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THE INFLUENCE OF MICROBIAL ENZYMES IN BIOREMEDIATION

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Summary

Applied microbiology is one of the fundamental branches of biotechnology, as it plays a role in the production and isolation of products obtained or used from microorganisms. The major products of microorganisms include microbial enzymes, microbial biomass, metabolites and compounds derived from microbial transformation. Large amounts of microbial enzymes are produced on an industrial scale, especially hydrolytic enzymes, i.e., amylases, proteinases, lipases, cellulases, and others. These enzymes are used in various industries, but due to increasing environmental pollution and the need to remediate contaminated soils, they are also successfully used in bioremediation process. In bioremediation, microorganisms (and their enzymes) are used to reduce the concentration of pollutants in the soil, usually by degrading them to less or non-harmful products (carbon dioxide, water and biomass).

Key words: bioremediation, microbial enzymes, soil pollution, heavy metals, pesticides

Introduction

During anthropogenic activity, the destructive effects of xenobiotic pollutants produced, such as azo dyes, phenols, pesticides, heavy metals, and others, cause deleterious effects on the entire ecosystem. Most of these chemicals cannot be biodegraded and are very persistent in the environment (Elekwachi et al., 2014). Respiratory diseases, allergies, perinatal disorders, mortality, cancer, cardiovascular and mental disorders are just some of the harmful effects these pollutants have on humans, but they also affect water, air, plants, animals, soil, sediment and all microorganisms. Conventional chemical and physical methods of removing these pollutants have not shown promising results due to their high economic cost, stringent requirements, and general public disapproval. The application of these unfriendly techniques often ended in the formation of even more toxic secondary pollutants (Rao et al., 2010; Karigar and Rao, 2011). The use of microbial enzymes is a good alternative because it is environmentally friendly, innovative, cost-effective, and promising (Tahri et al., 2013). Nevertheless, bioremediation has its limitations. It is a slow process that occurs in nature. Therefore, this approach has been improved and very successful by using genetically modified microorganisms that can produce larger amounts of the desired enzymes to degrade and catalyze xenobiotic pollutants. Microbial enzymes, unlike most microorganisms, can be used under extreme conditions, are effective against different concentrations of the pollutant, and are active even in the presence of antagonists and potential microbial enemies (Raveendran et al., 2018). They act only on their substrate

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and due to their smaller size, they are more mobile than microorganisms, which makes them a good alternative to overcome most of the disadvantages associated with microorganisms (Nannipieri and Bollag, 1991; Karam and Nicell, 1997; Nicell, 2001; Gianfreda and Bollag, 2002; Gianfreda and Rao, 2004). The aim of this article is to provide an overview of the enormous potential that microbial enzymes have in bioremediation in general for the future problems of waste disposal and environmental remediation.

Bacterial and fungal enzymes

Bacteria and many other fungi used for bioremediation are found in the biosphere. They grow in a wide variety of environmental conditions and retain their metabolic ability. There are many microbial enzymes that have been explored to date, but among the most representative enzymes derived from genetically modified microorganisms used in bioremediation processes are lacases, hydrolases, dehydrogenases, proteases, lipases, and others (Figure 1). These enzymes are mainly derived from aerobic bacteria (Table 1) such as *Mycobacterium*, *Pseudomonas*, *Rhodococcus*, *Sphingomonas*, and *Alcaligenes*. Anaerobic bacteria have been mainly used for bioremediation of chloroform, dechlorination of trichloroethylene (TCE) and polychlorinated biphenyls (PCBs) (Sharma, 2012).

White rot fungi produce different patterns of oxidative enzymes that are very effective and successfully used in bioremediation (Bumpous, 1993; Reddy, 1995; Pointing, 2001; Asgher et al., 2008; Rubilar et al., 2008). They tolerate higher concentrations of xenobiotic pollution than bacteria and are ubiquitous. Because they grow by hyphal dispersal, they can be found almost everywhere, whereas bacteria cannot. Because of their ability to grow with low-cost substrates, such as agricultural plant waste, they are good candidates for low-cost bioremediation. *Trametes versicolor*, the most common Basidiomycete with lygninolytic ability, produces laccase and takes agricultural waste as substrate (*e.g.*, wood crisps from corn and garden wheat compost). In contrast, the Ascomycete *Aspergillus niger* produces xylanases and cellulases, different enzymes, from the same agricultural wastes (Sánchez, 2009). Because carcinogenic azo dyes are commonly used in a variety of industries, they are a problem for the environment and human health. White rot fungi are good dye degraders and provide an attractive alternative for this growing problem (Asgher et al., 2008). Fungi produce laccases extracellularly as a secondary product of their metabolism during fermentation (Morozova, 2007). In addition to Basidiomycetes and Ascomycetes, Deuteromycetes are also a well-studied class of fungi for bioremediation (Gochev and Krastanov, 2007; Sadhasivam et al., 2008).

