DOI 10.66073/ReFocus-Conf/3010097

HYDROLYTIC ENZYME ACTIVITY AND SALT TOLERANCE OF *BACILLUS INAQUOSORUM* STRAIN J13 ISOLATED FROM OIL-CONTAMINATED SOIL

Abdullayeva Yulduz Alisher qizi[1], **Abdullayeva Jasmina A.**[2], **Axmedova Zahro Raxmatovna**[1]

[1] *Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan*

[2] *3rd-year student, General Medicine program, Faculty of International Education, Termez Branch of Tashkent State Medical University, Termez, Uzbekistan*

Abstract: This study investigated the hydrolytic enzyme activity and salt tolerance of *Bacillus inaquosorum* strain J13 isolated from oil-contaminated soil in Surkhandarya Region, Uzbekistan. The taxonomic affiliation of the strain was assessed by BLAST comparison of its 16S rRNA gene sequence. Activities of protease (47 mm), lipase (42 mm), xylanase (37 mm), cellulase (34 mm), and amylase (30 mm) were detected, whereas pectinase activity was not observed. The strain was able to grow at NaCl and Na_2SO_4 concentrations of up to 2 M. These findings support further evaluation of strain J13 as a candidate for bioremediation research.

Keywords: Bacillus inaquosorum J13, oil-contaminated soil, bioremediation, hydrolytic enzymes, lipase, protease, salt tolerance, hydrocarbons.

Annotatsiya: Ushbu tadqiqotda Surxondaryo viloyatining neft bilan ifloslangan tuprog‘idan ajratilgan *Bacillus inaquosorum* J13 shtammining gidrolitik ferment faolligi va tuzga chidamliligi o‘rganildi. Shtammning taksonomik mansubligi 16S rRNK geni ketma-ketligini BLAST orqali taqqoslash asosida baholandi. Proteaza (47 mm), lipaza (42 mm), ksilanaza (37 mm), sellulaza (34 mm) va amilaza (30 mm) faolligi qayd etildi, pektinaza faolligi aniqlanmadi. Shtamm NaCl va Na_2SO_4 ning 2 M gacha konsentratsiyalarida o‘sish qobiliyatini saqladi. Natijalar J13 izolyatini bioremediatsiya uchun istiqbolli nomzod sifatida keyingi tadqiqotlarda baholash zarurligini ko‘rsatadi.

Kalit so‘zlar: Bacillus inaquosorum J13, neft bilan ifloslangan tuproq, bioremediatsiya, gidrolitik fermentlar, lipaza, proteaza, tuzga chidamlilik, uglevodorodlar.

Аннотация: В исследовании изучены гидролитическая ферментативная активность и устойчивость к солям штамма *Bacillus inaquosorum* J13, выделенного из загрязнённой нефтью почвы Сурхандарьинской области. Таксономическую принадлежность оценивали путём сравнения последовательности гена 16S рРНК с использованием BLAST. Обнаружена активность протеазы (47 мм), липазы (42 мм), ксиланазы (37 мм), целлюлазы (34 мм) и амилазы (30 мм); активность пектиназы не выявлена. Штамм сохранял способность к росту при концентрациях NaCl и Na_2SO_4 до 2 М. Полученные результаты позволяют рассматривать изолят J13 как перспективный объект для дальнейшего изучения его потенциала в биоремедиации.

Ключевые слова: Bacillus inaquosorum J13, загрязнённая нефтью почва, биоремедиация, гидролитические ферменты, липаза, протеаза, солеустойчивость, углеводороды.

FOR CITATION
Abdullayeva Y.A., Abdullayeva J.A., Axmedova Z.R. Hydrolytic enzyme activity and salt tolerance of Bacillus inaquosorum strain J13 isolated from oil-contaminated soil // Proceedings of the Republican Scientific and Practical Conference “Status and Development Prospects of Fundamental and Applied Microbiology: The Viewpoint of Young Scientists”. – Tashkent, 2026. – P. 350–352.
DOI: https://doi.org/10.66073/ReFocus-Conf/3010097

Introduction

Contamination of soil with petroleum hydrocarbons during oil extraction, transportation, and refining adversely affects its physicochemical properties, fertility, and microbiological balance. One of the most promising approaches to the remediation of contaminated soils is bioremediation, which relies on the biodegradation potential of microorganisms [1]. Bacteria of the genus *Bacillus* are distinguished by their ability to form spores, their tolerance to adverse conditions, and, in some strains, the ability to synthesize biosurfactants [2, 3].

Soil lipase activity is one of the indicators used to assess biological processes in oil-contaminated environments [4]; however, enzyme activity alone does not prove the direct degradation of petroleum hydrocarbons. *Bacillus inaquosorum* was originally described as a subspecies of *B. subtilis* and was later elevated to species rank [5, 6]. Surfactin and other lipopeptides have been identified in *B. inaquosorum* strain KR2-7 [7]. The aim of the present study was to evaluate the taxonomic affiliation, hydrolytic enzyme activity, and salt tolerance of isolate J13 obtained from oil-contaminated soil.

