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TO EXPLORE THE BIOTECHNOLOGICAL POTENTIAL OF *OCEANOBACILLUS SP.* BRI 39

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ABSTRACT

Oceanobacillus sp. BRI 39 is *a* Gram-positive rod-shaped bacterium isolated from Antarctic seawater. The isolate was screened for various extracellular enzymes. Our results revealed the ability of the isolate to produce lipase, pectinase, cellulase, and urease. BRI 39 showed growth and enzyme production at a NaCl concentration of 10%. The activities of all the enzymes (crude extracts) were observed even at 10% NaCl concentration indicating their possible industrial applications. Moreover, the isolate produces bio-surfactant as observed from the hemolysis obtained on blood agar plates. Bio-surfactants have multiple applications in microbial-enhanced oil recovery, hydrocarbon degradation, etc. Overall, the study found that *Oceanobacillus* sp. BRI 39 might be used as a bioresource for a variety of biotechnological purposes.

Keywords: *Oceanobacillus sp. BRI 39, Lipase, Urease, Cellulase, Biosurfactants*

1. INTRODUCTION

Due to developments in the industrial, agricultural, and biotechnological sectors, it is now necessary to look for innovative microorganisms that may be applied in both science and industry [1]. Researchers are always looking for regions where the bacteria can live in harsh

environments. The Antarctic and marine areas of the Southern Ocean, Indian Ocean, etc. have shown to be great homes for these bacteria because of their ability to withstand cold temperatures [2]. Humans have been able to clarify several biological processes that apply to both aquatic and terrestrial creatures with the use of various molecular and biotechnological tools. For the past few years, research into natural marine products, including microbes, has grown significantly. As they generate a variety of antibiotics and other medically beneficial chemicals with a variety of biological actions, they are regarded as being extremely important [3]. The synthesis of commercial enzymes is only one of the numerous advantageous bioactivities that marine microorganisms have previously been shown to possess [4]. The ability of *Oceanobacilli* to create several enzymes, antibiotics, and exopolysaccharides is also well documented. Unfortunately, there are relatively few findings of biosurfactants from *Oceanobacilli* in the literature [5] [6]. Further analysis of the biosurfactant produced by the Antarctic sea water-isolated *Oceanobacillus* sp. BRI 10 [7]. But, attempting to comprehend that function is a difficult process. We may use experiments to try to comprehend the actions of bacteria and use them to our benefit in the industrial or bioremediation fields [8]. As they must adapt to harsh marine environmental circumstances including high or low temperature, alkaline or acidic water, high pressure, and restricted substrate in deep sea water, marine microorganisms have special characteristics [9]. As there is the possibility for marine microorganisms to be employed in industry, these specific properties have drawn numerous researchers to investigate in depth.

2. Materials and Method

2.1. Collection of Bacteria

Oceanobacillus sp. BRI 39 is gram-positive rod-shaped bacteria. This is obtained from the National chemical laboratory Pune (NCL), PUNE, and screened for various extracellular enzymes and biosurfactant production.

2.2. Isolation of Bacteria

The isolates were maintained on Marine salt Medium(MSM) for further studies (composition per 100 ml:- 3 g NaCl,0.7 g Yeast extract,0.96 g $MgSO_4$,0.7 g $MgCl_2$,0.5 g Protease-Peptone,0.2 g KCl, 0.1 g glucose, 0.036 g $CaCl_2$, 0.0026 g NaBr, and 1.8% g agar with pH adjusted to 7.0±0.2).

3. Production of Extracellular Enzyme

Using a straightforward quantitative plate test, bacteria were exposed to the screening of extracellular enzymatic activities including cellulase, lipase, urease, pectinase, and L-asparaginase.

3.1. Lipase activity

Lipase producing micro-organisms produced a zone of clearance (hydrolysis) when their

inappropriate dilutions were spread on the Tributyrin agar medium (TBA) [10] containing per liter of peptone 5 g; beef extract 3 g; tributyrin 10 ml and agar 20 g. The zone size was examined after 12, 24, 36, and 48 hour of incubation at room temp.

3.2. Cellulase activity

The plates were cultured at room temperature for 24 hours after the cultures were streaked on carboxymethyl cellulose (CMC) agar plates. The plates were then submerged in 10% NaCl for 10 minutes and 1% Congo red for 10 minutes. The clear area surrounding the reddish backdrop denotes the tested bacteria's synthesis of cellulose [11].

