Effect of carbon source on the synthesis and antioxidant properties of exopolysaccharides of lactic acid bacteria

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Abstract. The present study analyses the impacts of varying carbon sources (glucose, maltose, lactose, sucrose, raffinose) on the development, exopolysaccharide synthesis, and antioxidant activity of silage-derived lactic acid bacteria, encompassing Limosilactobacillus fermentum AG8, Lactiplantibacillus plantarum AG1, AG9, AG10, and Lacticaseibacillus rhamnosus AG16, with Lactobacillus bulgaricus serving as a control. The lactic acid bacteria were cultivated in MRS broth and growth was measured via optical density. The exopolysaccharide yield was quantified using the phenol–sulfuric acid method, and the antioxidant activity was assessed via DPPH radical-scavenging assays. The results showed that disaccharides (maltose and sucrose) promoted optimal growth, with AG9 and AG10 exhibiting the highest biomass. exopolysaccharide production peaked in sucrose media, particularly for L. bulgaricus and L. rhamnosus AG16. The highest antioxidant activity per total exopolysaccharide was found in sucrose-derived exopolysaccharide, but recalculating the results per mg revealed superior activity in exopolysaccharide cultured in maltose and raffinose. AG16 exopolysaccharide exhibited the greatest specific radical-scavenging activity (10.58 %/mg) in the presence of maltose. These findings emphasise the importance of selecting the correct carbon source for maximising lactic acid bacteria functionality. Sucrose enhances exopolysaccharide yield, while maltose and raffinose improve antioxidant efficiency. This study highlights the potential of silage-derived lactic acid bacteria for producing bioactive exopolysaccharide for use in the food and health industries.

1 Introduction

Lactic acid bacteria demonstrate a wide distribution range and numerous natural ecological niches in which they reside. The high diversity of lactic acid bacteria in different ecological conditions is evidence of the ability of lactic acid bacteria to adapt to ecosystems with changing conditions. Lactic acid bacteria are found in soil, water, plants, the gastrointestinal tract of animals and humans, and fermented products. They play a key role in the breakdown of organic matter (for example, in composting), which helps to recycle nutrients. They live in a symbiotic relationship with plants and animals, synthesising

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substances that suppress pathogens and improving the growth of both. Within the intestinal tracts of both animals and humans, they contribute to the maintenance of a healthy microbiome by competing with pathogens.

There are many different types of lactic acid bacteria, and a special place is taken by probiotic bacteria, which have a number of beneficial properties for humans. In recent times, probiotic research has increased in popularity due to their potential to improve health and treat some diseases in combination with other therapies. Antidiabetic [1] and antioxidant [2] effects of lactic acid bacteria have been described. In addition, probiotics are considered as an alternative to antibiotics due to the spread of antibiotic resistance in pathogenic bacteria [3]. Studies on the effects of probiotics on human health have focused on digestive tract diseases, inflammatory skin diseases, allergies, respiratory tract infections, obesity, metabolic diseases (particularly type 2 diabetes), cardiovascular, cognitive and mental health, bone health, liver disease, burn wounds and gynaecological diseases [4]. Probiotic additions containing L. reuteri, L. casei, L. paracasei, L. bulgaricus, L. acidophilus and B. subtilis can improve bone health by significantly increasing calcium levels [5].

Lactic acid bacteria produce a large number of metabolites. These are both protein and carbohydrate substances. Researchers have focused on a number of compounds, with lactic acid bacteria exopolysaccharides being among the most notable. Exopolysaccharides are involved in a wide range of biological functions, such as protection against drying, and are responsible for the attachment of cells to surfaces and the formation of biofilms. The ability of lactic acid cultures, which are used as starter cultures in the production of fermented dairy products, to produce exopolysaccharide significantly improves the texture and flavour of the final product, as well as its stability. The stability and properties of the exopolysaccharide in food products, especially in fermented dairy products, can be highly influenced by interactions with other ingredients, among which various natural additives attract particular attention due to their wide variety of structures, the possibility of fine modulation of lactic acid bacteria activity and properties, and biosafety.