Table 1 The most common microbial enzymes produced by various bacteria and their application
Tablica 1. Najčešći mikrobni enzimi koje proizvode razne bakterije i njihova primjena

Microorganism/enzyme	Application	Reference
Pseudomonas putida F6/ laccase	Degradation of synthetic dyes	McMahon *et al.,* 2007
Streptomyces cyaneus/ laccase	Oxidation of micropollutants (BPA, DFC, MFA)	Margot *et al.,* 2013
Ancylobacter aquaticus/ dehalogenase	Degradation of halogen acid ester	Kumar *et al.,* 2016
Pseudomonas sp. TL/ dehalogenase	Degradation of halogen acid	Liu *et al.,* 1994
Bacillus subtilis/ serine protease	Degradation of casein and feather	Suh and Lee., 2001
Bacillus pumilus/ Keratinolytic proteases	Complete degradation of feathers	El-Refai *et al.,* 2005
Bacillus subtilis/ protease	Deproteinization of crustacean wastes	Yang *et al.,* 2000
Bacillus subtilis/ lipase	Bioremediation of wastewater	Haniya *et al.,* 2017
Bacillus pumilus/ lipase	Degradation of palm oil containing industrial wastewater	Saranya *et al.,* 2019
Bacillus subtilis/ lipase	Removal of trough oil or grease stains from detergent	Saraswat *et al.,* 2017

Laccases

Of all oxidative enzymes, laccases (benzenediol oxygen oxidoreductases, EC 1.10.3.2) have the best catalytic properties. They are multicopper oxidases composed of monomeric, dimeric, and tetrameric glycoproteins found in plants, bacteria, and fungi (Shraddha et al., 2011). They are commonly known as blue oxidases and hold great potential for bioremediation because of their ability to oxidize phenolic compounds, aromatic amines, and ascorbate. Laccases have different substrate preference depending on the type of microorganism producing the enzyme (Giardina et al., 2010; Madhavi and Lele, 2009). The catalytic capacity is based on the oxidation of substrates and their functional groups. Two water molecules are formed with simultaneous electron loss of a single oxygen molecule (Chandra and Chowdhary, 2015) (Figure 1). The most interesting biochemical property of laccases is their high stability under various environmental conditions: pH, salt concentration, temperature, and organic solvents (Guan et al., 2018). Nutrient content can affect the synthesis and secretion of laccases in a culture medium. Laccase expression has been reported to be regulated by a variety of factors that act in a synergistic manner (Terrón et al., 2004; Sóle et al., 2008; Galhaup et al., 2002). Metals (*e.g.*, copper) regulate transcript levels in *T. versicolor* (Collins and Dobson, 1997). The oxidative state of manganese, silver, and cadmium has a strong influence on the transcription levels of laccase (Soden and Dobson, 2001; Baldrian and Gabriel, 2002).

