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Review on microbial identification for Phenol Degradation - GEN III Microlog

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Abstract

Contamination of soil, surface water, and underground water by aromatic organic pollutants such as phenol and its derivatives has caused great concern worldwide. A large number of microorganisms including bacteria, fungi, and algae30 are capable of degrading phenol. The biodegradation of phenol and its derivatives by bacteria has been extensively studied and a large number of phenol-degrading bacteria have been isolated and characterized at the physiological and genetic levels. Biolog's powerful carbon source utilization technology accurately identifies environmental and pathogenic microorganisms by producing a characteristic pattern or "metabolic fingerprint" from discrete test reactions. Biolog's single panel is easy to use, and identifies 4 times more species than alternative systems. GENIII dissects and analyzes the ability of the cell to metabolize all major classes of biochemicals, in addition to determining other important physiological properties such as pH, salt, and lactic acid tolerance, reducing power, and chemical sensitivity.

Keywords: Aerobic bacteria; Biodegradation; GEN-III; Identification; Metabolic pathway; Phenol.

1. Introduction

1.1 Phenol

Phenol is the basic structural unit of a wide varietyof synthetic organics [1, 2]. It is a listed priority pollutant by the U.S. Environmental Protection Agency and Agency for Toxic Substances and Disease Registry [3]. Phenolic pollutants are generated from several sources, like the partial degradation of phenoxy herbicides, the use of wood preservatives and the generation of wastes by petroleum-related industries such as petroleum refineries, gas and coke oven industries, pharmaceuticals, explosive manufacture, phenol-formaldehyde resin manufacture, plastic and varnish industries and related metallurgical operations, etc. [4, 5, 6].

Contamination of soil, surface water, and underground water by aromatic organic pollutants such as phenol and its derivatives has caused great concern worldwide. Phenols are well known for their high toxicity for human life, aquatic life, and others [7, 8, 9]. They are considered to be among the most hazardous contaminants, and they are certainly the most difficult to remove [10].

Phenol is a pollutant that is usually found in many industrial effluents such as wastewaters from coal processing plants, oil refineries, pulp and paper manufacturing plants, resins and coke manufacturing, steel industries, pharmaceutical industries, plastic and varnish industries, textile units, pesticide plants, tannery, and smelting and related metallurgical operations [11, 12].

Because of the aromatic structure of phenol, it is resistant to natural biodegradation and phenolic compounds have been reported to have high stability due to the difficulty of cleaving the benzene ring. Several microorganisms can tolerate phenol and use it as a carbon and energy source [13].

Acute exposure to phenol by the oral route leads to damage to blood, liver, kidney and cardiac toxicity, including weak pulse, cardiac depression, and reduced blood pressure. Various physicochemical methods like ionization, adsorption, reverse osmosis, electrolytic oxidation, H_2O_2, photocatalysis, etc. have been used for the elimination of phenol from contaminated waters.

1.2 Microbial Degradation of Phenol

Microbial degradation of chemicals in the environment is a route for their removal. The microbial degradation of these compounds is a complex series of biochemical reactions and often different when different microorganisms are involved. Microbial degradation of pollutants is crucial in order to predict their longevity and long term effects and also important in the actual remediation process. Depending on the type of bacteria that are responsible for the degradation i.e., in the presence of free oxygen or oxygen in combined state, bioremediation is classified as “aerobic” or “anaerobic”.

In aerobic respiration, oxygen acts as the electron acceptor. Molecular oxygen is a reactant for oxygenase enzymes and is incorporated into the final products. In anaerobic respiration, different inorganic electron acceptors are possible such as SO_4^{2-},CO_2 and Fe^{3+}. Most of the biodegradation is aerobic as anaerobic process is relatively slow and is difficult to maintain for bioremediation process. It is preferred where reduction is favored over oxidation as in the case of chlorinated compounds. Many synthetic compounds accumulate in nature because the release rates exceed the rates of microbial and chemical degradation.