3.3. Urease Activity

3.3.1. Stuart's urea broth: -Stuart's urea broth was inoculated with 24 hour pure heavy culture inoculum and Shaken the tube gently to suspend the bacteria after that tubes with loosens caps were put in an incubator at room temp. The color change was observed from 48 hours to 7 days. The broth has a vivid pink (fuchsia) hue that indicates urease activity [12].

3.3.2. Christensen's urea agar - Christensen's Urea agar slant was streaked with a heavy inoculum from 18-24 hours of the well-isolated colony and incubated the tube at 37˚c for 48 hours to 7 days. The agar plate has a vivid pink (fuchsia) hue that indicates urease activity. [12] [13].

3.3.3. L-asparaginase- Modified Czapek Dox medium was prepared, containing: 6 g/L Na2HPO4;2g/L KH2PO4; 0.5 g/L NaCl; 20 g/L L-asparagine; 2 g/L glycerol; 0.2 g/L MgSO4.7H2O; 0.005 g/L CaCl2.2H2O; pH 5.5. This medium was supplemented with 2% agar and 0.007% Bromothymol blue (BTB), respectively for the preparation of plates. Plates were inoculated with test cultures and incubated at room temp for 24 and 72 hour, respectively [14].

3.3.4. Pectinase

Petri plates containing autoclaved modified MS medium containing 0.3% KH2PO4, 0.6% Na2HPO4, 02% NH4Cl, 0.5% NaCl, 0.01% MgSO4. 7H2O, 1.5% agar supplemented with 0.2% pectin was prepared. After the solidification of the medium, four wells of 5 mm in diameter were cut into the agar plate. Each well was filled with 25 µL of cell supernatant [15]. After incubating for 24 hours at RT, plates were observed for pectinase activity by flooding them with an iodine solution containing 0.25% iodine, 0.5% potassium iodide, and 31 ml of 20% ethanol. The enzyme activity was observed by measuring the diameter of the clear zone around the well.

4. Biosurfactant Activity

The first screening test for the identification and isolation of Bio-surfactant producing bacteria was the hemolysis test. Isolate was streaked on blood agar medium and incubated at room temp for 24-48 hours to assay for hemolytic activity. The plates were visually examined for areas of clearing surrounding the colonies, a sign of the formation of biosurfactants [16].

5. Activity of crude extract at the high salt concentration (3-10%)

MSM supplemented with 3% NaCl prepared and autoclaved. After cooling the medium was inoculated with BRI 39 and incubated for 48 hour at room temp. After that cell supernatant was prepared and divided into the supernatant tubes containing 3-10% NaCl supplemented with different NaCl concentrations and kept for 1 hour and used for further work. Different media

was prepared and autoclaved for screening of enzymes (lipase and pectinase) after the solidification of the medium. Wells of 5 mm in diameter was cut in the agar plate. Cell supernatant in the amount of 25 L was placed in each well. Plates exhibit full enzyme activity after 48 hours of room-temperature incubation. [17].

6. Results

A. Production of extracellular enzyme

The capacity of *Oceanobacillus* sp. BRI 39 to produce various enzymatic activities is tested (Table 1). Five enzyme activities were shown to have favorable results, whereas four exhibited negative results, according to the findings.

Sl.no	Enzyme produced	Ability to produce enzymatic activity
1	Lipase	+
2	Protease	-
3	Amylase	-
4	Cellulase	+
5	Chitinase	-
6	L-asparaginase	+
7	Urease	+
8	Xylanase	-
9	Pectinase	+

Table.1 (Production of extracellular enzymes)

A.1. Cellulase activity

Bacterial culture was streaked on CMC agar and incubated for 48 hours at room temp and then flooded with Congo red and NaCl showing a zone of inhibition around it.