Materials and Methods

The object of the study was bacterial isolate J13, obtained from oil-contaminated soil in Surkhandarya Region. Colony morphology, cell shape, and Gram reaction were examined. Taxonomic affiliation was assessed by BLAST comparison of the 16S rRNA gene sequence (1,501 bp) with sequences in the NCBI database. Amylase, cellulase, xylanase, pectinase, protease, and lipase activities were tested by spot inoculation on solid media containing the corresponding substrates. The plates were incubated at 30 °C for 72 h. Amylase activity was detected with iodine solution, and cellulase, xylanase, and pectinase activities with 0.5% Congo red. Protease activity was assessed on skim milk medium, and lipase activity on medium containing Tween 80 or tributyrin. Activity was expressed as the diameter of the hydrolysis zone (mm); gelatinase activity was determined by liquefaction of gelatin medium. Salt tolerance was assessed by growth on nutrient media containing various concentrations of NaCl and Na_2SO_4. The highest concentration at which growth was observed was recorded as the salt tolerance of the strain under laboratory conditions.

Results and Discussion

On meat-peptone agar, isolate J13 exhibited colony characteristics typical of the genus *Bacillus*. The cells were rod-shaped, Gram-positive, and spore-forming. Based on BLAST comparison of the 16S rRNA gene sequence, the isolate was identified as *Bacillus inaquosorum* J13. Confirmation by additional phylogenetic analysis is advisable to reliably distinguish closely related species within the *B. subtilis* species complex.

In the enzymatic assays, the largest hydrolysis zones were recorded for protease (47 mm) and lipase (42 mm). Xylanase, cellulase, and amylase activities produced zones of 37, 34, and 30 mm, respectively. Gelatinase activity was positive, whereas pectinase activity was not detected. Protease and lipase activities indicate the potential of the strain to hydrolyze the corresponding organic substrates. Although lipase activity is a useful preliminary indicator in studies of oil-contaminated environments, the extent of oil degradation needs to be determined in a separate experiment [4].

The detection of biosurfactants in *B. inaquosorum* KR2-7 [7] provides a basis for testing whether isolate J13 also produces such compounds; however, this property was not directly measured in the present study. Isolate J13 retained the ability to grow at NaCl and Na_2SO_4 concentrations of up to 2 M. This result indicates potential tolerance to high salt concentrations, but bioremediation efficiency in naturally saline soils must be confirmed in separate trials.

DOI: 10.66073/ReFocus-Conf/3010097

Conclusion

Isolate J13, obtained from oil-contaminated soil in Surkhandarya Region, was identified as *Bacillus inaquosorum*. The strain exhibited protease (47 mm), lipase (42 mm), xylanase (37 mm), cellulase (34 mm), and amylase (30 mm) activities, while pectinase activity was not detected. In addition, the isolate grew at NaCl and Na_2SO_4 concentrations of up to 2 M. These properties provide a basis for further evaluation of isolate J13 as a promising candidate for bioremediation. Future studies should directly determine the extent of petroleum hydrocarbon degradation, biosurfactant synthesis, and the stability of the strain under environmental conditions.

References

1. Xu X., Liu W., Tian S. et al. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. Frontiers in Microbiology. 2018;9:2885. https://doi.org/10.3389/fmicb.2018.02885
2. Das K., Mukherjee A.K. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresource Technology. 2007;98(7):1339–1345. https://doi.org/10.1016/j.biortech.2006.05.032
3. Montagnolli R.N., Lopes P.R.M., Bidoia E.D. Assessing *Bacillus subtilis* biosurfactant effects on the biodegradation of petroleum products. Environmental Monitoring and Assessment. 2015;187:4116. https://doi.org/10.1007/s10661-014-4116-8
4. Margesin R., Zimmerbauer A., Schinner F. Soil lipase activity – a useful indicator of oil biodegradation. Biotechnology Techniques. 1999;13:859–863. https://doi.org/10.1023/A:1008928308695
5. Rooney A.P., Price N.P.J., Ehrhardt C., Swezey J.L., Bannan J.D. Phylogeny and molecular taxonomy of the *Bacillus subtilis* species complex and description of *Bacillus subtilis* subsp. *inaquosorum* subsp. nov. International Journal of Systematic and Evolutionary Microbiology. 2009;59:2429–2436. https://doi.org/10.1099/ijs.0.009126-0
6. Dunlap C.A., Bowman M.J., Zeigler D.R. Promotion of *Bacillus subtilis* subsp. *inaquosorum*, *Bacillus subtilis* subsp. *spizizenii* and *Bacillus subtilis* subsp. *stercoris* to species status. Antonie van Leeuwenhoek. 2020;113(1):1–12. https://doi.org/10.1007/s10482-019-01354-9
7. Kamali M., Guo D., Naeimi S., Ahmadi J. Perception of biocontrol potential of *Bacillus inaquosorum* KR2-7 against tomato Fusarium wilt through merging genome mining with chemical analysis. Biology. 2022;11(1):137. https://doi.org/10.3390/biology11010137