2. Degradation of phenol through aerobic pathway:

Because of widespread occurrence of phenol in the environment many microorganisms utilizes phenol as the sole carbon and energy source which includes both aerobic and anaerobic microorganisms.

2.1 Aerobic biodegradation of phenol:

Aerobic biodegradation has been studied in the early 19th centuries. In the first step of the aerobic pathway for the biodegradation of phenol, molecular oxygen is used by the enzyme phenol hydroxylase to add a second hydroxyl group in ortho-position to the one already present in which reaction requires a reduced pyridine nucleotide ($NADH_2$). The resulting catechol (1, 2-dihydroxybenzene) molecule can then be degraded via two alternative pathways depending on the responsible microorganism. In the ortho- or -ketoadipate pathway, the aromatic ring is cleaved between the catechol hydroxyls by a catechol 1, 2-dioxygenase (intradiol fission) [14, 15].

Preliminary evidence for the production of -ketoadipate during the degradation of phenol by strain *'Vibrio 01'* was first presented by Evans and Kilby [16, 17]. The resulting cis, cis muconate is further metabolized via -ketoadipate to Krebs cycle intermediates. In the meta-pathway, ring fission occurs adjacent to the two hydroxyl groups of catechol (extradiol fission). The enzyme catechol 2, 3-dioxygenase transforms catechol to 2-hydroxymuconic semialdehyde. This compound is metabolized further to intermediates of the Krebs cycle. The organisms which utilize phenol by aerobic pathway are *Acientobacter calcoceticus, Pseudomonas* species and *Candida tropicalis* and most of the eukaryotes typically employ ortho pathway. The aerobic genus *Pseudomonas* species have been subject to various studies and its versatility to utilize a wide spread of aromatic substrates makes it an attractive organism for use in waste water treatment applications.

3. Identification of isolates:

The identification of isolates were carried out by biochemical characterization and morphological observation which involves tests like gelatinase production,citrate utilization, gram staining, amylase and, indole test etc [18].

To determine the bacteriology Bergey's manual of determinative was used as a reference to identify the isolates. Phase-contrast microscopy and electron microscopy were used to determine the cell morphology. The pH range and optimum pH for growth for each strain were determined by monitoring the residual phenol concentrations in cultures inoculated into medium having different initial pH values (pH 6.5 to 8.5) and were inoculated. The final pH was measured as well. The highest concentration of phenol at which each isolate initiated growth (i.e., the phenol tolerance) was determined by monitoring the optical densities of cultures growing on phenol at initial concentrations ranging from 0.5 to 4 mM. The enzyme phenol hydroxylase (PH), plays a key role in the phenol degradation pathway, and it has the capability to identify the different samples contaminated with phenol. It is used as a molecular tool [19].

4. GEN III Biolog:

Biolog's latest generation redox chemistry enables testing and identification of aerobic Gram-negative and Gram-positive bacteria in the same test panel. Gram stain and other pre-tests are no longer needed. A simple, one minute setup protocol is used for each sample. The expanded GENIII database is designed to meet the needs of Biolog's broad customer base covering diverse disciplines of microbiology.

All Biolog Microbial Identification Systems — manual, semi-automated or fully automated— use the powerful new GENIII MicroPlate, allowing users to determine the most appropriate system to fit their current budget and level of throughput. Should needs change, all systems can be upgraded and expanded to meet new capacity requirements. Earlier generation Biolog instruments can be easily upgraded to GENIII without purchasing new equipment.

The new GEN III redox chemistry is applicable to an unprecedented range of both Gram-negative and Gram-positive bacteria. GEN III dissects and analyzes the ability of the cell to metabolize all major classes of biochemicals, in addition to determining other important physiological properties such as pH, salt, and lactic acid tolerance, reducing power, and chemical sensitivity.