Bacterial exopolysaccharides are known for their diverse structures, which are often compatible with living things and can break down naturally. They are attracted to water and often display a range of important biological properties. The beneficial effects of exopolysaccharide lactic acid bacteria include antioxidant, anti-tumour, anti-ulcer and cholesterol-lowering properties [6]. The antioxidant activity of exopolysaccharide, particularly its radical-binding activity, may be due to the presence of hydroxyl and other functional groups in both exopolysaccharide strains. These groups can transfer electrons to reduce radicals to a more stable form, or react with free radicals to stop the chain reaction. Synthesis of exopolysaccharide by lactic acid bacteria is vital for intestinal homeostasis. These bacteria form the basis of the intestinal mucosa and influence the immune system through cytokines synthesised into the blood, thereby regulating protective immunity.

The usefulness of exopolysaccharide is being taken into account by some scientists, who are now aiming to isolate exopolysaccharide lactic acid bacteria as a commercial polysaccharide with the prospect of further use in food technology. The aim is to find the conditions that will produce the highest exopolysaccharide yield. It has been shown that glucose, lactose, sucrose and mannose, can be used by different lactic acid bacteria strains as a carbon source for exopolysaccharide synthesis [7]. Imran et al. (2016) showed that the highest exopolysaccharide yield of Lactobacillus plantarum strains was found when cultured on glucose as a carbon source [8]. The yield, molecular weight, monosaccharide composition and biological functions of exopolysaccharide depend on the strain and the nutritional medium. The chemical structure of the exopolysaccharide dictates the molar weight and stiffness, which are the key parameters for their ability to intensify viscosity.

We previously isolated five strains from silage and characterised them for their probiotic properties. Their ability to synthesise of exopolysaccharide in yoghurt and de Man, Rogosa, and Sharpe medium was demonstrated. This study was aimed at identifying the effect of carbon source, in particular different carbohydrates on exopolysaccharide synthesis by Lastobacilli, which were isolated previously from silage. In addition, the antioxidant properties of exopolysaccharide isolated from nutrient medium with different sugars were evaluated.

2 Materials and methods

2.1 Strains and cultivation

A commercial variant of Lactobacillus delbrueckii subsp. bulgaricus ("Lactosintez", Moscow, Russia) was used in the study as a control strain. de Man, Rogosa, and Sharpe broth was used for cultivation. Commercial de Man, Rogosa, and Sharpe broth was used to prepare the overnight culture (Himedia, India). To obtain a working cultures, a 100 μL aliquot of each culture was individually transferred into MRS broth and grown 18 h under static conditions at 37 °C. After that, a working culture of each strain was transferred to medium with different carbon sources and cultured for 72 hours at 37 °C. During this time, the growth of the culture was recorded by the change in optical density, priming was carried out at 610 nm using a spectrophotometer SF-2000 (Russia).

2.2 Exopolysaccharide isolation and quantification

Isolation and quantification of exopolysaccharides were carried out as follows. Exopolysaccharide concentration was determined using a glucose-based calibration curve and expressed as milligrams of dextrose equivalent per gram of sample.

3 Results

It is vital to study how lactic acid bacteria grow and how they respond synthetically when the carbon source is different, in order to predict how these bacteria might be used in industry in the future.

3.1 Bacterial growth on different substrates

The initial research stage revealed discrepancies in the response of various lactobacilli to different carbon sources. The strain AG1 (Figure 1) accumulated the largest biomass (about 2.5 Dopt) when glucose was used as a carbon source. Within 24 hours, strain AG16 grew to 2 Dopt; the other strains showed growth of 1.3–1.6 Dopt during this period.

