprs.pl; beata.chablowska@ibprs.pl

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SELECTION OF THE STRAINS FROM *Leuconostoc* GENUS IN RESPECT OF BIOTECHNOLOGICAL PROPERTIES, ENABLING THEIR APPLICATION IN THE PROCESS OF ENSILING THE EDIBLE MUSHROOM *Agaricus bisporus*

Summary

The application value of the preparation, tested in the food industry is connected not only with therein contained strains of lactic acid bacteria but also with technological properties, determining the possibilities of obtaining the preparation in commercial form. The aim of the studies was to perform a preliminary evaluation of panel of 15 strains of lactic acid bacteria from Leuconostoc genus, being found in the collection of the Institute (IBPRS) in aspect of the possibility of their application in commercial starters for ensiling the common mushrooms A. bisporus. The conducted studies included cultures of bacteria in the laboratory conditions at temperature of 15°C and A. bisporus in MRS medium, separation of biomass from culture medium and preservation of the obtained biomass by lyophilisation method. The quantity of the obtained biomass and losses during separation process were specified, the concentration of lactic acid and acetic acid in supernatant was determined as well as survivability of bacteria during dehydration was recorded. The obtained results allowed performing the preliminary selection of the tested bacteria in aspect of their suitability for technological processes, connected with the ensiling of the mentioned mushrooms. Three LAB strains were eliminated from further studies as being technologically unsuitable whereas the remaining strains were qualified to the experiments which will be oriented to evaluation of organoleptic properties of the products, being ensiled with their participation.
***Key words**: ensiling of edible mushrooms A. bisporus , technological evaluation of parameters of LAB strains, bacterial preparations*

SELEKCJA SZCZEPÓW Z RODZAJU *Leuconostoc* W ZALEŻNOŚCI OD WŁAŚCIWOŚCI BIOTECHNOLOGICZNYCH UMOŻLIWIAJĄCYCH ICH STOSOWANIE W PROCESIE ZAKISZANIA PIECZARKI DWUZARODNIKOWEJ (*Agaricus bisporus)*

Streszczenie

Wartość zastosowanego preparatu testowanego w przemyśle spożywczym wynika nie tylko z zawartych w nim szczepów bakterii kwasu mlekowego lecz również z jego właściwości technologicznych, określających możliwości uzyskania produktu w formie handlowej. Celem badań było przeprowadzenie wstępnej oceny panelu 15 szczepów bakterii kwasu mlekowego z rodzaju Leuconostoc, będących w kolekcji Instytutu (IBPRS) w aspekcie możliwości ich zastosowania w komercyjnych starterach do zakiszania pieczarki A. bisporus. Przeprowadzone badania objęły oddzielenie biomasy z kultury medium oraz konserwację otrzymanej biomasy metodą liofilizacji z wykorzystaniem kultury bakterii w warunkach laboratoryjnych przy temperaturze 15°C z A. bisporus jako medium MRS. Określono: ilość uzyskanej biomasy, straty powstałe w trakcie procesu separacji, koncentracja kwasu mlekowego i octowego w supernatancie, przeżywalność bakterii w czasie dehydratacji. Uzyskane wyniki pozwoliły na wykonanie wstępnej selekcji badanych bakterii w aspekcie ich użyteczności w procesach technologicznych związanych z zakiszaniem wspomnianych grzybów. Trzy szczepy LAB zostały wyeliminowane z dalszych prac jako technologicznie nieodpowiednie, a pozostałe szczepy zakwalifikowano do eksperymentu nakierowanego na ocenę własności organoleptycznych produktów zakiszonych z ich udziałem.
***Słowa kluczowe**: zakiszanie grzybów jadalnych A. bisporus, technologiczna ocena parametrów szczepów LAB, preparaty bakteryjne*

1. Introduction

Edible mushrooms are a valuable diet component due to the content of many precious nutritive constituents such as fibre, mineral salts, vitamins and also, due to their attractive taste and flavour. From among breeding mushrooms, common mushrooms *Agaricus bisporus* deserve our attention; they may be treated as functional food due to their exceptional health-promoting properties. Apart from valuable protein (they contain almost all amino acids), well assimilable carbohydrates and fats (including beneficial polyunsaturated fatty acids), the mentioned mushrooms are a good source of potassium, copper and selenium; they contain iodine, fibre, vitamins A, E, vitamins from B group (including folic acid) and even vitamin D. In mushrooms *A. bisporus*, the presence of ß-glucan – polysaccharide, classified into soluble fraction of dietary fibre was found, as well [1].