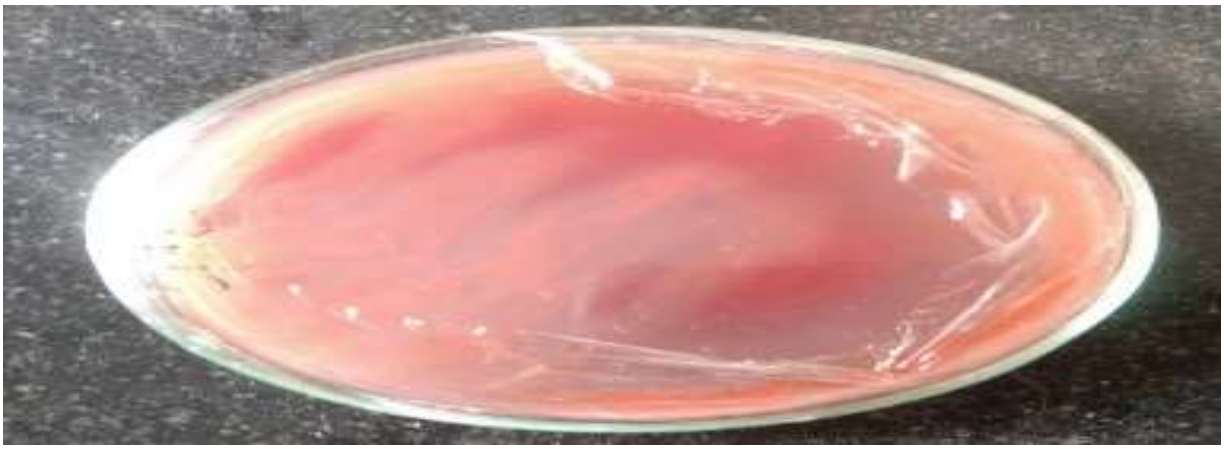

Fig: -1 (Cellulase activity)

A.2. Lipase activity

The tributyrin agar medium was streaked with bacterial colonies. After 12, 24, 36, and 48 hours of incubation at room temperature, the zone size was measured.

Fig: -2 (Lipase activity)

A.3. Urease Activity

A.3.1 Christensen's urea agar

The urea agar slant was streaked with a heavy inoculum from 18-24 hours of the well-isolated colony and Incubated in the tube at 37˚c for 48 hours to 7 days. The agar plate has a vivid pink (fuchsia) hue that indicates urease activity.

Fig: -3 (Urease activity on Christensen's urea agar after 15 to 48h to 7 days)

A.3.2. Stuart's Urea broth

Stuart's urea broth was inoculated with 24 hour pure heavy culture inoculum and Shake the tube gently to suspend the bacteria after that tubes with loosened caps were put in the incubator at room temp. The color change was observed from 48 hours to 7 days. The broth has a vivid pink (fuchsia) hue that indicates urease activity.

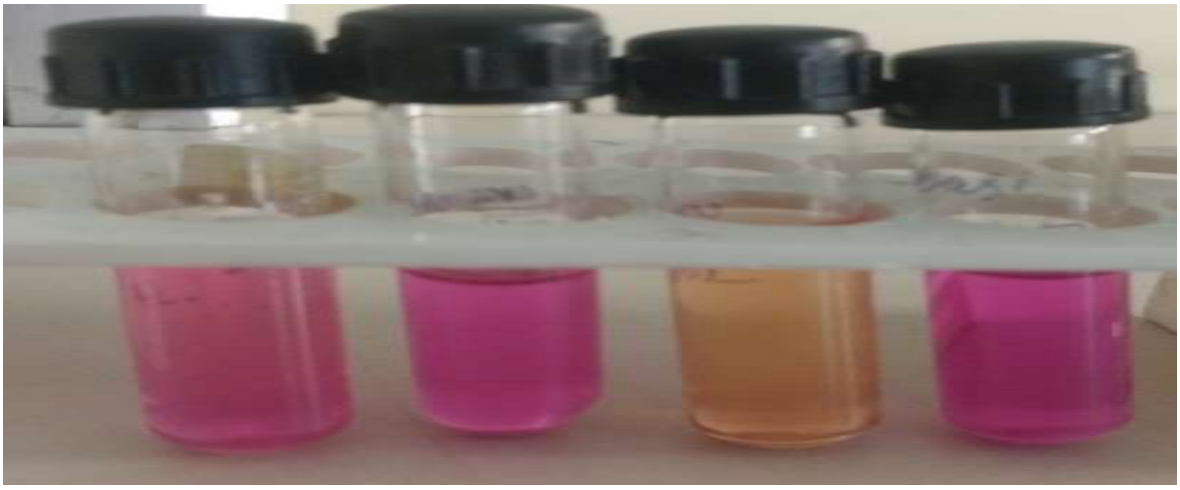

Fig: -4 (Urease activity on Stuart's urea broth after 7 days)

A.3.3. Pectinase

Petri plates containing autoclaved modified MS medium were prepared. After the solidification of the medium, four wells of 5 mm in diameter were cut into the agar plate. Cell supernatant in the amount of 25 µL was placed in each well. Show the area around the well at RT after the plate has been incubating for 48 hours. The diameter of the clear zone surrounding the well was measured to determine the enzyme activity.

