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Short communication

Evaluation of the ability to metabolize 1,2-propanediol by heterofermentative bacteria of the genus *Lactobacillus*

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ABSTRACT

Background: New directions of research on lactic acid bacteria include investigation of metabolic pathways for the synthesis and/or metabolism of 1,2-propanediol, commonly used in the food and chemical industry, medicine, pharmacy and cosmetology as well as agriculture. The objective of this study was to compare the capacity of strains representing three diverse heterofermentative species belonging to the genus *Lactobacillus* to synthesize and/or transform 1,2-PD as well as to suggest new directions of research aimed at commercial use of this metabolite.
Results: The novel strain of *Lactobacillus buchneri* A KKP 2047p, characterized as exhibiting an unusual trait for that species in the form of capacity to metabolize 1,2-PD, grew poorly in a medium containing 1,2-PD as a sole carbon source. The supplementation with glucose facilitated rapid growth of bacteria and use of 1,2-PD for the synthesis of propionic acid. A similar observation was noted for *Lactobacillus reuteri*. On the other hand, *Lactobacillus diolivorans* effectively metabolized 1,2-PD which was the sole carbon source in the medium, and the addition of glucose inhibited the synthesis of propionic acid. The experiments also investigated the effect of cobalamin as a diol dehydratase coenzyme involved in the propionic acid synthesis from 1,2-PD whose addition promoted the yield of the reaction in the case of all tested strains.
Conclusions: All tested isolates showed the ability to effectively metabolize 1,2-PD (in the presence of cobalamin) and its conversion to propionic acid, which reveals that investigated bacteria meet the essential requirements of microorganisms with a potential application.

1. Introduction

1,2-Propanediol (1,2-PD) is a widely-used chemical which has been increasingly utilized in anti-freeze and de-icing fluids, as well as a component of unsaturated polyester resins, liquid non-ionic detergents, and coolants and as an additive in cosmetics, nutritional products, medicines and dyes [1,2]. 1,2-PD is currently produced by chemical means but wide ranges of microorganisms (bacteria and yeast) are currently known to ferment sugars to 1,2-PD. The production of this diol has been reported from both yeast and bacteria: *Saccharomyces cerevisiae*, *Lactobacillus buchneri*, *Clostridium sphenoides*, *Clostridium thermobutyricum*, *Clostridium thermosaccharolyticum*, *Escherichia coli*, *Bacteroides ruminocola*, *Thermoanaerobacterium thermosaccharolyticum*, *Corynebacterium glutamicum*, *Salmonella typhimurium* and *Gluconobacter oxydans* [2,3].

It is seldom remarked that 1,2-PD is also an important component of silages, positively influencing their aerobic stability and quality [4]. This feature determines the significant role of the diol in animal breeding, beside such metabolites as lactic and acetic acids. 1,2-PD is a drug used in cattle ketosis treatment directly in case of animal health disorder or prophylactically added in feed. 1,2-PD can be added to the ensiled plant material as a chemical preservative or it can be detected as one of the metabolites synthesized by some heterofermentative species of lactobacilli [5]. Veiga da Cunha and Foster [6] were the first to report that *Lactobacillus brevis* and *L. buchneri* can produce 1,2-PD by degrading lactic acid, but it was not until Elferink et al. [7] published own research on the 1,2-PD production by the species that this new pathway by lactic acid degradation under anoxic conditions was described.

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Currently, apart from the species of *L. buchneri* and *Lactobacillus parabuchneri*, other heterofermentative species of the genus *Lactobacillus* are known to be capable of synthesis or metabolism of 1,2-PD, including *Lactobacillus diolivorans*, which is a relatively new species isolated and characterized by Krooneman et al. [8] as metabolizing 1,2-PD to 1-propanol and propionic acid, or *Lactobacillus reuteri*, which is capable of metabolizing 1,2-PD to propionaldehyde and propionic acid [8,9,10].

Three bacterial strains of the genus *Lactobacillus* identified on the basis of 16S sequence and named as *L. buchneri* A KKP 2047p, *L. diolivorans* K KKP 2057p and *L. reuteri* M KKP 2048p with confirmed ability to synthesize 1,2-PD and/or metabolize this compound to propionic acid were selected for the tests which are the subject of this work. The primary aim of the study was to compare the ability of *L. buchneri* A KKP 2047p, *L. diolivorans* K KKP 2057p and *L. reuteri* M KKP 2048p to synthesize and transform 1,2-PD in model conditions, as well as to identify new opportunities for their agricultural and industrial use.