The mushrooms *A. bisporus*, similarly as all edible mushrooms, are a quickly deteriorating food, and in connection with this fact, their storage period (shelf-life) in a fresh form is relatively short; their storage requires observing the specified technological regime. Directly after harvesting, to inhibit the breathing processes and also, to preserve the fleshiness of tissue and retain white colour, it is necessary to chill down the discussed mushrooms down to the temperature of 2-3°C, and the optimum conditions of

their storage are: temperature of 0 – 1°C and relative humidity of 90-95%. Under such conditions, the mushrooms may be stored for the period of 7 – 14 days [2]. The edible mushrooms are troublesome raw material for processing as they contain a high amount of water and are characterized by a high activity of enzymes, including oxidases which cause darkening of fruiting body during their processing and during the storage of final products [3].

The popular method of preserving these mushrooms consists in their pickling, that is the process of preserving food products, using, inter alia, vinegar, wine and oil. The process of pickling *A. bisporus* has been known for years and its technology has been developed in details; it is employed now on the industrial scale in many variants. Another method for preservation of fresh mushrooms, but being less popular and rather not used in the industrial scale, consists in ensiling, i.e. biological method of food conservation, based on the process of lactic acid fermentation, utilizing the sugars which occur in the raw material (or added) what lowers their energetic value. Lactic fermentation is considered as valuable method, being simple from biological viewpoint, the method which enables maintenance and/or improvement of safety, nutritive and sensory values and shelf-life of vegetables and fruits [4, 5, 6, 7]. Combining the mentioned method of biological preservation and the present biotechnological tools facilitates running the controlled fermentation processes [8].

Microbiota responsible for spontaneous fermentation of raw vegetables and fruits deserve a great interest as a potential instrument of improving the microbiological safety of fermented food [9, 10]. The capability of microorganisms to bio-preservation results mainly from synthesis of a wide range of primary and secondary metabolites, being antagonistic towards undesired bacteria. They include, inter alia, organic acids, carbon dioxide, ethanol, hydrogen peroxide, diacetyl, fungicidal compounds (e.g. fatty acids, phenyl acid and its derivates), bacteriocins and antibiotics [9]. Not all strains of lactic acid bacteria, as naturally present in vegetables and fruits, are capable to guarantee the same effectiveness during processing of the mentioned raw materials. Besides it, the "technological" properties of the strains which would allow their application in production are significant. Therefore, running the studies on the selection of the appropriate microorganisms for processing is indispensable. For instance, the capability of LAB strains to synthesize exopolysaccharides (EPS) is the important trait in shaping viscosity as well as nutritive properties of certain products [11]. Such property is characteristic of bacterial strains from *Leuconostoc mesenteroides*, which additionally acidify the environment mildly because their development is quickly inhibited by increasing concentration of lactic acid [12], giving by this the possibility of developing to the strains from natural microbiota of the raw material in further stages of fermentation. The discussed features of bacteria from *Leuconostoc mesenteroides* genus have inclined the authors of the present work to conduct the studies on ensiling *A. bisporus* mushrooms with the participation of the mentioned bacterial strains.

2. Methodology

During the conducted studies, a panel of fifteen LAB strains from *Leuconostoc* genus, coming mainly from the collection of the Department of Fermentation Technology of IBPRS (Institute of Agricultural and Food Biotechnology) was tested and presented in Table 1.