Fig:-5 (Pectinase activity)

A.3.4. L-asparaginase Activity

Modified Czapek Dox medium was prepared and Plates containing modified Czpeck dox medium were inoculated with BRI 39 and incubated at room temp for 24 hour. The dark blue color zone shows the zone of enzyme hydrolysis.

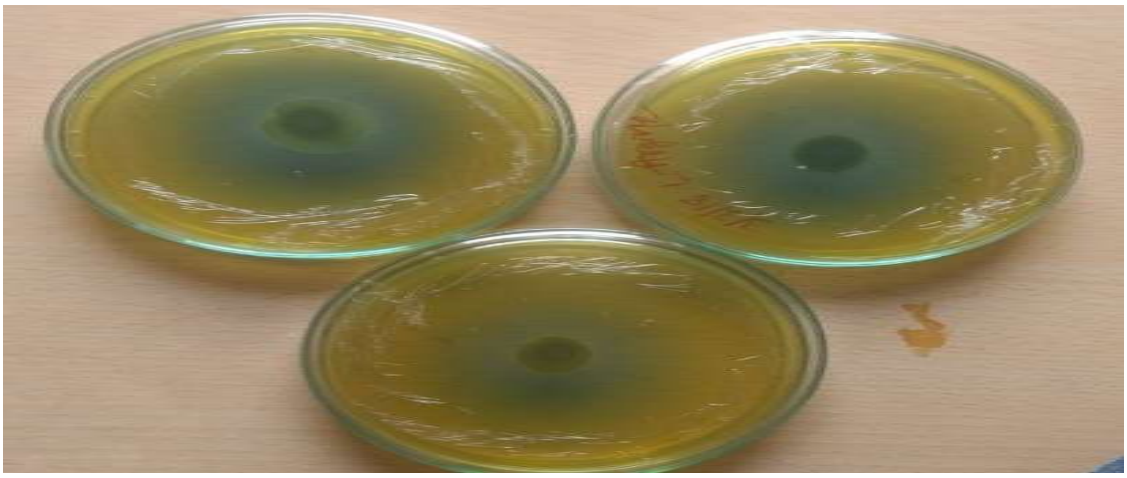

Fig:-6 (L-arginase activity)

B.1. Screening method for bio-surfactant production.

B.1.1. Hemolysis test

The first screening test for the identification and isolation of Bio-surfactant-producing bacteria was the hemolysis test. Isolate was streaked on blood agar medium and incubated at room temp for 24-48 hours to assay for hemolytic activity. The plates were visually examined for areas of clearing surrounding the colonies, a sign of the formation of biosurfactants.

Fig: -7 (Lysis of erythrocytes is indicated by the lytic zone around the colony)

B.2. Activity of crude extract at the high salt concentration (3-10%)

MSM supplemented with 3% NaCl prepared and autoclaved. After cooling the medium was inoculated with BRI 39 and incubated for 48h at room temp. After that cell supernatant was prepared and divided into the tubes containing 3-10% NaCl supplemented with different NaCl concentrations and used for further work. Different media was prepared autoclaved for screening of enzymes (lipase and pectinase). After the solidification of the medium. Wells of 5 mm in diameter was cut in the agar plate. Cell supernatant in the amount of 25 µL was placed in each well. After incubating for 48 hour at room temp, plates show all enzyme activity.