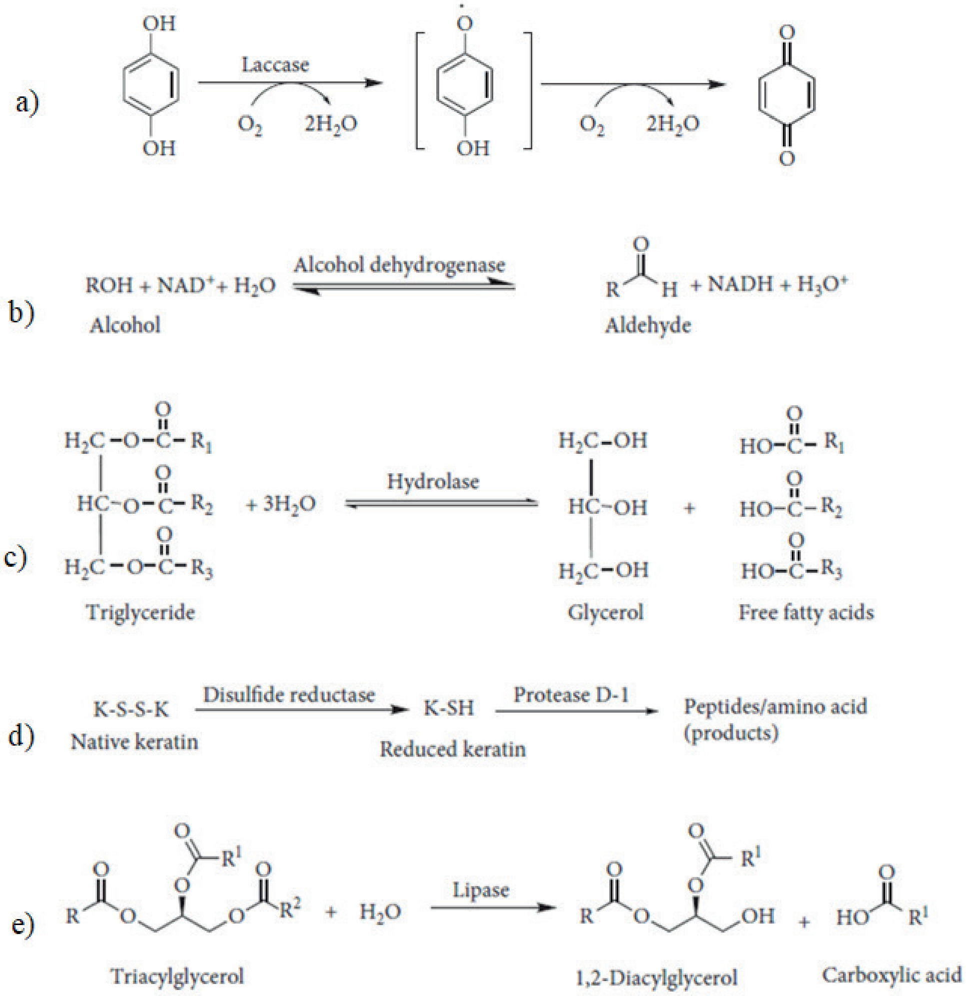

Figure 1. General enzymatic reaction catalysed by some microbial enzymes used in bioremediation: a) laccase, b) dehydrogenase, c) hydrolase, d) protease, and e) lipase. Figure was taken from the article S. Bhandari et al., 2021. with modifications by the author.

Dehalogenase

Microbial dehalogenase (EC 3.8.1.5) plays an important role in the bioremediation of chlorine-containing environments contaminated with halogens. It can cleave the carbon-halogen bond by three mechanisms (hydrolysis, reduction, and oxidation) to eliminate halogens (Wang et al., 2018a; Wang et al., 2018b). Dehalogenase replaces the halogen atom with a hydroxyl group from water and a hydrogen atom from a hydrogen molecule, which is appreciated. Microbially mediated reductive dehalogenation associated with growth is referred to as organohalide respiration. Dehalogenase has been successfully isolated from the bacterial genera *Dechalobacter*, *Pseudomonas*, and *Ancyclobacter* (Pandey et al., 2017). These bacterial genera have released amounts of this enzyme, which is capable of degrading various halogenated substrates, such as pentachlorophenol and other chlorinated phenols on 3,5-dichlorophenol (DCP), respectfully (Fricker et al., 2014).

Hydrolase

Hydrolases belong to a group of hydrolytic enzymes that cleave the chemical bonds of large toxic compounds to convert them to less toxic ones. These enzymes show high tolerance to water-miscible solvents and are readily available, environmentally friendly, and very inexpensive (Elekwachi, 2014). Microbial hydrolytic enzymes are used for the degradation of plastics, insecticides and pesticides, oil-contaminated soils, food production and processing wastes, etc. (Kumar and Sharma, 2019). Pollutants such as carbofuran, diazinon, and parathion can be degraded by hydrolysis using hydrolases produced by *Bacillus cereus*, *Achromobacter*, *Flavobacterium*, *Pseudomonas*, and *Nocardia* (Sutherland et al., 2002). One of the most used pesticides in agriculture are the organophosphate compounds (OP). OPs are a diverse group of chemicals used in the production of insecticides and nerve agents that are highly lethal neurotoxins. These pollutants can be degraded by the hydrolysis of phosphodiester bonds and, in the case of malathion and pyrethroids, by the hydrolysis of carboxylester bonds (Singh, 2014). The gene for organophosphorus hydrolase isolated from *Pseudomonas diminuta* (GenBank accession number M20392) was successfully expressed in *E. coli*. The results obtained with this modified microorganism were detoxification that reached 80% efficiency (Kapoor and Rajagopal, 2011). Another pesticide commonly used in agriculture, s-triazine, is degraded by *Pseudomonas sp.* through the production of hydrolases, cleaving the s-triazine ring (Seeger et al., 2010). Nitrile compounds like cyanide are highly toxic and lethal. The bacteria *Nocardia sp*. and *Rhodococcus sp*. produce enzymes that degrade these compounds almost completely in two steps, one of which is the direct hydrolysis of nitriles (Rao et al., 2010). Proteases and lipases belong to the group of extracellular hydrolytic enzymes with enhanced potential for bioremediation and are described in continuation.

Proteases

Proteases hydrolytically cleave peptide bonds between proteins and polypeptides. They can be isolated from animals, plants, bacteria and fungi. Based on the position of their active site in the peptide chain, peptides are divided into two groups: Endopeptidases and Exopeptidases (Rao et al., 1998). Endopeptidases act randomly and are found in the inner region of polypeptide chains, where they are further classified into six groups based on their active site.

