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The Process of Natural and Styrene–Butadiene Rubbers Biodegradation by *Lactobacillus plantarum*

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Abstract: The ability of *Lactobacillus plantarum* to degrade different mixtures of styrene–butadiene and natural rubbers was investigated. In experiments, 12 various compositions of rubber waste mixtures were investigated. *Lactobacillus plantarum* cultivation was carried out in a medium with the addition of individual mixtures at the temperature of 30 °C for 14 days. The presence of cellulose on the surface of bioreactors indicated the activity of bacteria. After cultivation, the rubber mixtures were tested again. Analyses of the elemental composition and mass balance of waste were carried out. The experiments showed that the culture media supplemented by various natural rubber mixtures allowed bacteria to produce bacterial cellulose. The mean thickness of bacterial cellulose was 3–6 mm after 14 days of culturing. In samples where the cellulose appeared, about 4% average mass loss was observed. The average carbon content in the rubber material used as the carbon source for bacteria, before the culture process, was about 89.07–95.40%. After 14 days of culturing, the carbon content was reduced from 31.15% to 56.45–65.7%.

Keywords: rubber waste; natural rubbers; styrene–butadiene rubbers; degradation process; *Lactobacillus plantarum*

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1. Introduction

About 1.3 billion tons of non-agricultural waste are generated in the European Union every year. Out of this, approximately 58 million tons are harmful for the natural environment. This amount means that the yearly average of waste production in highly developed countries is around 570 kg per person [1]. One of the most serious global environmental and economic problems is rubber waste. Rubber is commonly used in many applications. This results in a growing amount of rubber waste, which is very difficult to handle due to its long-term durability, propensity to self-ignition, and resistance to chemical and biological destruction [1,2]. It is very difficult to store and utilize waste from the car industry—used cars and end-of-life rubber elements. The basic process used for rubber waste utilization is the thermal method, mainly pyrolysis. This process requires the installation of expensive equipment that ensures the complete decomposition of waste and capture of toxic gases released during decomposition [3]. The main source of the waste is the automotive industry (about 80% of used rubber products are end-of-life tires) [4]. According to data gathered by the ETRMA (European Tyre and Rubber Manufacturer Association), 3.29 million metric tons of end-of-life tires were generated in Europe in 2016, up 100,000 tons from 2015, and their number is constantly growing [5–8].

The use of biological methods to degrade toxic compounds is the most environmentally friendly; although, this requires a long time. Microorganisms that can use waste as a source of carbon and energy in their metabolic pathways are present in waste disposal sites containing rubber waste [9,10].

The genus *Lactobacillus* is the largest group among lactic acid bacteria; it includes 145 species and 27 subspecies. *Lactobacillus* are common in the natural environment. They are characterized by a simple metabolism. Most of them are able to convert simple sugars into lactic acid. Most often, they occur in places that are rich in amino acids, carbohydrates, and nucleotide derivatives. On the other hand, these microorganisms can adapt to difficult conditions, which increases their occurrence in the environment. Natural habitats of Lactobacillus bacteria are healthy and rotting plants, milk and its products, as well as the genitourinary system and the gastrointestinal tract of humans and animals [11,12]. The genus *Lactobacillus* includes species with various glucose metabolism. The main product of metabolism is lactic acid, but formic acid, acetic acid, ethanol and carbon dioxide may also occur [11,13]. Microorganisms belonging to the genus *Lactobacillus* are aerotolerants or anaerobes, as well as acidophiles, but there are also species that tolerate acid environments well. There is no information in the literature about the formation of cellulose on the culture surface by *Lactobacillus* [12,14].

Suddenly, it occurred to us that the strain we studied, *Lactobacillus plantarum*, could produce cellulose. Moreover, it was able to degrade different mixtures of butadiene–styrene and natural rubbers, which were used in the culture medium as the main carbon source. That was why the authors decided to analyze this phenomenon in detail.

The aim of this study was to test the ability of a bacterial strain to degrade different mixtures of butadiene–styrene and natural rubbers, which were used as the only carbon source in the culture medium.