2. Materials and methods

2.1. Microorganisms and culture conditions

In the study five bacterial were used signet as A KKP 2047p, KKP 907p, C37, K KKP 2057p and M KKP 2048p. All strains have been isolated from corn silages with exception for M KKP 2048p which originated from wheat sourdough. After experiments described below strains have been deposited at the Collection of Industrial Microorganisms at the Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology in Warsaw (Poland).

MRS medium and three types of modified MRS media in which glucose was completely or partially replaced by 1,2-PD were used in the experiment: MRS, carbon source — glucose (20 g/L); MRS-MOD1, carbon source — 1,2-PD (8 g/L); MRS-MOD2, carbon source — glucose (10 g/L) and 1,2-PD (8 g/L); MRS-MOD3: carbon source — glucose (10 g/L) and 1,2-PD (8 g/L) and vitamin B_{12} (20 μg/L).

200 mL medium was inoculated with 1 mL of 24-h culture on MRS or MRS-MOD medium (inoculum concentration 9.2 log CFU/mL). Stationary cultures in flasks were carried out for 21 d at 30°C under aerobic conditions. The choice of culture time was based on previous studies, when the dynamics of concentration changes of this metabolite changed extensively between the 14th and 21st day of culturing [11].

2.2. Analytical methods

In order to determine the content of 1,2-PD and propionic acid, culture supernatant was separated from the bacterial biomass using a Jouan centrifuge (France) (10,000 rpm, 10 min, 15°C). Samples were purified by solid phase extraction on small SPE-C18 columns (Solid Phase Extraction SPE, Chromabond Macherey-Nagel, Germany). Methanol and water at ratio 1:2 were used for the extraction of 2 mL, flow rate 1 mL/min. The volume of eluate was 4 mL and was subjected to a gas chromatographic analysis using an Agilent Technologies 7890A gas chromatograph (Santa Clara, California, USA) equipped with a flame ionization detector (FID), capillary column with a diameter of 0.53 mm and length of 30 m with DB-FFAP (J&W Scientific Columns, USA). The carrier gas was helium with a flow rate of 85 mL/min, temperature program: 35°C (0.5 min), increase of 20°C/min to 90°C, and increase of 10°C/min to 200°C (0.5 min). The volume of injected sample was 1 μL, injector temperature was 170°C and detector temperature was 220°C. Identification of metabolites was based on peak retention times by comparing with the retention time of standard samples and the quantification was based on surface area of peak in Chemstation Software (Agilent Technologies, USA).

2.3. Genetic identification and differentiation of strains

Taxonomic affiliation of each strain was conducted using comparative analysis of 16S sequence. Genomic DNA was extracted with Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, Missouri, USA) following the manufacturer's instructions and PCR reaction with primer set (forward 5′ TGAGAGTTTGATCCTGGCTCAG 3′ and reverse 5′ GGCTAC CTTGTTACGACTTCAC 3′) flanking 16S gene was done as described by Zawadzka-Skomial et al. [12]. Amplicons were purified, sequenced in 3730 DNA Analyzer (Applied Biosystems, Genomed Sp. z.o.o.) and compared to sequences deposited in GenBank database using BLAST algorithm [13]. Sequences from experimental strains as well as belonging to the closest relatives and type strains were used to generate a phylogenetic tree from evolutionary distances from the multiple alignment by the neighbor-joining method in MEGA software [14,15,16].

2.4. Statistical analysis

Statistical analyses were performed by repeated measurements with one-way ANOVA in STATISTICA 10.0 (Statsoft, Poland), followed by Tukey's multiple comparison test. The Shapiro–Wilk test was used to determine whether the population was normally distributed. The Brown–Forsythe test was used to assess the equality of variances for a variable calculated for groups. P-values of $P < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Genetic identification of Lactobacillus strains and their diversity

LAB strains examined in the present study were identified according to a common procedure involving comparative analysis of 16S gene sequence. Strains KKP 2047p, KKP 907 and C 37 have been classified as *L. buchneri*; strains KKP 2048p and KKP 2057p have been classified as *L. reuteri* and *L. diolivorans* respectively; all with 99% of identities to reference strains deposited under numbers in GenBank database (NCBI) indicated as follows in Fig. 1. RAPD-PCR analysis with M13 primer was used to evaluate the genetic diversity among *L. buchneri* A KKP 2047p and *L. diolivorans* K KKP 2057p which phylogenetically represent the closest strains [8]. Mentioned strains were distinguished from each other on the basis of specific and reproducible RAPD profiles which clearly indicate that each strain is genetically diverse (data not shown).