Exopeptidases, on the other hand, act at the end of the aforementioned polypeptide chains (Li et al., 2013). Proteases are found in many fungal species, *e.g.*, *Aspergillus* or bacteria such as *Amycolatopsis* or *Bacillus sp.* (de Souza et al., 2015). Due to their high production, efficiency, and low cost, protease enzymes find their use in the pharmaceutical and food industries, as well as in the production of detergents and leather, and in wastewater treatment (Kieliszek et al., 2021). These enzymes can readily degrade polymers such as poly(hydroxybutyrate) (PHB) by breaking α-ester bonds, lipase ⍰-ω-bonds, and depolymerase-β-esters, indicating strong bioremediation abilities (Haider et al., 2019). Keratinase is one of the protease enzymes purified from the *Stenotrophomonas maltophilia* KB13 strain and has been successfully used for the biodegradation of all poultry waste containing insoluble keratin protein (Bhange et al., 2016). The keratinase enzymes found in bacteria such as *Bacillus sp*. and *Pseudomonas sp*. have shown significant degradation of poultry litter, especially feathers, releasing large amounts of antioxidants and amino acids. These products are used as fertilizers to promote plant growth and as dietary supplements (Mazotto et al., 2011; Jeong et al., 2010). Keratinase is also used in the leather industry for processes that have replaced the use of toxic chemicals (Na_2S and CaO) to avoid additional pollution (Akhter et al., 2020). Alkaline proteases produced by *Bacillus licheniformis* MP1 have shown 75% efficiency in deproteinization of crustacean wastes (Jellouli et al., 2011).

Lipases

Lipases catalyze the hydrolysis of the triglyceride ester bond into free fatty acids and glycerol (Casas-Godoy et al., 2012). They are derived from microorganisms, plants, and animals. Because lipases are widely distributed and can degrade hydrocarbons from contaminated soils and waters, they are an ideal candidate for bioremediation of organic pollutants. Due to their high stability, high substrate specificity, short processing time, low energy requirement, and low production cost, lipases are widely used in the paper, pulp, cosmetics, and petroleum industries (Arora et al., 2020; Gurung et al., 2013). They have high bioremediation potential and are effectively used for the degradation of wastewater, oil, and petroleum contaminants (Basheer et al., 2011). Lipase can be isolated from both bacteria and actinomycetes, mainly from the genera *Bacillus* and *Pseudomonas* (Priyanka et al., 2019). It has been reported that the toxicity of crude oil contamination was reduced by 80% within one week by using lipase from *Pseudomonas aeruginosa* (Amara and Salem, 2009; Verma et al., 2012). In the transesterification process, when alcohols such as methanol or ethanol (external carbon in the activated sludge) are present, Amano lipase from *Pseudomonas* was found to be crucial for the degradation of parabens (Wang et al., 2018a).

Conclusion

Enzyme-based processes are preferable to the traditionally used chemical processes. They are environmentally friendly, stable, more soluble, less costly, higher yielding, and their production is safe. Microbial enzymes are easier to produce on a large scale compared to enzymes derived from plants and animals. By using biotechnologically modified microorganisms, where the modified microorganisms can overexpress the desired gene, bioremediation processes can be made more effective and substrate-specific. This is extremely important given the contaminants occurring in nature that threaten the entire ecosystem. Unfortunately, the decline of human health is the main indicator of how xenobiotic contaminants can find their way into any life form. The use of enzymes of microbial origin in the degradation of toxic organic and inorganic chemicals is justified and acceptable when the product of the microbial-enzyme-mediated reaction is less toxic than the substrate itself. The potential of these enzymes is still poorly understood, as is the combined effect they might have in bioremediation. In summary, many studies consistently have been concluding that this class of enzymes are powerful green tools that every biotechnology industry will need to incorporate eventually.