The highest increase in lactic acid bacteria was seen in the maltose variant, in comparison to the other test variants. In this case, the leading strain was AG9, and slightly lower Dopt was shown by strains AG10 and AG1. The other strains showed a Dopt level of 2.5. Intensive growth of bacteria comparable to that on maltose was detected in the case of using sucrose as a carbon source. In this case, the highest number of cells after 24 hours was in strains AG10 and L.bulgaricus (2.7 Dopt), followed by strains AG1 and AG8. The lowest abundance was observed in strains AG9 and AG16, yet it is noteworthy that after 30 hours of cultivation, strain AG9 became the leader, attaining parity with the other strains in terms of abundance, while AG16 remained at the same level. A similar trend of cell accumulation during growth on lactose has been observed in some parts, as has been found

during growth on sucrose. In this case, leadership was taken by L.bulgaricus and strain AG1, with slightly lower values being recorded for AG8 and AG10. For strain AG9, as with sucrose, a delay in reaching the stationary phase (when the cells stop growing) after 30 hours of cultivation was revealed. This phase is when the cells accumulate the most biomass.

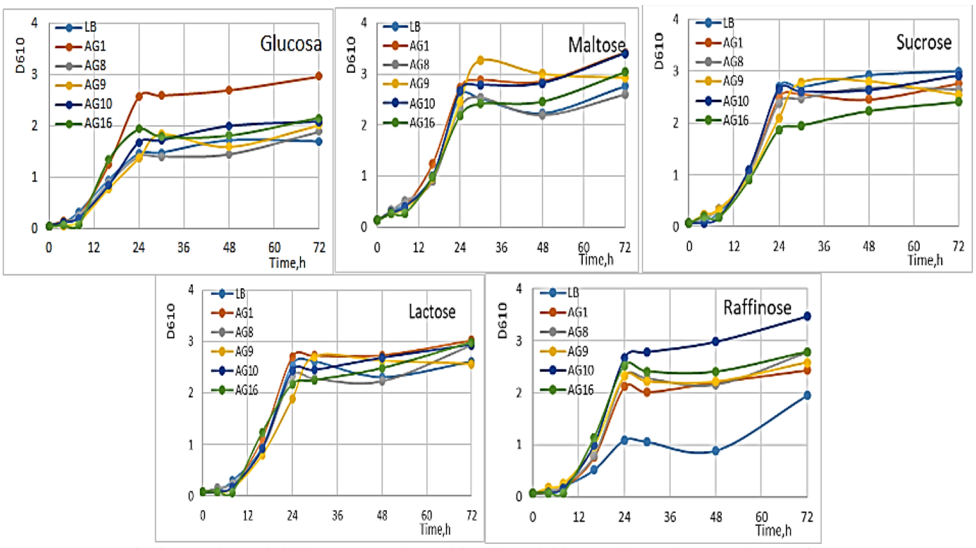

Fig. 1. Growth dynamics of different strains of lactic acid bacteria under varying substrates.

Raffinose is a trisaccharide, which means it has three sugars: galactose, glucose and fructose. The utilization of raffinose has led to diminished biomass accumulation in comparison to disaccharides. After 24 hours of cultivation, the highest optical densities were observed in strains AG10 and AG16, at 2.6 and 2.5 respectively. The commercial strain L. bulgaricus performed the worst on raffinose. A notable increase in abundance was seen for AG10 during the 72-hour cultivation period. This could be an indication of a more complex enzymatic ensemble, which might be better at processing complex polysaccharides.

In summary, the results obtained allow us to draw some conclusions about certain regularities. First of all, disaccharides are more advantageous than the monosaccharide glucose. Maltose, which has two glucose molecules in its composition, is the most favourable growth substrate as a carbon source. Replacing one glucose with fructose in sucrose or galactose in lactose leads to a slight decrease in biomass yield. The accumulation of biomass by the lactic acid bacteria used is less favourable when working with a trisaccharide that contains only one glucose. It should be noted that the homofermentative strain L. bulgaricus was found to be least adapted to consuming raffinose.