2. Materials and Methods

2.1. Biological Materials

The *Lactobacillus plantarum* bacterial strain was used for the biosynthesis of bacterial cellulose, which constitutes evidence of bacterial growth. This strain was included in the resources of the LOCK 105 Pure Industrial Cultures Collection at the Institute of Fermentation Technology and Microbiology, Lodz University of Technology, under the number LOCK 1145. The strain was also deposited at the Polish Microbiological Collection (PCM) at the Institute of Immunology and Experimental Therapy of the Polish Science Academy in Wroclaw (deposit number B/00174). The LOCK 1145 strain was isolated from plant silage. LOCK 1145 is a Gram-positive, immobile, non-sporulating rod. The physiological, biochemical, and genetic properties of this strain are described in the patent No. 238266 [15].

2.2. Culture Condition

The modified Schramm–Hestrin nutrient medium was used for the cultivation of bacteria. It was composed of 20 g of glucose, 5 g of yeast extract, 5 g of aminobak, 1.15 g of citric acid, 2.7 g of Na_2HPO_4, and 0.5 g of $MgSO_4$ per 1000 mL of water [16]. Because it is known from the literature that changing the carbon source in the culture media may result in a different cellulose structure, glucose was removed and replaced by other additives in all experiments [17,18]. In each case, the medium was inoculated with a pre-culture of the *Lactobacillus plantarum* LOCK 1145. The tests used 5 mL of bacterial inoculum per 100 mL of Schramm–Hestrin medium. Bacterial cellulose was cultivated in many different square-shaped culture plates.

All experiments were conducted with the use of different styrene–butadiene and natural rubber mixtures. The composition of the styrene–butadiene mixture was butadiene rubber 100 parts by weight, technical stearin 1 part by weight, zinc white 3 parts by weight, HAF carbon black (N330) 50 parts by weight, thiohexane CBS accelerator 1 part by weight, and grade II ground sulfur 2 parts by weight. The composition of the natural rubber mixture consisted of 100 parts by weight, technical stearin 2 parts by weight, zinc white 5 parts by weight, HAF carbon black (N330) 45 parts by weight, thiohexane CBS accelerator 0.5 parts by weight, and grade II ground sulfur 2.5 parts by weight. The mixtures were prepared according to the BN-72/BN 6031-03 standards [19]. In each case, different mixtures of natural rubber were added to the culture media, which was prepared without glucose. The following mixtures, which are shown in Table 1, were separately added to the glucose-free culture medium. The authors would like to emphasize that the liquid culture medium was glucose-free in all experiments. Rubber waste mixtures were the only carbon source for bacteria.

Table 1. Full names of the mixtures used in experiments as a culture medium and their abbreviations.

Abbreviation	Name of the Substance
SBR	vulcanizate of styrene–butadiene rubber
SBR + cls	sulfuric vulcanizate of styrene–butadiene rubber with an addition of cross-linking substance
SBR + CB	sulfuric vulcanizate of styrene–butadiene rubber with the addition of carbon black
SBR + CB + cls	sulfuric vulcanizate of styrene–butadiene rubber with the addition of carbon black with an addition of cross-linking substances
NR	sulfuric vulcanizate of natural rubber
NR + CB	sulfuric vulcanizate of natural rubber with an addition of carbon black
NR + cls	sulfuric vulcanizate of natural rubber with an addition of cross-linking substances
NR + CB + cls	sulfuric vulcanizate of natural rubber with an addition of carbon black and cross-linking substances
EPDM	sulfuric vulcanizate of ethylene–propylene–diene monomer rubber
EPDM + CB	sulfuric vulcanizate of ethylene–propylene–diene monomer rubber filled with carbon black
EPDM + CB + cls	sulfuric vulcanite of ethylene–propylene–diene rubber filled with carbon black, with an addition of cross-linking substances
EPDM Keltan	sulfuric vulcanizate of ethylene–propylene–diene rubber, KELTAN type
Ground rubber from transmissions tape	—

In each case, the cultivation of the *Lactobacillus plantarum* LOCK 1145 was continued in proper conditions (30 °C degrees, pH value 5.6–5.8 oxygen condition) for 14 days.