LAB strains were cultured in MRS medium, containing ca. 2.3% saccharose. The mushrooms A. *bisporus* contain about 0.4 g of sugar /100 g; so, the addition of sugar is necessary for initiation of lactic fermentation. The real ratio of weight of marinate to the weight of the mushrooms is ca. 30:70; hence, if 1% of sugar had fallen on the raw material in relation to its weight, the solution should contain ca. 2.3%. The cultures were carried on for 24 h, at temperature of 25°C. In the cultures, after their termination, the number of lactic acid bacteria was determined.

The number of lactic acid bacteria (LAB) was determined by the plate method on MRS medium according to standard PN-EN 15787:2009 [13].

Table1. Bacteria strains used in the studies
Tab. 1. Szczepy bakterii zastosowane w badaniach

No	Symbol and name of strain	Origin of strain
1.	*Leuconostoc citreum* (Ł 06)	Baker's rye starter
2.	*Leuconostoc lactis* (Ł 07)	Baker's rye starter
3.	*Leuconostoc sp.* 1	Baker's rye starter
4.	*Leuconostoc sp.* 2	Baker's rye starter
5.	*Leuconostoc citreum* C750(3)	Baker's rye starter
6.	*Leuconostoc mesenteroides/dex* 750G	Baker's rye starter
7.	*Leuconostoc mesenteroides* sz 1.2-7	Baker's rye starter
8.	*Leuconostoc citreum* sz 2.1-3	Baker's rye starter
9.	*Leuconostoc citreum* sz 2.1-7	Baker's rye starter
10.	*Leuconostoc mesenteroides* sz 2.1-6	Baker's rye starter
11.	*Leuconostoc mesenteroides* C	Cider
12.	*Leuconostoc mesenteroides* ZO	Fermented mushrooms *A. bisporus*
13.	*Leuconostoc mesenteroides* G4C	Baker's buckwheat starter
14.	*Leuconostoc mesenteroides* JIIF	Fermented apples
15.	*Leuconostoc mesenteroides* PIN	Fermented paprika

Source: own work / Źródło: opracowanie własne

The liquid remaining after termination of cultivation was separated in two portions. The first one, of volume 50 ml was separated at two rates of rotation: 5500 and 9000 rpm in order to determine the quantity of the obtained bacterial biomass. Then, the supernatant was collected and the remaining sediment was dried by convection method at ambient temperature for 12h. The remaining liquid was separated at 9000 rpm and the obtained biomass was lyophilized. The lyophilisation was carried out using laboratory lyophilising device Christ Alpha1-4 in which it is possible to conduct all three stages of sublimation drying: freezing, the main drying and additional drying; the temperature of the product, temperature of ice condenser and value of pressure may be controlled on any stage of the process. The final humidity of the preparations is 2-3%. As protective agents, glycerol and skimmed milk powder were used.

The survivability of bacteria during the lyophilisation process was expressed in % as ratio of bacterial count in lyophilisate and bacterial number in the amount of biomass from which a given lyophilisate was obtained.
In supernatant, the levels of lactic acid and acetic acid were determined in order to pre-determine the suitability of a given bacterial strain for ensiling of the examined mushrooms *A. bisporus.* The determination of lactic and acetic

acid content was carried out with the application of High Performance Liquid Chromatography (HPLC) with UV detection.

3. The results of the studies

In effect of LAB cultivation in MRS medium, the capability of bacteria to grow at temperature of 25°C was evaluated (temperature of environment at which the mushrooms will be ensiled on the industrial scale) as a model system in which the only one source of sugar was the additive of saccharose instead of glucose (standard MRS).

In Table 2, there were given the results concerning the effectiveness of the process of cultivation and of efficiency of biomass separation, expressed as log of the number of bacteria in the culture and in supernatant. The mentioned table contains also the mean weights of sediments, obtained as a result of culture separation and the data, specifying the synthesis of lactic acid and acetic acid.