Biolog's powerful carbon source utilization technology accurately identifies environmental and pathogenic microorganisms by producing a characteristic pattern or "metabolic fingerprint" from discrete test reactions performed within a 96 well microplate. Culture suspensions are tested with a panel of pre-selected assays, then incubated, read and compared to extensive databases of environmental organisms, human pathogens, veterinary pathogens and plant pathogens. The scope of the 96 assay reactions, coupled with sophisticated interpretation software, delivers a high level of accuracy that is comparable to molecular methods. The one minute per sample set up is much simpler and faster than DNA sequencing and the automated pattern matching eliminates the need for training and expertise in gene sequence interpretation.

5. Technologies available for Biolog include:

1. Microbial identification technology (species level) and
2. Phenotypic microarray technology (for characterization of microbes)

5.1 Biolog Microbial Identification System:

Biolog Inc., USA provides technology for the identification of the species of a new isolate among a wide range of aerobic/anaerobic bacteria, yeast and filamentous fungi. Fig 1 shows the anatomy of GEN III identification.

5.1.1 A Simple, straight forward procedure:

The identification of microorganisms in GEN III Biolog process involves the following process:

- Isolate a pure culture of bacteria on agar media
- Prepare inoculum at specified cell density
- Inoculate the Biolog Micro Plate overnight.
- Incubate the plate and observe the reaction pattern.
- Enter the reaction pattern to obtain ID result

5.1.2 Microbial Identification Databases for Biolog Systems:

Biolog's powerful carbon source utilization technology accurately identifies environmental and pathogenic microorganisms by producing a characteristic pattern or "metabolic fingerprint" from discrete test reactions performed within a 96 well microplate. Culture suspensions are tested with a panel of pre-selected carbon sources and compared against 2900+ identification profiles of environmental and fastidious organisms of interest in diverse fields of microbiology. Five databases are available for a broad spectrum of aerobic bacteria. The following table 1 shows some of the GEN III aerobic bacteria.

5.1.3. Application:

Clean room analysis for identification of microbes prevalent in environment; Industrial quality control in analysis of food and/or agricultural products; Plant disease diagnosis, Veterinary, Analysis of clinical samples including dangerous pathogens of human, animal & plant origin, Education & Research involving General & Applied Microbiology.

5.1.4 Uses:

1. Biolog has designed proprietary microplates for identification of a wide range of microbes upto species level, such as Gen III plate (for gram negative and gram positive aerobic bacteria), AN plate (for anaerobic bacteria), YT plate (for yeast) and FF plate (for filamentous fungi). Nearly 2550species are covered by Biolog for identification.
2. All Biolog Microbial Identification Systems are manual, semi-automated or fully automated. The powerful new GENIII MicroPlate allows users to determine the most accurate system to economy and level of throughput. All systems can be upgraded and expanded to meet new capacity requirements. Without purchasing new equipment earlier generation Biolog instruments can be easily upgraded to GENIII.
3. Biolog is one-minute-per-sample set up is much simpler and faster than DNA sequencing and the automated pattern matching eliminates the need for training and expertise in gene sequence interpretation.
4. GEN III MicroPlates for microbial identification is a one minute set-up process does not any gram staining technique and it is identify the both gram positive and gram negative bacteria in one panel, overall 1350 species are covered in single panel.

Conclusion

Biolog's advanced redox chemistry is used to enable identification and testing of aerobic Gram-positive and Gram-negative bacteria in the same test panel. Gram stain and other pre-tests are no longer needed. This GEN III database is designed to fulfill the needs of Biolog's users covering diverse disciplines of microbiology. All Biologs Microbial Identification Systems are manual, semi-automated or fully automated uses the new the powerful GEN III MicroPlate, to determine the most appropriate system allowing users to fit their current budget and level of throughput. Typical biochemical properties were also detected while testing the strains biochemically using the BIOLOG Micro Station system within one bacterial strain for a possible reclassification of strains, particularly when using modern methods of gene identification based on the comparison of their genomes.

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