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REFERENCES

1. Akhter, M., Wal Marzan, L., Akter, Y., & Shimizu, K. (2020): Microbial Bioremediation of Feather Waste for Keratinase Production: An Outstanding Solution for Leather Dehairing in Tanneries. Microbiology insights, 13, 1178636120913280. https://doi.org/10.1177/1178636120913280
2. Amara, A.A. and Salem, S.R. (2009): Degradation of Castor Oil and Lipase Production by Pseudomonas aeruginosa. American-Eurasian Journal of Agriculture and Environment, 5(4): 556-563.
3. Arora, N.k., Mishra, J. and Mishra, V. (2020): Microbial Enzymes: Roles and Applications in Industries in Microorganisms for Sustainability. Springer, Berlin, Germany.
4. Asgher, M., Bhatti, H. N., Ashraf, M., & Legge, R. L. (2008): Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. Biodegradation, 19(6): 771–783. doi:10.1007/s10532-008-9185-3
5. Baldrian, P., & Gabriel, J. (2002): Copper and cadmium increase laccase activity inPleurotus ostreatus. FEMS Microbiology Letters, 206(1): 69–74. doi:10.1111/j.1574-6968.2002.tb10988.x
6. Basheer, S. M., Chellappan, S., Beena, P. S., Sukumaran, R. K., Elyas, K. K., & Chandrasekaran, M. (2011): Lipase from marine Aspergillus awamori BTMFW032: Production, partial purification, and application in oil effluent treatment. New Biotechnology, 28(6): 627–638. doi:10.1016/j.nbt.2011.04.007
7. Bhandari, S., Kumar Poudel, D., Marahatha, R., Dawadi, S., Khadayat, K., Phuyal, S., Shrestha, S., Gaire, S., Basnet, K., Khadka, U., Parajuli, N. (2021): Microbial Enzymes Used in Bioremediation, Journal of Chemistry, 2021(8849512), 1-17. https://doi.org/10.1155/2021/8849512
8. Bhange, K., Chaturvedi, V., & Bhatt, R. (2016): Feather degradation potential of Stenotrophomonas maltophilia KB13 and feather protein hydrolysate (FPH) mediated reduction of hexavalent chromium. Biotech, 6(1). doi:10.1007/s13205-016-0370-5
9. Bumpus, J.A. (1993): White-rot fungi and their potential use in soil bioremediation processes. In J.-M. Bollag, & G. Stotzky (Eds), Soil Biochemistry (pp.65-100). Marcel Dekker, New York.
10. Casas-Godoy, L., Duquesne, S., Bordes, F., Sandoval, G., & Marty, A. (2012): Lipases: an overview. Methods in molecular biology (Clifton, N.J.), 861, 3–30. https://doi.org/10.1007/978-1-617
11. Chandra, R., & Chowdhary, P. (2015): Properties of bacterial laccases and their application in bioremediation of industrial wastes. Environmental science. Processes & impacts, 17(2): 326–342. https://doi.org/10.1039/c4em00627e

12. Collins, P.J. and Dobson, A. D. W. (1997): Regulation of Laccase Gene Transcript in Trametes versicolor. Applied and Environmental Microbiology, 63: 3444-3450.

13. de Souza, P. M., Bittencourt, M. L., Caprara, C. C., de Freitas, M., de Almeida, R. P., Silveira, D., Fonseca, Y. M., Ferreira Filho, E. X., Pessoa Junior, A., & Magalhães, P. O. (2015): A biotechnology perspective of fungal proteases. Brazilian journal of microbiology, (2): 337–346. https://doi.org/10.1590/S1517-838246220140359

14. Elekwachi, C.O., Andersen, J., Hodgman, T.C. (2014): Global use of biremediation technologies for decontamination of ecosystems. Journal of Bioremediation & Biodegradation, 5(4): 2-9.

15. El-Refai, H. A., AbdelNaby, M. A., Gaballa, A., El-Araby, M. H., & Abdel Fattah, A. F. (2005): Improvement of the newly isolated Bacillus pumilus FH9 keratinolytic activity. Process Biochemistry, 40(7): 2325–2332. doi:10.1016/j.procbio.2004.09.006

16. Fricker, A. D., LaRoe, S. L., Shea, M. E., & Bedard, D. L. (2014): Dehalococcoides mccartyi strain JNA dechlorinates multiple chlorinated phenols including pentachlorophenol and harbors at least 19 reductive dehalogenase homologous genes. Environmental science & technology, 48(24): 14300–14308. https://doi.org/10.1021/es503553f

17. Galhaup, C., Goller, S., Peterbauer, C. K., Strauss, J., & Haltrich, D. (2002): Characterization of the major laccase isoenzyme from Trametes pubescens and regulation of its synthesis by metal ions a. Microbiology, 148(7): 2159–2169. doi:10.1099/00221287-148-7-2159

18. Gianfreda, L., & Rao, M. A. (2004): Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzyme and Microbial Technology, 35(4): 339–354. doi:10.1016/j.enzmictec.2004.05.006

19. Gianfreda, L., & Bollag, J. M. (2002): Isolated enzymes for the for the transformation and detoxication of organic pollutants. In Burns, R.G and Dick R. (Eds), Enzymes in the Environment: Activity, Ecology and Applications. 491-538. Marcel Dekker, New York.

20. Giardina, P., Faraco, V., Pezzella, C., Piscitelli, A., Vanhulle, S., & Sannia, G. (2010): Laccases: a never-ending story. Cellular and molecular life sciences: CMLS, 67(3): 369–385. https://doi.org/10.1007/s00018-009-0169-1

21. Gochev, V. K. and Krastanov, A.I. (2007): Isolation of laccase producing Trichoderma spp. Bulgarian Journal of Agricultural Science, 13: 171-176.