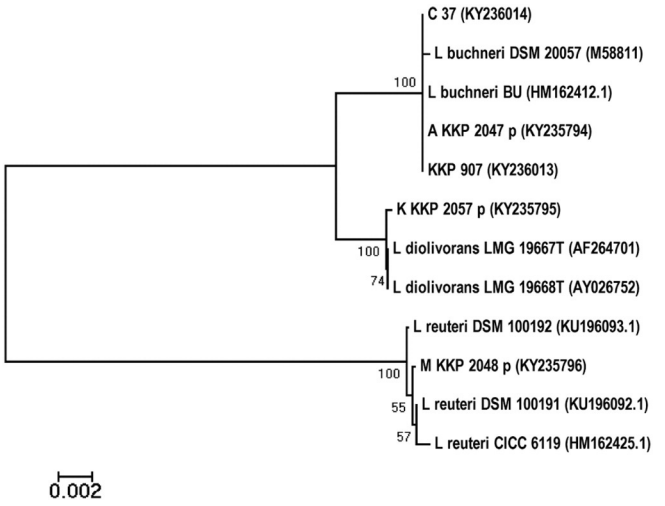

Fig. 1. Evolutionary relationships of 12 taxa. There were a total of 1373 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [16].

3.2. Metabolite profile of heterofermentative lactobacilli in flask cultures

In the first phase of the study, cultures were cultivated in MRS medium where glucose was the sole carbon source. Low levels of propionic acid were obtained as a result of the culture: 114.0 mg/L for the strain of *L. diolivorans*, 66.0 mg/L for the strain of *L. reuteri* and 113.0 mg/L for the strain of *L. buchneri*. The aim of the following stage of the study was to assess the metabolic ability of tested strains in the modified MRS medium. In that phase, the strain *L. diolivorans* K KKP 2057p in the modified MRS medium containing 1,2-PD as the sole carbon source (MOD1-MRS) was able to synthesize the highest concentration of propionic acid (1228.0 mg/L) as compared to other tested *Lactobacillus* strains. In the case where glucose was the carbon source next to 1,2-PD (MRS-MOD2), *L. buchneri* A KKP 2047p was the strain that stood out against other tested species. On the 21st day of culturing, the concentration of propionic acid in the culture solution amounted to 283.0 mg/L. The highest concentrations of propionic acid were obtained in the culture where in addition to 1,2-PD and glucose as the carbon source, the medium was enriched with cobalamin (MRS-MOD3). In this case, the culture of *L. reuteri* M KKP 2048p had the highest concentration of this metabolite (1845.0 mg/L). Research also revealed that an efficient synthesis of propionic acid is dependent on the presence of cobalamin.

Bacteria of the species *L. reuteri* are among the few that can synthesize cobalamin, which is a cofactor of diol dehydratase, an enzyme involved in the reactions of transforming glycerol to 1,3-propanediol and 1,2-PD to propionic acid. It was found that the maximum production of vitamin B_{12} by *L. reuteri* is reached during culture in MRS medium supplemented with glycerol or 1,2-PD [9,17]. In the present study, the strain *L. reuteri* M KKP 2048p showed more effective synthesis of propionic acid in medium supplemented with cobalamin, which meant that its addition was justifiable in the case of this culture.

Furthermore, it was found that only the strain of *L. diolivorans* KKP 2057p had the simultaneous ability to use 1,2-PD as a sole carbon source for the production of biomass [data unpublished] and synthesis of propionic acid. *L. diolivorans* used 70% of the substrate of MRS-MOD1 medium (Table 1). In the same conditions, other tested species of the strains of *L. reuteri* and *L. buchneri* exhibited very weak growth [data unpublished] and reduction in 1,2-PD content to only 3 to 10%. Supplementation of culture medium with glucose resulted in the inhibition of the metabolism of 1,2-PD to propionic acid in the case of this strain. As an easily assimilated source of carbon and energy, glucose was metabolized in the first place, as evidenced by the results of the percentage of 1,2-PD used which was added exogenously to the substrate. On the other hand, in cultures of the other two strains, its utilization rose to 50% (Table 1), enabled growth of bacteria and consequently facilitated an efficient synthesis of propionic acid.

