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Acinetobacter: Environmental and biotechnological applications

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Among microbial communities involved in different ecosystems such as soil, freshwater, wastewater and solid wastes, several strains belonging to the genus of *Acinetobacte*r have been attracting growing interest from medical, environmental and a biotechnological point of view. Bacteria of this genus are known to be involved in biodegradation, leaching and removal of several organic and inorganic man-made hazardous wastes. It is also well known that some of *Acinetobacter* strains produce important bioproducts. This review summarizes the usefulness and environmental applications of *Acinetobacter* strains.

Key words: *Acinetobacter*, biodegradation, xenobiotic, oil, heavy metals, bioproducts, lipases, polysaccharides.

INTRODUCTION

Acinetobacter spp. are widespread in nature, and can be obtained from water, soil, living organisms and even from human skins. They are oxidase-negative, non-motile, strictly aerobic and appear as gram-negative coccobacilli in pairs under the microscope. They can use various carbon sources for growth, and can be cultured on relatively simple media, including nutrient agar or trypticase soya agar. Also, most members of *Acinetobacter* show good growth on MacConkey agar with the exception of some *A. lwoffii* strains (Bergogne-Bérézin and Towner, 1996).

Species of *Acinetobacter* have been attracting increasing attention in both environmental and biotechnological applications. Some strains of this genus are known to be involved in biodegradation of a number of different pollutants such as biphenyl and chlorinated biphenyl, amino acids (analine), phenol, benzoate, crude oil, acetonitrile, and in the removal of phosphate or heavy metals. *Acinetobacter* strains are also well represented among fermentable bacteria for the production of a number of extra-and-intracellular economic products such as lipases, proteases, cyanophycine, bioemulsifiers and several kinds of biopolymers.

The focus of this review, therefore, concerns the use of several *Acinetobacter* strains as a biocatalyst to remediate various environmental contaminants and other biotechnological applications.

BIOREMEDIATION OF CONTAMINANTS

Several investigations have shown that many toxic compounds can enter the environment, disperse and persist to a great extent (van der Meer et al., 1992). The application of conventional physical or chemical processes to cleanup industrial sites and water supplies are extremely expensive and time consuming. In contrast, bioremediation is relatively inexpensive and can achieve the conversion of hazardous substances, using microorganisms, into forms that are less or non-toxic (Adams and Ribbons, 1988). Increase of bioremediation capacity is being fostered by the knowledge of specificity of microorganisms in biodegradation of certain chemicals. In the last two decades, the field of microbiology has increasingly focused on the use of microorganisms for environmental clean up. *Acinetobacter* is one of the bacteria can degrade and remove a wide range of organic and inorganic compounds.

Biodegradation of xenobiotics and halogens

Xenobiotics pollutants such as benzene, toluene, phenol and styrene, as well as halogenated organic compounds such as pentachlorophenol and polychlorinated biphenyls are often present in waste streams in fairly low concentrations. They may also be present in larger quantities as spills, or in the soil and water at abandoned industrial sites. These compounds are generally highly toxic and exceedingly difficult to dispose of.

A number of studies have focused on the biodegradation of phenol by various microorganisms. Among phenol degraders are several strains of *Acinetobacter*, which can use it as a sole energy and carbon source (Briganti et al., 1997; Chibata and Tosa, 1981). Recently, out of twelve bacterial phenol-degraders

isolated from different Egyptian ecosystems, four are closely related to *Acinetobacter* (Abd-El-Haleem et al., 2002a). One of these has been used in two different environmental application studies (Abd-El-Haleem et al., 2002c; Beshy et al., 2002). Other xenobiotic compounds such as toluene (Zilli et al., 2001), 4-hydroxybenzoate (Allende et al., 2000), 2-chloro-N-isopropylacetanilide (Martin et al., 1999), 4-hydroxymandelic and 4-hydroxy-3-methoxymandelic acids (Rusansky et al., 1987), benzoic and p-hydroxybenzoic (Delneri et al., 1995), 4-chlorobenzoate (Adriaens and Focht, 1991) and 3-chlorobenzoic acid (Zaitsev and Baskunov, 1985) can be metabolized to their corresponding benzoates by various *Acinetobacter* strains.

It has also been reported that certain *Acinetobacter* strains can utilize biphenyls including chlorinated biphenyls (Adriaens and Focht, 1991; Furukawa and Chakrabarty, 1982). Singer et al. (1985) observed that out of 36 pure isomers of polychlorinated biphenyls examined, 33 were metabolized by *Acinetobacter* sp. strain P6. Furthermore, some *Acinetobacter* spp. isolated from mixed cultures has proven to be proficient at complete mineralization of monohalogenated biphenyls (Shields et al., 1985). Strains of *Acinetobacter* have also been employed for degradation of lignin (Buchan et al., 2001; Crawford,. 1975; Mak et al., 1990) and amino acids (Kahng et al., 2002; Kim et al., 2001).

Degradation of oil

Several constituents of oily sludge are carcinogenic and potent immunotoxicants (Mishra et al., 2001). Oily sludge is a complex mixture of alkanes, aromatic compounds, NSO (nitrogen-, sulfur-, and oxygen-containing compounds), and asphaltene fractions (Bossert and Bartha, 1984). *Acinetobacter* strains are considered one of the most efficient oil degraders (Rusansky et al., 1987).