22. Guan, Z.-B., Luo, Q., Wang, H.-R., Chen, Y., & Liao, X.-R. (2018): Bacterial laccases: promising biological green tools for industrial applications. Cellular and Molecular Life Sciences. doi:10.1007/s00018-018-2883-z

23. Gurung, N., Ray, S., Bose, S., & Rai, V. (2013): A Broader View: Microbial Enzymes and Their Relevance in Industries, Medicine, and Beyond. BioMed Research International, 2013, 1–18. doi:10.1155/2013/329121

24. Haider, T., Völker, C., Kramm, J., Landfester, K., & Wurm, F. R. (2019): Plastics of the future? The impact of biodegradable polymers on the environment and on society. Angewandte Chemie International Edition. doi:10.1002/anie.201805766

25. Haniya, M., Naaz, A., Sakhawat, A., Amir, S., Zahid, H., & Syed, S. A. (2017): Optimized production of lipase from Bacillus subtilis PCSIRNL-39. African Journal of Biotechnology, 16(19): 1106–1115. doi:10.5897/ajb2017.15924

26. Jellouli, K., Ghorbel-Bellaaj, O., Ayed, H. B., Manni, L., Agrebi, R., & Nasri, M. (2011): Alkaline-protease from Bacillus licheniformis MP1: Purification, characterization and potential application as a detergent additive and for shrimp waste deproteinization. Process Biochemistry, 46(6): 1248–1256. doi:10.1016/j.procbio.2011.02.012

27. Jeong, J.-H., Lee, O.-M., Jeon, Y.-D., Kim, J.-D., Lee, N.-R., Lee, C.-Y., & Son, H.-J. (2010): Production of keratinolytic enzyme by a newly isolated feather-degrading Stenotrophomonas maltophilia that produces plant growth-promoting activity. Process Biochemistry, 45(10): 1738–1745. doi:10.1016/j.procbio.2010.07.020
28. Kapoor, M., & Rajagopal, R. (2011): Enzymatic bioremediation of organophosphorus insecticides by recombinant organophosphorous hydrolase. International Biodeterioration & Biodegradation, 65(6): 896–901. doi:10.1016/j.ibiod.2010.12.017
29. Karigar, C. S., & Rao, S. S. (2011): Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review. Enzyme Research, 2011: 1–11. doi:10.4061/2011/805187
30. Karam, J. & Nicell, J.A. (1997): Potential Applications of Enzymes in Waste Treatment. Journal of Chemistry Technology and Biotechnology. 69: 141-153.
31. Kieliszek, M., Pobiega, K., Piwowarek, K., & Kot, A. M. (2021): Characteristics of Proteolytic Enzymes Produced by Lactic Acid Bacteria. Molecules (Basel, Switzerland), 26(7): 1858. https://doi.org/10.3390/molecules26071858
32. Kumar, A & Sharma, S. (2019): Microbes and Enzymes in Soil Health and Bioremediation in Microorganisms for Sustainability. Springer, Berlin, Germany.
33. Kumar, A., Pillay, B., & Olaniran, A. O. (2016): l-2-Haloacid dehalogenase from Ancylobacter aquaticus UV5: Sequence determination and structure prediction. International Journal of Biological Macromolecules, 83: 216–225. doi:10.1016/j.ijbiomac.2015.11.066
34. Li, Q., Yi, L., Marek, P., & Iverson, B. L. (2013): Commercial proteases: present and future. FEBS letters, 587(8): 1155–1163. https://doi.org/10.1016/j.febslet.2012.12.019
35. Liu, J. Q., Kurihara, T., Hasan, A. K., Nardi-Dei, V., Koshikawa, H., Esaki, N., & Soda, K. (1994): Purification and characterization of thermostable and nonthermostable 2-haloacid dehalogenases with different stereospecificities from Pseudomonas sp. strain YL. Applied and environmental microbiology, 60(7): 2389–2393. https://doi.org/10.1128/aem.60.7.2389-2393.1994
36. Madhavi, V and Lele, S.S. (2009): Laccase: Properties and applications. BioResources. 4(4): 1694-1717.
37. Margot, J., Bennati-Granier, C., Maillard, J., Blánquez, P., Barry, D. A., & Holliger, C. (2013): Bacterial versus fungal laccase: potential for micropollutant degradation. AMB Express, 3(1): 63. doi:10.1186/2191-0855-3-63
38. Mazotto, A. M., de Melo, A. C., Macrae, A., Rosado, A. S., Peixoto, R., Cedrola, S. M., Couri, S., Zingali, R. B., Villa, A. L., Rabinovitch, L., Chaves, J. Q., & Vermelho, A. B. (2011): Biodegradation of feather waste by extracellular keratinases and gelatinases from Bacillus spp. World journal of microbiology & biotechnology, 27(6): 1355–1365. https://doi.org/10.1007/s11274-010-0586-1
39. McMahon, A. M., Doyle, E. M., Brooks, S., & O'Connor, K. E. (2007): Biochemical characterisation of the coexisting tyrosinase and laccase in the soil bacterium Pseudomonas putida F6. Enzyme and Microbial Technology. 40(5): 1435–1441. doi:10.1016/j.enzmictec.2006.10.020
40. Morozova, O. V., Shumakovich, G. P., Shleev, S. V., & Yaropolov, Y. I. (2007): Laccase-mediator systems and their applications: A review. Applied Biochemistry and Microbiology, 43(5): 523–535. doi:10.1134/s0003683807050055
41. Nannipieri, P., & Bollag, J.-M. (1991): Use of Enzymes to Detoxify Pesticide-Contaminated Soils and Waters. Journal of Environment Quality, 20(3): 510. doi: 10.2134/jeq1991.00472425002000030002
42. Nicell, J.A. (2001): Environmental applications of enzymes. Interdisciplinary Environment Review 3, 14-41.