At the end, a precise rinse process was executed. Cellulose membranes were rinsed with water, then with 1% NaOH, again with water, then with 1% CH_3COOH, and finally, with water again. Each step took at least 24 h. After the rinsing process was completed, BNCs were sliced, lyophilized, and used for preparing paper-layered compositions. The presence of cellulose on the surface of the bioreactors indicated the activity of bacteria and their metabolic processes, during which rubber waste was biodegraded. An analysis of the elemental composition of rubber waste was conducted with a statistical analysis. During this, the average carbon content in samples before and after the culture processes was the most important factor. In experiments where bacterial activity was observed, mass balances were carried out. We checked the weight of rubber waste before and after the culturing process.

2.3. Statistics

In order to answer the research questions, statistical analyzes were performed using IBM SPSS Statistics, version 25. Basic descriptive statistics and the Mann–Whitney test were calculated using this software. The level of significance in this study was assumed to be $\alpha = 0.05$. Results are reported mean results with the ±SD. All the tests were conducted with at least two repetitions.

3. Results and Discussion

The majority of the experiments showed that an addition of different types of butadiene--styrene and natural rubber mixtures to the culture medium allowed bacteria to produce bacterial cellulose, showing bacterial activity, and thus its carbon uptake from the rubber substrate. This demonstrated that *Lactobacillus plantarum* could obtain the glucose necessary for its metabolic processes from butadiene–styrene and natural rubbers.

When it comes to mean growth dynamics in the case of sulfuric vulcanizate of natural rubber (NR), 6 mm of cellulose was obtained after 14 days. Similar results were achieved for two mixtures: vulcanizate of natural rubber with an addition of carbon black (NR + CB) and sulfuric vulcanizate of natural rubber with an addition of cross-linking substances (NR + cross-linking substances). An addition of these two mixtures resulted in obtaining 5 mm-thick cellulose membranes in each case. The slowest growth was observed for the culture medium containing the mixture of sulfuric vulcanizate of natural rubber with an addition of carbon black and cross-linking substances (NR + CB + cross-linking substances). In this case, 4 mm of a cellulose membrane was produced by the bacterial strain after 14 days of the culture process.

Regarding SBRs, the best results were observed for the mixture of styrene–butadiene rubber with an addition of carbon black (SBR + CB), Figure 1a. The growth dynamics for this substance was the highest, and 6 mm of bacterial cellulose was obtained after 14 days of culturing. An addition of vulcanizate of styrene–butadiene rubber (SBR) made it possible to obtain 5 mm of cellulose. The slowest growth was observed for the culture medium containing the mixture of sulfuric vulcanizate of styrene–butadiene rubber (SBR + cross-linking substances), Figure 1b. In this case, 3 mm of a cellulose membrane was produced by the bacterial strain after 14 days of the culture process. Positive results were also observed for sulfuric vulcanizate rubber EPDM (ethylene propylene diene monomer) filled with carbon black. In this case, 5 mm of a cellulose membrane was obtained after 14 days of the culture process. The same result was achieved for ground rubber from transmission tapes. The growth rate of cellulose on the bioreactor surface is correlated with an amount of carbon taken from the waste. For tests with the best efficiency (6 mm cellulose), further research is being carried out for the optimization of selected parameters (Figure 2).

(**a**) (**b**)

Figure 1. Thickness of bacterial cellulose (red arrows) cultured on: (**a**) SBR + CB; (**b**) SBR + cross-linking substances.