Removal of phosphate and heavy metals

Biological phosphate removal from wastewater is an efficient cost- effective alternative to chemical phosphorus precipitation. This biological process is obtained by recycling the sludge through anaerobic and aerobic zones. It is dependent on the enrichment of activated sludge with polyphosphate accumulating strictly aerobic *Acinetobacter* sp. which could absorb phosphate up to 100 mg phosphorus per gram of dry biomass during aerobic conditions and release it anaerobically (van Groenestijn et al., 1989; Timmerman, 1984). It was confirmed that *Acinetobacter* are primarily responsible for biological phosphate removal (Auling et al., 1991; Wagner et al., 1994). *Acinetobacter* strains also play an important role in the removal of heavy metals (Boswell et al., 2001; Francisco et al., 2002).

BIO-PRODUCTS FROM *ACINETOBACTER*

Bioemulsifiers

Bioemulsifiers, which contain both hydrophobic and hydrophilic groups, are used widely in the food, agrochemical, cosmetic, and pharmaceutical industries. Several microorganisms including *Acinetobacter* strains can synthesize a wide variety of bioemulsifiers (Rosenberg and Ron, 1998). Among the so-called "bioemulsans," the most studied are those produced by *A. calcoaceticus* RAG-1 (Rosenberg and Ron, 1998), *A. calcoaceticus* BD4 (Kaplan et al., 1987), and *A. radioresistens* KA53 (Navon-Venezia et al., 1995). The low-molecular-mass bioemulsifiers are generally glycolipids, such as trehalose lipids, sophorolipids, and rhamnolipids, or lipopeptides, such as surfactin, gramicidin S, and polymyxin. The high-molecular-mass bioemulsifiers are amphipathic polysaccharides, proteins, lipopolysaccharides, lipoproteins, or complex mixtures of these biopolymers (Toren et al., 2001).

A. calcoaceticus RAG-1 is an industrially important strain which has been extensively characterized with respect to its growth on hydrocarbons and its production of a high molecular mass bioemulsifier, emulsan (Rosenberg and Ron, 1998). The RAG-1 emulsan is a non-covalentlylinkedcomplexofa lipoheteropolysaccharide and protein. The polysaccharide, called apoemulsan, has a molecular weight of about 990 kD. *A. calcoaceticus* BD4, initially isolated by Taylor and Juni (1961), produces a large polysaccharide capsule. When released into the medium, the capsular polysaccharide forms a complex with proteins which then becomes an effective emulsifier. The BD4 emulsan apparently derives its amphipathic properties from the association of an anionic hydrophilic polysaccharide with proteins (Bryan et al., 1986). *A. radioresistens* strain KA53 (Navon-Venezia et al., 1995) produce a bioemulsifier designated alasan. It has a molecular weight of 100 to 200 kD, and an emulsifying activity which increases with preheating at 60 to 90°C (Toren et al., 2001, 2002).

Polysaccharides, polyesters and lipases

Several strains of *Acinetobacter* produces extracellular polysaccharides with sizes up to several million Daltons. These polysaccharides can consist of D-galactose, D-2-acetamido-2-deoxy-D-glucose, 3-(L-2-hydroxypropionamido)-3,6-dideoxy-D-galactose (Haseley et al., 1997), rhamnose, 3-deoxy-3-(D-3-hydroxybutyramido)-D-quinovose, S-(+)-2-(4'-Isobutylphenyl)propionic acid or lipopolysaccharide (Haseley et al., 1997; Kunii et al., 2001; Yamamoto et al., 1990) . Furthermore, some *Acinetobacter* strains are able to grow on ethanol and synthesize exopolysaccharides called ethapolan (Johri et al., 2002; Pirog et al., 2002; Pyroh et al., 2002).

Several *Acinetobacter* strains are also known to accumulate wax esters, polyhydroxyalkalonic acids and cyanophycin (Krehenbrink et al., 2002; Pirog et al., 2002; Spiekermann et al., 1999; Vinogradov et al., 2002). Various types of these biopolymer are widely used in the manufacture of fine chemicals such as cosmetics, candles, printing inks, lubricants, and coating.

Furthermore, a vast number of *Acinetobacter* lipases have a wide range of potential applications in the hydrolysis, esterification, and transesterification of triglycerides, and in the chiral selective synthesis of esters (Chen et al., 1999; Li, et al. 2000, 2001).

ACINETOBACTER AS A BIOREPORTER

The use of bioluminescent bioreporter as an inexpensive and real-time method for detecting and monitoring contaminants in the environment is one of the most promising nano-technologies. Bioreporters refer to intact, living microbial cells that have been genetically engineered to produce a measurable signal in response to a specific chemical or physical agent. The genetic construct consists of an inducible promoter gene fused to a reporter gene such as luciferase and green fluorescent protein (Applegate et al., 1998; Hay et al., 2000)

Recently, we (Abd-El-Haleem et al., 2002b) were able to construct a bioluminescent reporter strain, *Acinetobacter* sp. DF4 for the detection of phenol by inserting the phenol 3-hydroxyacyl-CoA-dehydrogenase (*3-hcd*) promoter upstream of the bioluminescence genes *luxCDABE*. When it was introduced into the chromosome of *Acinetobacter* sp. DF4, the resulting strain, produced a sensitive bioluminescence response to phenol at concentrations ranging from 5.0 to 100 ppm. With such specificity, *Acinetobacter* strains are prime candidates for whole-cell bioreporter monitoring of phenol.

CONCLUSIONS AND PERSPECTIVE

There are numerous applications of *Acinetobacter* strains in hazardous waste treatment or as producers of economically important bio-products. Potential improvements are expected from genetic engineering of *Acinetobacter* strains from natural environments with increased robustness for environmental and industrial applications.

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