43. Nnolim, N. E., Okoh, A. I., & Nwodo, U. U. (2020): Bacillus sp. FPF-1 Produced Keratinase with High Potential for Chicken Feather Degradation. Molecules, 25(7): 1505. doi:10.3390/molecules25071505
44. Pandey, K., Singh, B., Pandey, A.K., Badruddin, I.J., Pandey, S., Mishra, V.K., & Jain, P.K. (2017): Application of Microbial Enzymes in Industrial Wastewater Treatment. International Journal of Current Microbiology and Applied Sciences, 6, 1243-1254. https://doi.org/10.20546/ijcmas.2017.608.151
45. Pointing S. B. (2001): Feasibility of bioremediation by white-rot fungi. Applied microbiology and biotechnology, 57(1-2): 20–33. https://doi.org/10.1007/s002530100745
46. Priyanka, P., Kinsella, G., Henehan, G. T., & Ryan, B. J. (2019): Isolation, purification, and characterization of a novel solvent stable lipase from Pseudomonas reinekei. Protein expression and purification, 153: 121–130. https://doi.org/10.1016/j.pep.2018.08.007
47. Rao, M. B., Tanksale, A. M., Ghatge, M. S., & Deshpande, V. V. (1998): Molecular and Biotechnological Aspects of Microbial Proteases. Microbiology and Molecular Biology Reviews, 62(3): 597–635. doi:10.1128/mmbr.62.3.597-635.1998
48. Rao, M., Scelza, R., Scotti, R., & Gianfreda, L. (2010): Role of enzymes in the remediation of polluted environments. Journal of Soil Science and Plant Nutrition, 10(3): 333-353. doi:10.4067/s0718-95162010000100008
49. Raveendran, S., Parameswaran, B., Ummalyma, S. B., Abraham, A., Mathew, A. K., Madhavan, A., Rebello, S., & Pandey, A. (2018): Applications of Microbial Enzymes in Food Industry. Food technology and biotechnology, 56(1): 16–30. https://doi.org/10.17113/ftb.56.01.18.5491
50. Reddy, C. (1995): The potential for white-rot fungi in the treatment of pollutants. Current Opinion in Biotechnology, 6(3): 320–328. doi:10.1016/0958-1669(95)80054-9
51. Rubilar, O., Diez, M. C., & Gianfreda, L. (2008): Transformation of Chlorinated Phenolic Compounds by White Rot Fungi. Critical Reviews in Environmental Science and Technology, 38(4): 227–268. doi:10.1080/10643380701413351
52. Sadhasivam, S., Savitha, S., Swaminathan, K., & Lin, F.-H. (2008): Production, purification and characterization of mid-redox potential laccase from a newly isolated Trichoderma harzianum WL1. Process Biochemistry, 43(7): 736–742. doi:10.1016/j.procbio.2008.02.017
53. Sánchez, C. (2009): Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnology Advances, 27(2): 185–194. doi:10.1016/j.biotechadv.2008.11.001
54. Saranya, P., Selvi, P. K., & Sekaran, G. (2019): Integrated thermophilic enzyme-immobilized reactor and high-rate biological reactors for treatment of palm oil-containing wastewater without sludge production. Bioprocess and Biosystems Engineering. doi:10.1007/s00449-019-02104-x
55. Saraswat, R., Verma, V., Sistla, S., & Bhushan, I. (2017): Evaluation of alkali and thermotolerant lipase from an indigenous isolated Bacillus strain for detergent formulation. Electronic Journal of Biotechnology, 30: 33–38. doi:10.1016/j.ejbt.2017.08.007
56. Seeger, M., Hernández, M., Méndez, V., Ponce, B., Córdova, M., & González, M. (2010): Bacterial degradation and bioremediation of chlorinated herbicides and biphenyls. Journal of Soil Science and Plant Nutrition, 10(3). doi:10.4067/s0718-95162010000100007
57. Sharma, S. (2012): Bioremediation Features, Strategies, and Applications. Asian Journal of Pharmacy and Life Science, 2: 202-213.
58. Shraddha, Shekher, R., Sehgal, S., Kamthania, M., & Kumar, A. (2011): Laccase: microbial sources, production, purification, and potential biotechnological applications. Enzyme research, 2011: 217861. https://doi.org/10.4061/2011/217861