The new species of *L. diolivorans* was isolated, characterized and described for the first time in 2002 by Krooneman et al. [8]. The authors found that in anaerobic conditions, in a medium containing 1,2-PD as a sole carbon source, *L. diolivorans* metabolizes this substrate by hydrolysis catalyzed by cobalamin-dependent diol dehydratase to 1-propanol and propionic acid, similar to bacteria of the family *Enterobacteriaceae* [8,17]. The main difference between the species of *L. buchneri* and *L. diolivarans* lies in the ability to grow in a medium containing 1,2-PD as a sole carbon source. It is worth noting that until 2014, it was considered that strains of the species of *L. buchneri* are characterized by the ability to synthesize 1,2-PD but do not metabolize this compound to propionic acid. The first findings about this feature of the strain of *L. buchneri* A KKP 2047p obtained from the natural environment were presented by Zielińska et al. [11], a finding which was also confirmed in the present study.

On the basis of experiments, it can be concluded that all tested strains demonstrate potential in the context of synthesis of propionic acid using 1,2-PD as a carbon source. 1,2-PD, whose presence in fermented feeds is highly desirable, may provide valuable information for the development of composition of bacterial preparations' starter cultures designed to preserve food and feeds and having a stimulating effect, for example on the synthesis of 1,2-PD and propionic acid. The presence of these metabolites in fermented feeds increases the aerobic stability of silage and enables long-term preservation of plant material intended for feeding animals. This is also the reason why in order to strengthen the effect of a particular preparation's starter culture in fermenting renewable raw materials for the production of biofuels, in addition to the species of *L. buchneri*, including the strain A KKP 2047p, which has exceptional abilities, it is also important to ensure the participation of strains of the species of *L. diolivorans* and *L. reuteri*. According to the authors of the present finding, joining the biochemical properties of these selected species in the starter culture can bring measurable positive effects for the quality of obtained feed and for animal welfare, as well as for the efficiency of biogas plants and a rational management of materials stored in such plants, an example of which can be the starter culture for corn grain conservation developed by our team [11].

4. Conclusions

Metabolism of lactic acid bacteria makes it possible to obtain valuable metabolites derived thus far by chemical synthesis. The present research work focused mainly on evaluating the metabolic ability of selected bacteria of the genus *Lactobacillus* isolated from the natural environment. All isolates tested demonstrated the ability to effectively metabolize 1,2-PD (in the presence of cobalamin) and convert it to propionic acid, which means that the described microorganisms meet the basic requirements set forth for microorganisms with a potential for application. Further detailed biotechnological characterization of strains attractive to the industry will certainly offer a possibility of their wider application in the future.

Table 1
Final concentrations of propionic acid obtained in the culture of strains of *L. buchneri*, *L. reuteri* and *L. diolivorans* in various culture media, and the ability of the strains to metabolize 1,2-PD.

	Medium	*L. diolivorans* K KKP 2057p	*L. reuteri* M KKP 2048p	*L. buchneri* A KKP 2047p
Propionic acid content [mg/100 mL]	MRS	11.4 ± 0.6 (a)	6.6 ± 1.9 (b)	11.3 ± 2.1 (a)
	MRS-MOD1	122.8 ± 4.6 (a)	10.0[a] ± 1.7 (b)	9.4[a] ± 1.4 (b)
	MRS-MOD2	16.4 ± 3.6 (a)	17.6 ± 1.5 (a)	28.3 ± 2.7 (b)
	MRS-MOD3	125.4 ± 5.0 (a)	184.5 ± 5.6 (b)	174.9 ± 5.5 (b)
Utilization of 1,2-PD from the growth medium after 21 d of culturing bacteria, %	MRS-MOD1	70.5 ± 3.0 (a)	4.0[a] ± 1.0 (b)	8.0 ± 2.0[a] (b)
	MRS-MOD2	40.9 ± 2.8 (a)	50.7 ± 3.1 (b)	54.8 ± 2.3 (b)
	MRS-MOD3	84.6 ± 1.0 (a)	98.4 ± 0.3 (c)	87.0 ± 1.5 (b)

There was a standard deviation for each measurement (after the symbol "±").
Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).
[a] Very weak bacterial growth.

Conflict of interest

The authors declare that they have no conflict of interest.

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