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Keywords Degradation of cellulose; Cellulosomes; Scanning microscopy; Cellulases; Paenibacillus polymyxa

Introduction

Cellulose is a ubiquitous polysaccharide that is degraded by microorganisms belonging to various systematic groups regardless of the abiotic conditions (humidity, oxygenation, pH etc.) in the environment. Strains of bacteria belonged to the genus Paenibacillus are among the isolated microorganisms capable of hydrolyzing cellulose.

Paenibacillus polymyxa is a spore-forming, Gram-positive, facultaively anaerobic, heterotrophic bacterium that lives in various environments such as: soil, waters, the surface and interior of roots and the above-ground of plants [1]. It utilizes various organic and mineral compounds as a source of nitrogen. Some strains synthesize nitrogenase and are able to assimilate molecular nitrogen [2,3]. P. polymyxa produces lectins, heteroauxins, antibiotics (polymyxin) and other biologically active compounds, as a result of which it is included into plant growth promoting bacteria [4,5]. P. polymyxa strains are able to hydrolyse cellulose, both its amorphous and crystalline regions, by synthesizing a cellulase complex composed of endo-1,4 -glucanase (3.2.1.4), exocellobiohydrolase (3.2.1.91), cellobiose phosphorylase (2.4.1.20) and cellodextrins (2.4.1.49). e main role in the degradation of cellulose is played by endo- -1,4-glucanase and exocellobiohydrolase. Endo- -1,4-glucanase hydrolyzes amorphous regions of cellulose to cellodextrins and cellotetrose, which are then degraded by exocellobiohydrolase to cellobiose [6-9].

Some microorganisms produce exogenous cellulases located in surface structures termed cellulosomes [10-13]. Cellulosomes were rst observed in Clostridium thermocellum, which degrades cellulose under thermophilic conditions [10,11]. ere are only a few articles on the formation of cellulosomes or minicellulosomes on the surface of bacterial cells belonging to the genera Bacillus and Paenibacillus, which are capable of degrading cellulose [9,14,15].

So far there have been few studies involving microscopic analysis regarding the colonization of cellulose by P. polymyxa bacteria. e problem is important as that the mechanism of cellulose degradation in the environment considerably di ers depending on the taxonomic group of microorganisms carrying it out. Fungi do not require direct contact between the hyphae and substrate to hydrolyze cellulose, whereas in the case of bacterial cells, adhesion and the colonization of cellulose are necessary for the synthesis of cellulases to be initiated particularly in the initial stages of cellulolysis.

*Corresponding author: 3 D Z H á . R Z D O F] \ N % L R Q L F X P / 7 ' & K H á P : D U V D Z 3 R O D Q G 7 H O S D Z H O Ń P 2 O F] \ N # E L R Q L F X

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5'-CCTCCAGCTCTCCATGGTGATCG-3'. e size of the obtained products of ampli cation con rmed the presence in the genome of both studied strains of nitrogenase iron protein subunit NifH gene

presents P. polymyxa AB 808 725 (EG14) on cellulose. Non-confocal images obtained in transmitted light are designated with the symbol A, whereas B images are the same fields of vision-confocal images of acridine orange fluorescence. Images C and D are examples of the control under stronger magnification. The presented Figures 3 and 4 show the presence of bacteria on the surface of the cellulose fibres and crystals as well as in other parts of the field of vision, which indicates the colonization of the substrate-cellulose.

Figure 5 presents an acridine orange stained preparation prepared from P. polymyxa AB808724 (EG2) culture in Park's medium containing microcrystalline Avicel cellulose. The individual figures were taken using laser scanning confocal microscopy of the cell surface (0 µ) and of the following layers of the preparation: 2.4 µ; 4,8 µ; 7.2 µ; 8.5 µ and 11.9 µ deep. Based on observations and computer analyses of the obtained images the penetration of bacterial cells into the Avicel cellulose crystals can be concluded.

The results of microscopic observations of cellulosomes on the surface of the cells of the studied bacterial strains are presented in Figures 6-11. The studied cells were treated with ferritin and analyzed by scanning (Figures 6b and 7b) and transmission electron microscopy (Figures 9 and 11). Around the cells not treated with ferritin and not grown with cellobiose, both in scanning and transmission electron microscope no structures, known in the literature as cellulosomes, were observed (Figures 6a, 7a, 8 and 10).

Figure 6b presents a scanning electron microscope image of ferritin-treated P. polymyxa AB808724 cells. On the surface of the cells „protuberances" resembling cellulosomes are visible. An analogous picture from P. polymyxa AB808725 culture is presented in Figure 7b. In preparations of P. polymyxa

B. polymyxa (currently Paenibacillus polymyxa) and B. macerans as well as two species B. azotoxans and B. psychrosaccharolyticus included in Bergey's bacterial taxonomy [31].

were able to degrade carboxymethylcellulose. e isolated cellulosome e ciently degraded lignocellulose.

Van Dyk et al. [32] and Mayer et al. [33] demonstrated that B. licheniformisstrain SVD1 synthesizes multi-enzyme complexes (MEC) with hemi-cellulolytic activity. e total molecular mass of the complex was approximately 2000 kDa. Other enzymes forming MEC hydrolyzed xylan, mannose, pectin and carboxymethylcellulose. However, no avicellase activity was found, which precludes calling this multi-enzyme complex a cellulosome. However, it is possible to use genetic modi cations that would allow the use of the MEC in many areas of the biodegradation of natural wastes.

Conclusions

- ‡ e investigated spore-forming bacteria belong to the speP. polymyxa.
- ‡ e two strains P. polymyxaare diasotrophic bacteria because showing of nitrogen xing ability.
- ‡ ese isolates can to degradate the amorphous and crystalline regions in cellulose.
- ‡ e cells of investigated bacteria colonise of cellulose bers during the degradation.

ese bacteria when cultured in medium with lter paper form spherical structures resembling cellulosomes on the surface of the bacteria such as anaerobic, cellulolytic bacteria of the GenusClostridium

References

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