Fig:-8 (Lipase activity at 6-10%)

7. Discussion

The marine environment is the largest habitat on Earth, representing more than 70% of the surface of our planet [18]. Marine environmental conditions are tremendously different from terrestrial ones. The biggest temperature, light, and pressure extremes that life has to deal with are found in the oceans. These extremes boost marine bacterial ecosystem richness and cause great variation in their structure and activity [19]. As a result, it is possible to think of marine bacterial ecology as a superb source of novel bioactive substances with potential uses in

industry, the environment, medicine, and pharmaceuticals [20]. The progress of bio-discovery research is significantly hampered by the paucity of laboratory cultures of the microorganisms that are most prevalent in the environment, despite the enormous diversity of bacteria [21]. Today only a limited scale of research was made on the bioactivity of marine microbes; hence an effort was made to explore the potent strain from a water sample collected from Antarctic seawater. Baharum et al. (2010) concentrated on marine microorganisms that have biotechnological uses in the pharmaceutical and enzyme industries. They also gave a general review of the difficulties researchers confront in exploring and using the marine reserve [9]. One of the biotechnological uses for which isolates were assessed, according to Hala Ezzat [22], was their potential to produce extracellular enzymes such as protease, lipase, amylase, and cellulase as well as the ability to degrade crude oil and chemical dyes and solubilize phosphate. Salt seawater may offer microbial compounds, particularly enzymes, that might be used therapeutically on people without causing any negative side effects [23]. In this present study, obtained microbial strains were checked for the presence of 5 enzymes *viz.,* cellulose, urease, pectinase, lipase, and L-asparaginase. As a result, bacteria from such hostile habitats may possess potential traits that can be used for biotechnological purposes.

8. Conclusion

The highlights of the present work such as cellulase activity, L-asparaginase activity, lipase activity, and pectinase activity indicate its potential for application in the textile industry, chemotherapeutic agent, dairy industry, and clarification of juice. Its applicability for the production of bio-surfactant may be used in the recovery of oil. To describe such action, more research is required.

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10. References

[1] R. Franco-Duarte *et al.*, “Advances in Chemical and Biological Methods to Identify Microorganisms—From Past to Present,” *Microorganisms*, vol. 7, no. 5, p. 130, May 2019, doi: 10.3390/microorganisms7050130.

[2] A. Poli, I. Finore, I. Romano, A. Gioiello, L. Lama, and B. Nicolaus, “Microbial Diversity in Extreme Marine Habitats and Their Biomolecules,” *Microorganisms*, vol. 5, no. 2, p. 25, May 2017, doi: 10.3390/microorganisms5020025.

[3] S. V. Jagannathan, E. M. Manemann, S. E. Rowe, M. C. Callender, and W. Soto, “Marine Actinomycetes, New Sources of Biotechnological Products,” *Marine Drugs*, vol. 19, no. 7, p. 365, Jun. 2021, doi: 10.3390/md19070365.