59. Singh, Baljinder. (2014): Review on microbial carboxylesterase: general characteristic and role in organophosphate pesticides degradation. Biochemistry & Molecular Biology. 2: 1-6. 10.12966/bmb.03.01.2014.
60. Soden, D. M., & Dobson, A. D. W. (2001): Differential regulation of laccase gene expression in Pleurotus sajor-caju. Microbiology (Reading, England), 147: 1755–1763. https://doi.org/10.1099/00221287-147-7-1755
61. Solé, M., Kellner, H., Brock, S., Buscot, F., & Schlosser, D. (2008): Extracellular laccase activity and transcript levels of putative laccase genes during removal of the xenoestrogen technical nonylphenol by the aquatic hyphomycete Clavariopsis aquatica. FEMS microbiology letters, 288(1): 47–54. https://doi.org/10.1111/j.1574-6968.2008.01333.x
62. Suh, H. J., & Lee, H. K. (2001): Characterization of a Keratinolytic Serine Protease from Bacillus subtilis KS-1. Journal of Protein Chemistry, 20(2): 165–169. doi:10.1023/a:1011075707553
63. Sutherland, T., Russell, R., & Selleck, M. (2002): Using enzymes to clean up pesticide residues. Pesticide Outlook, 13(4): 149–151. doi:10.1039/b206783h
64. Tahri, N., Bahafid, W., Sayel, H., & El Ghachtouli, N. (2013): Biodegradation: Involved Microorganisms and Genetically Engineered Microorganisms. Biodegradation - Life of Science. doi:10.5772/56194
65. Terrón, M. C., González, T., Carbajo, J. M., Yagüe, S., Arana-Cuenca, A., Téllez, A., Dobson, A. D., & González, A. E. (2004): Structural close-related aromatic compounds have different effects on laccase activity and on lcc gene expression in the ligninolytic fungus Trametes sp. I-62. Fungal genetics and biology: FG & B, 41(10): 954–962. https://doi.org/10.1016/j.fgb.2004.07.002
66. Verma, S., Saxena, J., Prasanna, R., Sharma, V., & Nain, L. (2012): Medium optimization for a novel crude-oil degrading lipase from Pseudomonas aeruginosa SL-72 using statistical approaches for bioremediation of crude-oil. Biocatalysis and Agricultural Biotechnology, 1(4): 321–329. doi:10.1016/j.bcab.2012.07.002
67. Wang, D., Li, A., Han, H., Liu, T., & Yang, Q. (2018a): A potent chitinase from Bacillus subtilis for the efficient bioconversion of chitin-containing wastes. International journal of biological macromolecules, 116: 863–868. https://doi.org/10.1016/j.ijbiomac.2018.05.122
68. Wang, Y., Feng, Y., Cao, X., Liu, Y., & Xue, S. (2018b): Insights into the molecular mechanism of dehalogenation catalyzed by D-2-haloacid dehalogenase from crystal structures. Scientific Reports, 8(1). doi:10.1038/s41598-017-19050-x
69. Yang, J.-K., Shih, I.-L., Tzeng, Y.-M., & Wang, S.-L. (2000): Production and purification of protease from a Bacillus subtilis that can deproteinize crustacean wastes⍰. Enzyme and Microbial Technology, 26(5-6): 406–413. doi:10.1016/s0141-0229(99)00164-7

UPOTREBA MIKROBNIH ENZIMA U BIOREMEDIJACIJI

SAŽETAK

Primijenjena mikrobiologija jedna je od temeljnih grana biotehnologije jer ima ulogu u proizvodnji i izolaciji proizvoda dobivenih iz mikroorganizama ili pomoću njih. Među najvažnijim proizvodima mikroorganizama su mikrobni enzimi, mikrobna biomasa, metaboliti te spojevi proizišli iz mikrobne transformacije. Velika količina mikrobnih enzima proizvodi se na industrijskoj razini, posebice hidrolitički enzimi kao što su; amilaze, proteinaze, lipaze, celulaze i drugi. Ovi enzimi imaju široku primjenu u različitim industrijama, no zbog ubrzanog onečišćenja okoliša i potrebe za sanacijom onečišćenog tla, uspješno se primjenjuju u procesu bioremedijacije. Bioremedijacija koristi mikroorganizme (njihove enzime) da bi smanjila koncentracija štetnih tvari u tlu, najčešće tako da ih se razgrađuje do produkata koji su manje ili nisu uopće štetni (ugljikov dioksid, vodu i biomasu). Cilj ovog članka je pružiti pregled golemog potencijala koji mikrobni enzimi imaju u bioremedijaciji za buduće probleme zbrinjavanja otpada i sanacije okoliša.

Ključne riječi: bioremedijacija, mikrobni enzimi, onečišćenje tla, teški metali, pesticidi

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