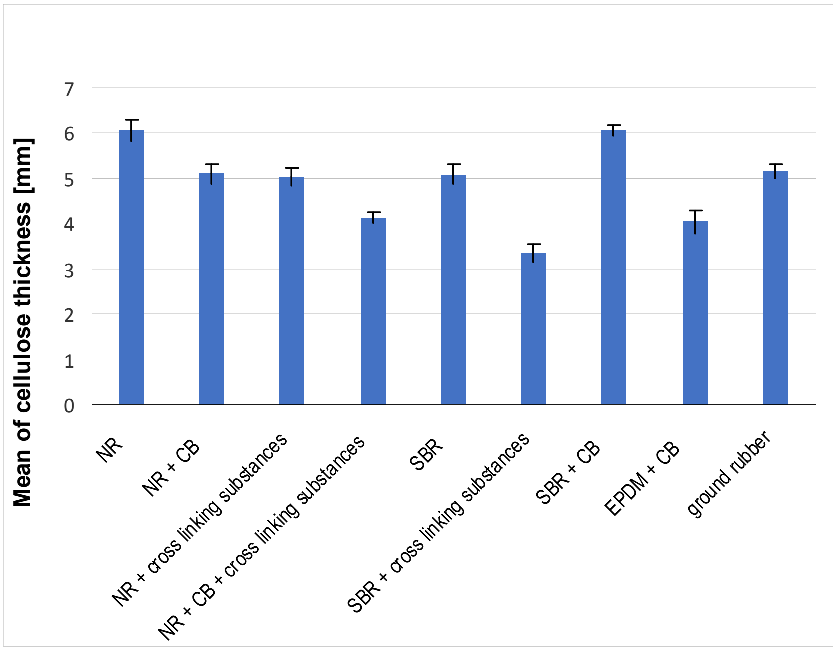

Figure 2. The mean thickness of bacterial cellulose after 14 days of culturing with an addition of different carbon sources.

Low activities of the investigated bacterial strains were observed in some bioreactors. This conclusion was withdrawn after analysis of bacterial cellulose thickness after the experiments. The lowest thickness of cellulose was observed in the case of three types of samples, i.e., SBR + cross linking substances, NR + CB + cross linking substances, and EPDM + CB. Some additives in the mixtures, which were used as the main carbon source may have been harmful for the bacteria and prevented them from growing. The authors analyzed these cases to obtain optimal results.

To confirm the preliminary results, that the bacterial strain can gain glucose from styrene–butadiene and natural rubbers to use it for its metabolic processes, an analysis of the elemental composition was conducted. The average carbon content in the rubber material used as the carbon source for bacteria was 89.07—95.40%. After 14 days of culturing, the content ranged between 56.45 and 65.7%.

Due to the characteristics of the distribution of the percentage of carbon content in the measurement before and after cultivation, it was decided that the non-parametric Mann–Whitney U test should be used. The independent test was used because the study failed to combine the pre- and post-culture measurements corresponding to one gum fragment. The test results turned out to be statistically significant and the effect size measured with the use of eth-square (η^2) was 0.74, which indicates a very strong difference between the measurements. The average carbon content (%) after the experiment was significantly lower than before the experiment. The exact values of the discussed analysis are presented in Tables 2 and 3, as well as Figure 3.

Moreover, before being used as a carbon source for bacteria, styrene–butadiene rubbers mainly consisted of carbon and zinc, and sometimes oxygen was visible during analysis. After the culturing process, the presence of many more different elements was observed. This fact proved that the bacteria conducted their metabolic processes. Additional elements found in the elemental composition analysis showed that the bacteria not only used carbon for themselves, but also produced metabolites during the culturing process.

Table 2. An average carbon content in the rubber material before and after realizing the experiments (30 control samples) with basic descriptive statistics: mean, median, standard deviation, skewness, kurtosis, minimum, and maximum value, respectively.

Average Carbon Content (%)	M	Me	SD	Sk.	Kurt.	Min.	Max.
Before the experiment	91.49	95.08	7.51	−2.55	7.21	65.12	96.58
After the experiment	59.71	56.88	6.68	1.20	1.32	51.17	78.03

Table 3. The results of the Mann–Whitney U test checking the differences between the measurement before and after the cultivation in the mean carbon content (%).

	Before the Experiment (n = 21)			After the Experiment (n = 21)					
	M_{rank}	Mdn	IQR	M_{rank}	Mdn	IQR	Z	p	η^2
Average carbon content (%)	31.81	95.08	5.23	11.19	56.88	8.01	−5.51	<0.001	0.74

