ANALYSIS OF BIOMASS ACCUMULATION BY LACTIC ACID BACTERIA ISOLATED FROM PLANT RAW MATERIALS

Holubchyk D.[1,2], Dugan O.[1], Potemska O.[2], Khablenko A.[1,2]
[1]Igor Sikorsky Kyiv Polytechnic Institute, khronosuranovich@gmail.com
[2]Institute of Food Resources of the NAAS of Ukraine

Abstract

These theses are devoted to the analysis of dry weight biomass in potential probiotic lactic acid bacterial strains isolated from maize, barley, and oat sourdoughs. The results show that the sample weights are 2,04 ± 0,16, 2,16 ± 0,05, and 2,12 ± 0,11 g/L, with no statistically significant differences among them. Further research should focus on determining the number of viable cells (CFU/mL) in these cultures and optimizing the nutrient media to suit their needs.

Keywords: *Lactobacillaceae, probiotics, biomass, dry weight analysis.*

Introduction. The increasing public awareness of probiotics is driving a growing interest in this topic among representatives of various social groups and age categories, largely due to the popularization of science, the promotion of a healthy lifestyle, and similar factors [1]. As of 2023, the probiotic market was valued at 5,39 billion USD and is projected to grow at an average annual rate of 4,2 % until 2032. Functional food products – and primarily dietary supplements – play a key role in driving the demand for probiotics [2]. To meet human needs, it is necessary not only to continuously search for new potential probiotic microorganisms but also to assess their ability to accumulate biomass, which in turn determines their potential for efficient industrial application.

Among probiotic strains that have demonstrated beneficial effects on the human body (for example, reducing plasma glucose levels in individuals with diabetes), lactic acid bacteria (LAB) predominate [3], making them particularly promising for further study and practical use.

The aim of this study is to assess and compare the biomass accumulation capacity of LAB isolates obtained from sourdoughs made with different types of flour.

Materials and methods. Three LAB isolates obtained from maize, barley, and oat sourdoughs were used in this study. Prior to the experiment, each culture was cultivated in liquid MRS (De Man-Rogosa-Sharpe) medium at 37 °C for 24 hours to reach the stationary growth phase and, consequently, the maximum amount of physiologically active biomass [4]. To obtain the cell mass, the samples were centrifuged at 3,500 g for 15 minutes [5] and washed twice with distilled water. The resulting biomass was then transferred into pre-labeled, pre-dried (in a drying oven), and pre-weighed aluminum weighing boxes. The LAB cells were dried at 105 °C until a constant weight was reached, after which the boxes were placed in a desiccator for cooling and subsequently weighed using an analytical balance with an accuracy of four decimal places [6].

All measurements were performed in triplicate. The results were considered statistically significant at a significance level of $p < 0,05$.

Results and discussion. The conducted study made it possible to determine the amount of biomass accumulated by the three examined cultures (the isolate from maize

sourdough – M, from barley – B, and from oat – O) during 24 hours of cultivation in liquid MRS medium (Fig. 1).

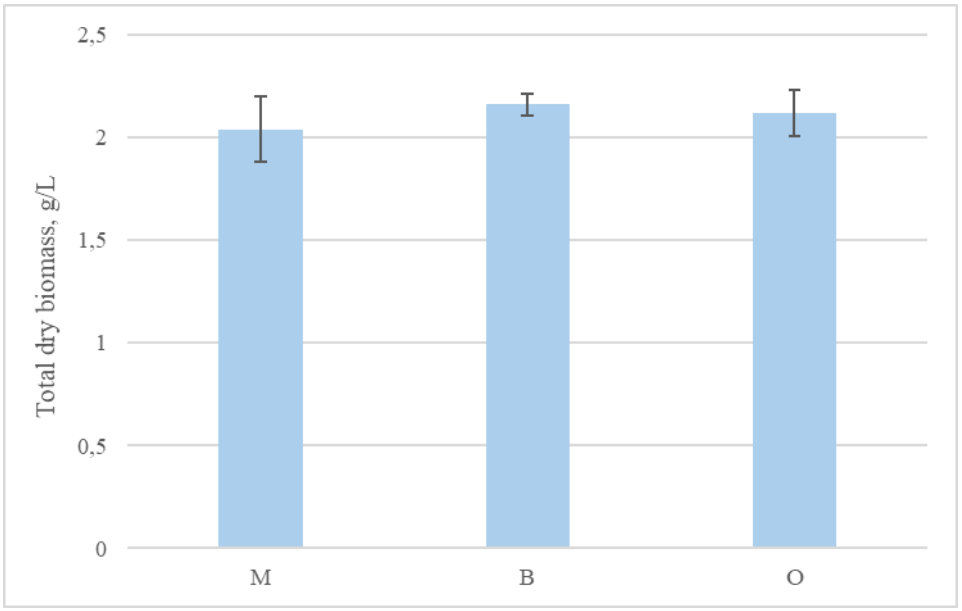

Fig. 1. Total dry biomass of lactic acid bacteria isolated from different sources.

Analysis of the obtained data shows that there is no statistically significant difference in the amount of biomass accumulated by the cultures isolated from sourdoughs based on different types of flour after 24 hours of cultivation in MRS medium.

