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CHARACTERIZATION OF *Bacillus* STRAINS PRODUCING BIOSURFACTANTS

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Abstract

Biosurfactants are natural compounds that have proven valuable in various industrial applications including environmental restoration. This work was aimed at the characterization of recently isolated *Bacillus* strains (T-1, T'-1 and I'-1a) producing biosurfactants. They were isolated from 100-year-old oil refinery sludge in Czechowice-Dziedzice (Poland). The aged sludge was acidic (pH 2) and highly contaminated with polycyclic aromatic hydrocarbons and heavy metals. The *Bacillus* strains were identified by three different methods: 16S rDNA gene sequences, BIOLOG system and fatty acid analysis (FAME). Morphological and biochemical characterization was done by traditional microbiological methods, API ZYM and BIOLOG analyses. Bacterial susceptibility to antibacterial agents (both antibiotics and biocides) was evaluated by the disc diffusion method. Three isolates were resistant only to two antibiotics: amoxicillin and nalidixic acid. All tested biocides (13) inhibited microbial growth of all three cultures. The effect of carbon nanotubes on growth and biosurfactant-production by these *Bacillus* strains was also investigated. Isolated strains grew very well in liquid and solid medium with different concentrations of the carbon nanotubes. Although no change in growth properties was observed exposure with the carbon nanotubes, stimulation of endospore production occurred. Biosurfactants produced by *Bacillus* strains in liquid medium were observed to cause dispersion of the carbon nanotubes. The three *Bacillus* strains were cultured on brewery effluents and molasses to produce biosurfactants.
Blood agar lysis, oil spreading and drop collapsing tests, lipase activity, measuring of surface and interfacial tensions, biosurfactants genes analysis, isolation and identification of biosurfactants were applied to evaluate biosurfactants production.
Antifungal activity of *Bacillus* strains and associated surfactant contained medium was tested utilizing 10 plant fungal pathogens. The results demonstrated the ability of the *Bacillus* strains and biosurfactant containing cell-free supernatants to inhibit mycelial growth of fungi *Botrytis cinerea* A 258, *Phomopsis viticola* W 977, *Septoria carvi* K 2082, *Colletotrichum gloeosporioides* A 259, *Phoma complanata* A 233 and *Phoma exigua var. exigua* A 175. The results provide the utilization of *Bacillus spp*. for control of important phytopathogenic fungi that demonstrate important potential for agricultural applications. These applications may include replacement of current potentially hazardous chemical biocides used for control of plant pathogens with safe, effective, natural biosurfactant treatments.