3.2 Exopolysaccharide syntheses

During cultivation in the presence of varying carbon sources, lactic acid bacteria accumulated different amounts of exopolysaccharides. Importantly, no strict correlation was found between bacterial numbers and exopolysaccharide amounts. The commercial strain of L. bulgaricus and strains from silage accumulated the highest amount when grown on sucrose. Sucrose was the best substrate for increasing exopolysaccharide. Furthermore, the amount of accumulated L. bulgaricus exopolysaccharide decreased in the following order: lactose > glucose > maltose > raffinose (Figure 2). The strains L. plantarum AG1, L. plantarum AG10 and L. fermentum AG8 synthesised almost the same amount of

exopolysaccharide on maltose- or lactose-containing media; however, the amount of exopolysaccharide on these media was lower than on the sucrose-containing medium. The lowest accumulation of exopolysaccharide was shown by the L. plantarum AG1, L. plantarum AG10 and L. fermentum AG8 strains in the variants of cultivation on media containing glucose or raffinose. For L. plantarum AG9, the following series in terms of exopolysaccharide accumulation was identified: sucrose > lactose > glucose > maltose > raffinose. This trend coincides with that of L. bulgaricus. The largest amount of exopolysaccharide was accumulated by L. rhamnosus AG16, like all other strains, on sucrose, with lactose being the second highest sucrose substitute. When grown in a medium containing glucose or raffinose, AG16 synthesised approximately 4 mg/ml of exopolysaccharide. However, in a medium containing maltose AG16 accumulated only 2 mg/ml of exopolysaccharide, which was the lowest value observed among all the analysed variants.

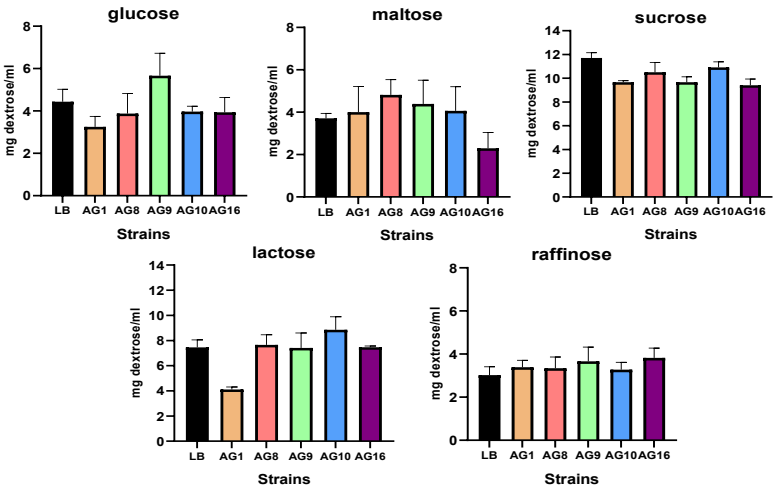

Fig. 2. The amount of exopolysaccharide in different lactic acid bacteria strains when growing on different carbon sources after 72 hours of cultivation.

3.3 Antioxidant activity of exopolysaccharide

The antioxidant activity was analysed using the 2,2-diphenyl-1-picrylhydrazyl method, which enables the radical-scavenging activity of the substrate in the complex to be evaluated. Exopolysaccharide systems evaluated the activity in the initial aliquot, resulting in the data shown in Figure 3.

As expected, the highest radical-scavenging activity was detected in exopolysaccharide synthesised with sucrose, given the highest exopolysaccharide yield in this case. However, exopolysaccharide from strains AG1 and AG8 exhibited the greatest activity. AG16 had the lowest activity, despite the comparable amount of exopolysaccharide synthesised. The radical-scavenging activity of exopolysaccharide obtained by bacterial growth on lactose was slightly lower. The most active strain was L. plantarum AG9, as well as L. bulgaricus. The other strains exhibited 12-3 percent lower exopolysaccharide activity. Similar exopolysaccharide activity was detected in L. bulgaricus strains L. plantarum AG1, L. fermentum AG8 and L. plantarum AG9 when cultured on glucose or maltose. However, the exopolysaccharide activity obtained from L. plantarum AG10 and L. rhamnosus AG16 after

cultivation on maltose was higher than that obtained from glucose. The lowest exopolysaccharide activity was detected in exopolysaccharide obtained from strains grown on raffinose. This is primarily because the yield of exopolysaccharide is lower when using raffinose as a carbon source.