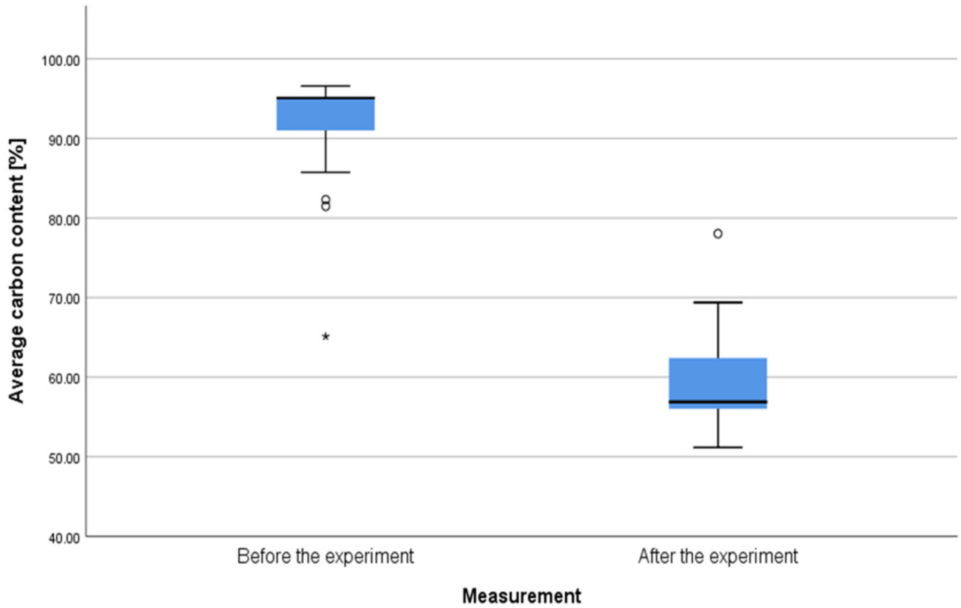

Figure 3. Box plots for the average carbon content (%) broken down into measurements before and after the experiment. *—before the experiment, °—after the experiment.

Moreover, an analysis of natural rubber samples after the end of the culturing process, which was performed under a scanning electron microscope, showed the presence of pores in the rubber structure. Figure 4 presents the surface of sulfuric vulcanizate of natural rubber with an addition of carbon black and cross-linking substances (NR + CB + cross-linking substances) after 14 days of cultivation using the *Lactobacillus plantarum* LOCK 1145. Apart from pores (red arrows) on the sample surface, bacteria were observed (green arrows). They were shaped like short rods (4–5 µm long and up to 1 µm wide).

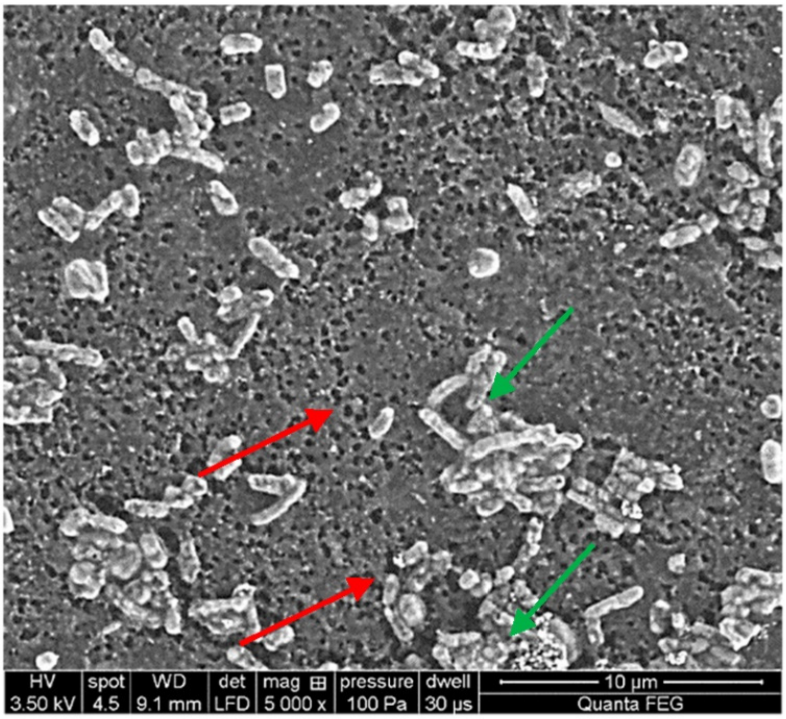

Figure 4. Visible pores in rubber structure (red arrows) and bacteria (green arrows) at the surface of NR + CB + cross-linking substances, after 14 days of cultivation using the *Lactobacillus plantarum*.

In experiments where bacterial activity was observed, mass balances were carried out. The rubber used as the carbon source was weighed before and after experiments. In each case, a mass loss of about 1–5% in relation to the initial weight was observed. The appearance of the rubber material without using a microscope did not change. Under a scanning electron microscope, the presence of pores in rubber structure was observed. The decrease in rubber mass is due to the fact that bacteria, which had grown in the bioreactor, took the carbon necessary for their metabolic processes from it. Statistical analysis (basic descriptive statistics were calculated for the gum mass before and after cultivation (g) and the gum mass loss in (g) and (%) divided into three pH values: 5.6, 5.7, and 5.8) for average weight loss is presented in Table 4. The authors present the results for the most optimal pH values.