A comparison of our results with similar studies on other LAB reveals significant variability in biomass accumulation, which is often related, among other factors, to the intended use of a specific strain. For example, *Ligilactobacillus agilis* LPB 56, adapted for lactic acid production, accumulates 0,6811 g/L of biomass after 24 hours of cultivation in MRS medium [7], whereas the probiotic *Lacticaseibacillus rhamnosus* E/N accumulates 21 g/L after 18 hours of cultivation [8]. These values differ significantly in terms of dry biomass content from the LAB isolates obtained from sourdough. On the other hand, strains that are still under investigation for various potential applications tend to show biomass accumulation levels more comparable to those of the studied cultures. For instance, *Lcb. rhamnosus* BRM 029693, which is being considered for use in cheese production, can accumulate 3,2 g/L of dry biomass [9], while *Lactiplantibacillus plantarum* 200655, isolated from kimchi and regarded as a promising probiotic due to its range of beneficial effects on human health, accumulates 2,504 g/L [10].

Although the LAB strains examined in this study accumulate relatively low amounts of biomass, a more important factor remains the number of viable cells present in the medium – measured in CFU/mL. Determining their concentration should be the focus of future research. In addition, while MRS is a widely accepted medium for cultivating LAB, different strains have varying nutritional requirements and therefore require optimization of the growth medium to maximize biomass yield [11]. This approach can lead to an increase in productivity by 2,5 times or more [12].

Conclusions. It was established that LAB isolates from sourdoughs based on maize, barley, and oat flour accumulate 2,04 ± 0,16, 2,16 ± 0,05, and 2,12 ± 0,11 g/L of biomass, respectively, and these values do not differ significantly from one another. Overall, the obtained values are lower than those reported for similar microorganisms; however, a more important factor – which should be the focus of future research – is the number of colony-forming units generated by the cultures. Additionally, optimization of the growth media may be a viable approach to increase biomass yield.

References:

1. Shaping the future of probiotics and prebiotics / M. Cunningham et al. *Trends in Microbiology*. 2021. Vol. 29, no. 8. P. 667–685. URL: https://doi.org/10.1016/j.tim.2021.01.003.

2. Probiotic Ingredients Market Report. Straits Research. 2023. URL: https://straitsresearch.com/report/probiotic-ingredients-market.

3. Probiotics: supplements, food, pharmaceutical industry / S. Putta et al. *Therapeutic, Probiotic, and Unconventional Foods*. 2018. P. 15–25. URL: https://doi.org/10.1016/b978-0-12-814625-5.00002-9.

4. Hydrogen sulfide effects on the survival of lactobacilli with emphasis on the development of inflammatory bowel diseases / I. Kushkevych et al. *Biomolecules*. 2019. Vol. 9, no. 12. P. 752. URL: https://doi.org/10.3390/biom9120752.

5. The probiotic properties of *Lactiplantibacillus plantarum* isolated from plant material / D. S. Holubchyk et al. *Biotechnologia Acta*. 2025. Vol. 18, no. 1. P. 38–43. URL: https://doi.org/10.15407/biotech18.01.038.

6. Regulation of cultivation temperature on biomass and activity of *Bifidobacterium breve* B2798 / K. Liu et al. *Fermentation*. 2024. Vol. 10, no. 11. P. 553. URL: https://doi.org/10.3390/fermentation10110553.

7. Production of L-lactic acid from the soybean vinasse / S. G. Karp et al. 2009. URL: http://hdl.handle.net/1884/18456.

8. Application of response surface methodology to enhancement of biomass production by *Lactobacillus rhamnosus* E/N / M. Polak-Berecka et al. *Brazilian Journal of Microbiology*. 2011. Vol. 42, no. 4. P. 1485–1494. URL: https://doi.org/10.1590/s1517-83822011000400035.

9. Production of *Lactobacillus rhamnosus* BRM 029693 in feed-batch fermentation / R. M. d. Freitas et al. *Research, Society and Development*. 2020. Vol. 9, no. 7. P. e531974280. URL: https://doi.org/10.33448/rsd-v9i7.4280.

10. Choi G.-H., Lee N.-K., Paik H.-D. Optimization of medium composition for biomass production of *Lactobacillus plantarum* 200655 using response surface methodology. *Journal of Microbiology and Biotechnology*. 2021. Vol. 31, no. 5. P. 717–725. URL: https://doi.org/10.4014/jmb.2103.03018.

11. Chang C. P., Liew S. L. Growth medium optimization for biomass production of a probiotic bacterium, *Lactobacillus rhamnosus* ATCC 7469. *Journal of Food Biochemistry*. 2012. Vol. 37, no. 5. P. 536–543. URL: https://doi.org/10.1111/jfbc.12004.

12. Pilot-plant scale biomass production by *Lactobacillus rhamnosus* GG ATCC 53103: a comparison between batch and fed-batch fermentation / M. Armand et al. *Journal of microbiology, biotechnology and food sciences*. 2023. P. e9718. URL: https://doi.org/10.55251/jmbfs.9718.