The weight of dry sediment (biomass together with EPS) varied within the limits of 0.25-0.115 g·50 ml^{-1} of culture for majority of the strains, only in the case of strain no 10 (*Leuconostoc mesenteroides* sz 2.1-6), no 12 (*Leuconostoc mesenteroides* ZO) and no 15 (*Leuconostoc mesenteroides* PIN) amounted to 0.050; 0.045 and 0.060 g·50 ml^{-1}, respectively what is an evidence of worse capability of growth under the conditions of the conducted studies.

In the case of culture of the most of the strains, log of bacteria count was found within the limits of 9.93-9.38; only in the case of strain no 10 (*Leuconostoc mesenteroides* Sz. 2.1-6), the mentioned log was equal to 8.82 and strain no 15 (*Leuconostoc mesenteroides* PON) did not reveal any capability to grow under the examined conditions. (log of bacteria count was 2). Considerably more differentiated results were in the case of evaluation of the possibilities of obtaining bacteria from the liquid by separation where the range of log of bacterial number was found in the interval of 6.04 – 3.65 and only in the case of culture of strain no 12 (*Leuconostoc mesenteroides* ZO) the mentioned log was equal to 7.00 what is an evidence of worse efficiency of secreting the biomass of this strain even by 4 rows of the size and it is overlapped with the relatively low weight of the obtained sediment. In the case of strains no 10 (*Leuconostoc mesenteroides* Sz 2.1-6) and no 15 (*Leuconostoc mesenteroides* PIN), a low bacterial count in the supernatant (log value amounting to 4.00 and 1.00, respectively) was correlated with the weak capability of growing in the examined conditions – the weight of the obtained biomass was equal to 0.05 and 0.06 g · 50 ml^{-1}.

The highest concentrations of lactic acid were obtained in the case of the strains, marked with numbers 1, 3, 8, 9 and 11 whereas the lowest ones were found in the case of strains 10, 12 and 15. In the case of strains 1, 2, 3, 6, 8, 9, 10, 11, 13 and 14, the was found the synthesising of small quantities of acetic acid – the most quantity was recorded in the case of strain 6-1,1 g·l^{-1} while strains no 4, 5, 7, 12 and 15 did not synthesise acetic acid at all.

To evaluate the suitability of bacterial biomass of the particular strains for obtaining commercial starter cultures, the experiments were carried out, consisting in preservation of bacterial biomass by freeze-drying (sublimation) in the laboratory scale. The results have been given in Table 3. Almost all bacterial strains, as used in the discussed experiment, were characterized by good or very good survivability (near to or considerably above 80%) during dehydration process what – in aspect of this criterion – qualifies them as material which may be used in commercial starter cultures destined for ensiling mushrooms s; only one strain marked with no 15 was distinctly different in respect of survivability as compared to other strains (39%).

The discussed results included technological criteria such as intensity of bacterial growth in the conditions appropriate for ensiling the mushrooms, i.e. at ambient temperature, efficiency (easiness) of separating the biomass from breeding medium, quantity of the synthesized lactic acid and acetic acids, what will affect the rate of ensiling process and the quality of the obtained products and survivability of bacteria during preservation of biomass by dehydration method (what determines the possibility of producing the commercial preparations).

Table 2. Evaluation of effects of culture of the selected strains from *Leuconostoc* genus
Tab. 2. Ocena efektów hodowli wybranych szczepów z rodzaju Leuconostoc