Table 4. Basic descriptive statistics for mass losses of rubber waste during the 14-day breeding process.

pH	Variables	M	Me	SD	Min.	Max.
5.6	Rubber mass before (g)	4.37	4.32	0.22	4.19	4.61
	Rubber mass after (g)	4.19	4.18	0.19	4.01	4.38
	Average mass loss (g)	0.18	0.18	0.05	0.14	0.23
	Average mass loss (%)	4.17	4.29	0.88	3.24	4.99
5.7	Rubber mass before (g)	4.75	4.85	0.22	4.50	4.90
	Rubber mass after (g)	4.51	4.62	0.20	4.28	4.64
	Average mass loss (g)	0.24	0.23	0.02	0.22	0.26
	Average mass loss (%)	4.98	4.89	0.30	4.74	5.31
5.8	Rubber mass before (g)	4.18	4.12	0.15	4.06	4.35
	Rubber mass after (g)	4.00	3.98	0.16	3.86	4.17
	Average mass loss (g)	0.17	0.18	0.03	0.14	0.20
	Average mass loss (%)	4.32	4.14	0.57	3.86	4.96

In conclusion, the experiments which were carried out have promising results. Most of the experiments demonstrated that an addition of different types of natural and styrene–

butadiene rubber mixtures to the culture medium allowed bacteria to carry out their metabolic processes. This showed that the *Lactobacillus plantarum* LOCK 1145 is able to obtain glucose from rubber mixtures and takes part in the degradation process of these rubbers. In one cycle of bacterial culture, we obtained an average of more than a 4% reduction in rubber waste mass. By repeating the process several times, we could achieve a significant reduction of over 80% of the waste output weight on an annual basis. To make use of bacterial cellulose formed on the surface of the reactor, the authors are working on paper-layered composites that can be used as packaging material. Some tests in this field were conducted, and the results are very promising. Due to the lack of literature data on the ability of *Lactobacillus plantarum* to produce cellulose, the authors plan to conduct an analysis of bacterial metabolites by HPLC method. Genetic identification of the bacterial strain was conducted and the strain has been deposited at the Polish Microbiological Collection (PCM) at the Institute of Immunology and Experimental Therapy of the Polish Science Academy in Wroclaw (deposit number B/00174). With the above data, we would like to emphasize the practical aspect of the experiments that was investigated by the authors. By repeating the process several times, we could achieve a significant reduction of over 80% of the waste output weight on an annual basis. This provides us with the opportunity to significantly reduce pollution in an alternative way, obtaining a material that can be used for further processing, without the need for a combustion process. During this process, in addition to CO_2, toxic substances such as POPs, PAH, VOCs, Cd, and Pb are released into the atmosphere. That is why the authors are also interested in designing a bioreactor model (batch reactor) for the utilization of rubber waste with the use of bacteria on a semi-industrial scale.

4. Conclusions

The study found that the *Lactobacillus plantarum* strain could grow on a substrate where rubber waste was the only carbon source for bacteria. The authors proved that this strain takes part in the degradation process of these rubbers. The presence of cellulose on the surface of bioreactors indicated the activity of bacteria and their metabolic processes, during which rubber waste was biodegraded. The average carbon content in the rubber material used as the carbon source for bacteria was 89.07–95.40%. After 14 days of culturing, the content decreased and ranged between 56.45 and 65.7%. In one cycle of bacterial culture, we obtained an average of more than a 4% reduction in rubber waste mass. Repeating the process several times, we can achieve a significant reduction of over 80% of the waste output weight on an annual basis.

5. Patents

Patent No. 237035 "The layered cellulose-paper composite the method of producing the composite"; Polish Patent Office, Warsaw, November 2020.

Patent No. 238266 "The strain of lactic bacteria *Lactobacillus plantarum* LOCK 1145 and a method of producing bacterial cellulose by culturing a strain *Lactobacillus plantarum* LOCK 1145"; Polish Patent Office, Warsaw, May 2021.

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