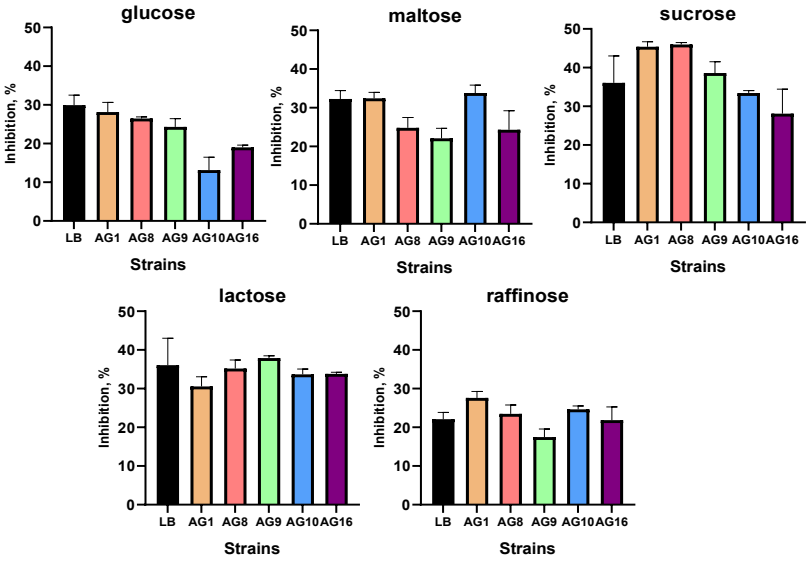

Fig. 3. The radical-scavenging activity of different exopolysaccharide obtained from lactic acid bacteria strains grown on different carbon sources after 72 hours of cultivation is shown. The radical-scavenging activity of the obtained exopolysaccharide mixtures is shown, independent of amount.

The above results led us to conclude that the activity needed to be recalculated based on the number of exopolysaccharide. The radical-scavenging activity per mg of exopolysaccharide was then recalculated, as shown in Table 1.

Recalculating the data produced a different result to that shown in Figure 3. Generally speaking, v synthesised on sucrose exhibited the lowest activity in all strains. The strain was most active in terms of antioxidant activity (7-8 %/mg v) when cultivated on maltose and raffinose. L. plantarum AG1 exopolysaccharide exhibited high activity in the presence of glucose, maltose, and raffinose (8–8.5 %/mg exopolysaccharide). The activity of L. fermentum AG8 exopolysaccharide was found to be high on raffinose (7 %/mg exopolysaccharide). The AG9 strain of L. plantarum had one of the lowest levels of radical-scavenging activity exopolysaccharide, at between 4-5 %/mg v, and this was the case for all substrates. L. plantarum AG10 synthesised v with high radical-scavenging activity levels on media with raffinose and maltose. L. rhamnosus AG16 produced the highest levels of exopolysaccharide with antioxidant activity of 10.58%/mg exopolysaccharide when cultivated on maltose; other carbohydrates resulted in significantly lower exopolysaccharide activity.

Table 1. Radical-binding activity of exopolysaccharide per 1 g of polysaccharide obtained by growing lactic acid bacteria by varying the carbon source.

Carbon source	**Glucose**	**Maltose**	**Sucrose**	**Lactose**	**Raffinosa**
Strains	% inhibition/mg exopolysaccharide				
Lb	6.75±0.58	8.69±0.58	3.08±0.59	4.84±0.40	7.31±0.58
AG1	8.67±0.76	8.11±0.39	4.70±0.13	6.71±0.42	8.14±0.49
AG8	6.83±0.10	5.15±0.55	4.38±0.05	4.95±0.08	7.03±0.68

AG9	4.30±0.38	5.04±0.59	4.00±0.30	4.54±0.19	4.77±0.58
AG10	3.31±0.85	8.32±0.50	3.06±0.06	3.82±0.05	7.51±0.27
AG16	4.83±0.16	10.58±2.14	2.99±0.67	3.86±0.22	5.71±0.89

4 Discussion

This study investigates the influence of different carbon sources on the growth, exopolysaccharide production, and antioxidant activity of lactic acid bacteria strains isolated from silage, including Limosilactobacillus fermentum AG8, Lactiplantibacillus plantarum AG1, AG9, AG10, and Lacticaseibacillus rhamnosus AG16, with Lactobacillus delbrueckii subsp. bulgaricus as a control. The lactic acid bacteria strains were cultivated in MRS broth supplemented with glucose, maltose, lactose, sucrose, or raffinose as carbon sources.