Symbol and name of strain	Weight of sediment, g·50 ml^{-1}	LAB count in culture, log CFU · ml^{-1}	LAB count in supernatant, log CFU · ml^{-1}	Lactic acid, g · l^{-1}	Acetic acid, g · l^{-1}
1. *Leuconostoc citreum* (Ł 06)	0,190	9,89	5,11	10,9	0,2
2. *Leuconostoc lactis* (Ł 07)	0,115	9,62	4,48	4,1	0,9
3. *Leuconostoc* sp. 1	0,135	9,72	3,92	9,4	0,4
4. *Leuconostoc* sp. 2	0,150	9,89	3,65	4,3	-
5. *Leuconostoc citreum* C750(3)	0,200	9,93	5,38	6,6	-
6. *Leuconostoc mesenteroides/dex* 750G	0,250	9,67	6,04	6,3	1,1
7. *Leuconostoc mesenteroides* sz 1.2-7	0,115	9,45	5,38	5	-
8. *Leuconostoc citreum sz* 2.1-3	0,180	9,88	6,00	10,6	0,3
9. *Leuconostoc citreum sz* 2.1-7	0,200	9,83	5,00	9,7	0,2
10. *Leuconostoc mesenteroides sz* 2.1-6	0,050	8,82	4,00	1	0,1
11. *Leuconostoc mesenteroides* C	0,210	9,89	5,04	10,3	0,3
12. *Leuconostoc mesenteroides* ZO	0,045	9,38	7,00	0,4	-
13. *Leuconostoc mesenteroides* G4C	0,200	9,52	3,95	4,2	0,7
14. *Leuconostoc mesenteroides* JIIF	0,195	9,65	5,73	4,6	0,3
15. *Leuconostoc mesenteroides* PIN	0,060	2,00	1,00	2,1	-

Source: own work / Źródło: opracowanie własne

Table 3. Survivability of bacteria during sublimation (freeze-drying)
Tab. 3. Przeżywalność bakterii w procesie suszenia sublimacyjnego

Symbol and name of strain		Survivability, %
1.	*Leuconostoc citreum* (Ł 06)	78
2.	*Leuconostoc lactis* (Ł 07)	93
3.	*Leuconostoc* sp. 1	90
4.	*Leuconostoc* sp. 2	88
5.	*Leuconostoc citreum* C750(3)	90
6.	*Leuconostoc mesenteroides/dex* 750G	93
7.	*Leuconostoc mesenteroides* sz 1.2-7	91
8.	*Leuconostoc citreum* sz 2.1-3	85
9.	*Leuconostoc citreum* sz 2.1-7	77
10.	*Leuconostoc mesenteroides* sz 2.1-6	90
11.	*Leuconostoc mesenteroides* C	92
12.	*Leuconostoc mesenteroides* ZO	82
13.	*Leuconostoc mesenteroides* G4C	90
14.	*Leuconostoc mesenteroides* JIIF	89
15.	*Leuconostoc mesenteroides* PIN	39

Source: own work / Źródło: opracowanie własne

It was found that from among the tested strains, the strains marked with numbers 10, 12 and 15 did not satisfy the criteria necessary for their application in starters for ensiling the discussed mushrooms. The remaining strains require laboratory studies in a wider range and then, trials, covering sensory analysis of the silages, produced with their application; their results will constitute the ultimate criterion, verifying the possibility of applying the selected bacteria in food industry.

4. Conclusions

1. Most of the tested strains was characterized by a good growth on MRS medium at temperature of 25^{o}C, only strain no 10 (*Leuconostoc mesenteroides* Sz. 2.1-6) and strain no 15 (*Leuconostoc mesenteroides* PIN) did not meet the mentioned criterion.
2. In supernatant after separation of bacteria of strain 12 (*Leuconostoc mesenteroides* ZO cha) the highest number of bacteria was remained (on the level of 10^{7}) what is an evidence of a low suitability of the discussed strain for multiplication of biomass in the industrial conditions.
3. The lowest concentration of lactic acid in supernatant was determined in the case of strains: no 10 (*Leuconostoc mesenteroides* Sz.2.1-6), no 12 (*Leuconostoc mesenteroides* ZO) and strain no 15 (*Leuconostoc mesenteroides* PIN) what may indicate their lower suitability as components of starter cultures for ensiling mushrooms A. *bisporus* due to a weaker capability of ensiling the environment.
4. Almost all bacteria, employed in the discussed experiment were characterized by good or very good survivability (near to or considerably above 80%) during the dehydration process, excluding strain with number 15.
5. Determination of the possibility of employing the tested colonies in the commercial starters for ensiling mushrooms *A. bisporus* requires further studies, including sensory analyses of the ensiled products, made with their application.

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