[4] T. H. Cheng, N. Ismail, N. Kamaruding, J. Saidin, and M. Danish-Daniel, "Industrial enzymes-producing marine bacteria from marine resources," *Biotechnology Reports*, vol. 27, p. e00482, Sep. 2020, doi: 10.1016/j.btre.2020.e00482.
[5] R. Margesin and F. Schinner, "Potential of halotolerant and halophilic microorganisms for biotechnology," *Extremophiles*, vol. 5, no. 2, pp. 73–83, Apr. 2001, doi: 10.1007/s007920100184.
[6] J. Lu, Y. Nogi, and H. Takami, "Oceanobacillus iheyensis gen. nov., sp. nov., a deep-sea extremely halotolerant and alkaliphilic species isolated from a depth of 1050 m on the Iheya Ridge," *FEMS Microbiology Letters*, vol. 205, no. 2, pp. 291–297, Dec. 2001, doi: 10.1111/j.1574-6968.2001.tb10963.x.
[7] V. V. Jadhav *et al.*, "Studies on biosurfactant from Oceanobacillus sp. BRI 10 isolated from Antarctic sea water," *Desalination*, vol. 318, pp. 64–71, Jun. 2013, doi: 10.1016/j.desal.2013.03.017.
[8] O. Ojuederie and O. Babalola, "Microbial and Plant-Assisted Bioremediation of Heavy Metal Polluted Environments: A Review," *IJERPH*, vol. 14, no. 12, p. 1504, Dec. 2017, doi: 10.3390/ijerph14121504.
[9] S. N. Baharum, E. K. Beng, and M. A. A. Mokhtar, "Marine Microorganisms: Potential Application and Challenges," *J. of Biological Sciences*, vol. 10, no. 6, pp. 555–564, Aug. 2010, doi: 10.3923/jbs.2010.555.564.
[10] D. Kumar, L. Kumar, S. Nagar, C. Raina, R. Parshad, and V. K. Gupta, "Screening, isolation and production of lipase/esterase producing Bacillus sp. strain DVL2 and its potential evaluation in esterification and resolution reactions," 2012.
[11] R. C. Kasana, R. Salwan, H. Dhar, S. Dutt, and A. Gulati, "A Rapid and Easy Method for the Detection of Microbial Cellulases on Agar Plates Using Gram's Iodine," *Curr Microbiol*, vol. 57, no. 5, pp. 503–507, Nov. 2008, doi: 10.1007/s00284-008-9276-8.
[12] M. J. Zimbro, Ed., *Difco & BBL Manual: manual of microbiological culture media*, 2. ed. Sparks, MD: Becton, Dickinson, 2009.
[13] C. A. Stuart, E. Van Stratum, and R. Rustigian, "Further Studies on Urease Production by *Proteus* and Related Organisms," *J Bacteriol*, vol. 49, no. 5, pp. 437–444, May 1945, doi: 10.1128/jb.49.5.437-444.1945.
[14] R. V. Mahajan, S. Saran, R. K. Saxena, and A. K. Srivastava, "A rapid, efficient and sensitive plate assay for detection and screening of L -asparaginase-producing microorganisms," *FEMS Microbiol Lett*, vol. 341, no. 2, pp. 122–126, Apr. 2013, doi: 10.1111/1574-6968.12100.
[15] A. Tariq and Z. Latif, "Isolation and biochemical characterization of bacterial isolates producing different levels of polygalacturonases from various sources".
[16] V. Walter, C. Syldatk, and R. Hausmann, "Screening Concepts for the Isolation of Biosurfactant Producing Microorganisms," in *Biosurfactants*, vol. 672, R. Sen, Ed. New York, NY: Springer New York, 2010, pp. 1–13. doi: 10.1007/978-1-4419-5979-9_1.
[17] Computer Aided Drug Designing and Molecular Modeling Lab, Department of Bioinformatics, Alagappa University, Karaikudi-630 003, Tamil Nadu, India *et al.*, "Screening, isolation and characterization of biosurfactant producing Bacillus subtilis strain

ANSKLAB03," *Bioinformation*, vol. 14, no. 06, pp. 304–314, Jun. 2018, doi: 10.6026/97320630014304.

[18] M. J. Costello and C. Chaudhary, "Marine Biodiversity, Biogeography, Deep-Sea Gradients, and Conservation," *Current Biology*, vol. 27, no. 11, pp. R511–R527, Jun. 2017, doi: 10.1016/j.cub.2017.04.060.

[19] S. C. Doney *et al.*, "Climate Change Impacts on Marine Ecosystems," *Annu. Rev. Mar. Sci.*, vol. 4, no. 1, pp. 11–37, Jan. 2012, doi: 10.1146/annurev-marine-041911-111611.

[20] A. Beygmoradi and A. Homaei, "Marine microbes as a valuable resource for brand new industrial biocatalysts," *Biocatalysis and Agricultural Biotechnology*, vol. 11, pp. 131–152, Jul. 2017, doi: 10.1016/j.bcab.2017.06.013.

[21] I. Joint, M. Mühling, and J. Querellou, "Culturing marine bacteria - an essential prerequisite for biodiscovery: Culturing marine bacteria," *Microbial Biotechnology*, vol. 3, no. 5, pp. 564–575, Sep. 2010, doi: 10.1111/j.1751-7915.2010.00188.x.

[22] H. E. Abou El-Hassayeb, "Screening of Isolated Marine Bacteria for Multiple Biotechnological Applications," *Int.J.Curr.Microbiol.App.Sci*, vol. 5, no. 7, pp. 424–430, Jul. 2016, doi: 10.20546/ijcmas.2016.507.046.

[23] F. Izadpanah Qeshmi, A. Homaei, P. Fernandes, and S. Javadpour, "Marine microbial L-asparaginase: Biochemistry, molecular approaches and applications in tumor therapy and in food industry," *Microbiological Research*, vol. 208, pp. 99–112, Mar. 2018, doi: 10.1016/j.micres.2018.01.011.