Results indicated that disaccharides, particularly maltose and sucrose, supported the highest biomass accumulation, with strain AG9 showing optimal growth on maltose and AG10 on sucrose. exopolysaccharide production was most efficient on sucrose, with L. bulgaricus and AG16 yielding the highest amounts. However, no direct correlation was observed between biomass and exopolysaccharide yield. Antioxidant activity analysis revealed that exopolysaccharide from sucrose cultures exhibited the highest total radical-scavenging activity, but recalculation per mg of exopolysaccharide showed that maltose and raffinose-derived exopolysaccharide had superior specific activity. Notably, AG16 exopolysaccharide displayed the highest radical-scavenging activity (10.58%/mg) when grown on maltose.

Many studies have reported on the diversity of exopolysaccharides synthesised by different strains of the same Lactobacillus species. For instance, certain types of L. fermentum can produce a sugar-rich substance called exopolysaccharide, which is mostly made up of glucose, galactose, and/or mannose. However, the exopolysaccharide produced by L. fermentum S1 is particularly rich in mannose. These exopolysaccharides may comprise varying proportions of galactose and glucose, with certain exopolysaccharide variants also comprising a substantial proportion of rhamnose (up to 21%) and a negligible proportion of arabinose [10], as observed in L. fermentum strain YL-11 [11]. Conversely, some L. plantarum strains only form exopolysaccharide from glucose and galactose [12]. Conversely, the exopolysaccharide of L. fermentum MTCC 25067 does not change significantly when the bacterium is cultured on different nutrient media with glucose, galactose, lactose or sucrose as the carbon source [13]. When glucose is used as the carbon source, different L. plantarum strains produce an extremely wide range of exopolysaccharide. Most of the characterised L. plantarum strains produce exopolysaccharide with a high proportion of mannose, glucose and/or galactose.

The findings highlight the critical role of carbon source selection in optimizing lactic acid bacteria growth, exopolysaccharide yield, and functionality. Sucrose emerged as the preferred substrate for maximal exopolysaccharide production, while maltose and raffinose enhanced specific antioxidant properties. These insights are valuable for industrial applications, particularly in developing functional foods with tailored probiotic and prebiotic benefits. The study underscores the potential of silage-derived lactic acid bacteria strains as sustainable sources of bioactive exopolysaccharide for food technology and health-related industries.

5 Conclusion

This study shows that the type of carbon source used affects how well lactic acid bacteria isolated from silage grow, make exopolysaccharide and have antioxidant activity. The

greatest biomass was attained with disaccharides (maltose and sucrose), while the maximum exopolysaccharide yield was seen in the medium with sucrose, particularly for L. bulgaricus and L. rhamnosus strains AG16. Conversely, the cultures grown on maltose and raffinose exhibited the highest specific antioxidant activity of exopolysaccharide, with a record value of 10.58 %/mg for strain AG16. These results highlight the importance of optimising cultivation conditions to obtain targeted bioactive compounds. Lactic acid bacteria are a source of great promise for biotechnology because of their ecological diversity and ability to adapt to different substrates. Utilising lactic acid bacteria strains from natural sources, such as silage, enables the development of sustainable and cost-effective processes for producing functional food ingredients, probiotics, and biologics with defined properties. Further research in this direction can contribute to the development of green technologies and increase the application of lactic acid bacteria in the food, medical and agricultural industries. The work therefore helps to increase understanding of the metabolic potential of lactic acid bacteria, and provides practical solutions for their use in biotechnology. These solutions combine environmental safety with high efficiency. Consequently, lactic acid bacteria and their exopolysaccharides and exopolysaccharides are pivotal components of ecosystems that contribute to the stability of biogeochemical cycles. Their subsequent research and implementation in the field of biotechnology, most notably in the realm of food technology, stands to contribute to the evolution of an ecologically sustainable economic framework.

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