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STUDIES ON THE ISOLATION AND CHARACTERIZATION OF PROPOXUR-DEGRADING ORGANISM

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ABSTRACT

The enrichment culture method was used to extract a bacterial strain from soil that could use propoxur as its only carbon and nitrogen source. Various aromatic chemicals are used by the isolated bacterium that destroys propoxur, and the process of isolating, characterising, and identifying this organism is detailed in this work. The pH was brought down to 7.0 after the medium was heated, filtered, and mixed. Autoclaving it for 20 minutes at 15 psi was the next step after dosing it 100 ml into 500 ml Erlenmeyer flasks. The use of photocatalysts for the breakdown of organic pollutants may soon reach new heights with the help of nano-based technologies.

Keywords: Isolation, Propoxur, Biodegradation, Bacteria, Organism

INTRODUCTION

The extensive usage and manufacture of synthetic organic chemicals in both agricultural and industrial contexts has led to environmental pollution. One example of an industrial chemical that is intentionally or unintentionally discharged into the environment is lubricants. Other examples include insulators, hydraulic fluids, solvents, plasticizers, dyes, detergents, medicines, and synthetic waste. Toxic chemicals are released into the environment by most industrial facilities.

This has led to their environmental concentrations increasing at an alarming rate. Many of these chemicals and their biotransformation products have raised concerns about their potential carcinogenic, genetically harmful, or poisonous effects on humans and other animals. If these compounds are not broken down, they can accumulate in the soil and cause serious pollution and ecological concerns. Soil seepage by various aromatic chemical groups, such as polychlorinated biphenyls, nitroaromatics, sulphoaromatics, and haloaromatics, contributes to environmental pollution. Toxicological effects are only one of many detrimental consequences of chemicals in the environment on ecosystem components. Since these dangerous chemicals had not previously exist in the biosphere, they may be considered alien to nature.

The biodegradation of environmental pollutants is facilitated by a varied microbial community. A complicated mechanism enables native microbial populations in water and soil to degrade a broad range of SOCs into smaller compounds that may be used by other metabolic processes. The natural carbon cycle cannot continue without the mineralization of organic molecules. Due to their significance as agents for the breakdown of manmade chemicals in nature, research on the biotechnological potential of microbes for decontaminating polluted areas and effluents has garnered significant interest.

Microbes in water and soil are crucial players in biogeochemical cycles because they decompose or alter a diverse array of aromatic chemicals, both naturally occurring and artificially produced. Fungi and bacteria have acquired the art of metabolising aromatic

compounds. Micrococcus, Pseudomonas, Rhodococcus, Alcaligenes, Nocardia, Flavobacterium, Azotobacter, Acinetobacter, and Mycobacterium are all types of bacteria that fall under this category. Aspergillus and Cunninghamella, both belonging to the Phanerochaete family, are among the most versatile fungal genera. The metabolic rate of heterotrophic bacteria and fungus in a given environment is usually far greater than that of aerobic organisms like yeasts, algae, diatoms, and higher plants and animals, even though aerobic species like these can metabolise a broad variety of compounds. Therefore, populations of microbes in soil and water are mostly responsible for biodegrading environmental pollutants.

LITERATURE REVIEW

Dewi, Tirta & Imamuddin, Hartati & Antonius, Sarjiya. (2015). The carbamate pesticide propoxur (2-isopropoxyphenyl-N-methylcarbamate) is harmful to ecosystems. In the majority of soils, propoxur is quickly broken down by soil microbes. The presence of more pesticide-degrading bacteria in the soil leads to a greater rate of propoxur environmental degradation. This research intends to screen soil samples for bacteria with a high propensity to breakdown propoxur and isolate them. The enrichment culture method was used for isolating and selecting the samples.Soil samples were taken from five separate sites in a rice field in Ngawi, and bacteria that break down propoxyur were identified. High Performance Liquid Chromatography (HPLC) was used to investigate the biodegradation of propoxur by isolated bacteria. A total of ten consorsia were chosen and then cleaned. As its only carbon source, one isolate grew on propoxur at a concentration of 3000 ppm. Findings indicated that 2-isopropoxyphenol and methylamine were hydrolyzed from propoxur by the H2-NG isolate.

Kim, Hyun & Dong-Uk, Kim & Lee, Hyo Sun & Yun, Jungpyo & Ka, Jong-Ok. (2017). Three sets of syntrophic bacteria were found in agricultural soils; these bacteria break down the N-methylcarbamate pesticide propoxur. Propoxur served as an adequate carbon and energy supply for every pair. We picked SP1 for more research since it destroyed propoxur the quickest of the pairings. 16S rRNA gene sequence analysis revealed a close relationship between SP1 members and those of the Pseudaminobacter and Nocardioides genera. Through syntrophic interaction, SP1 entirely destroyed propoxur, leaving no detectable byproducts. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that the syntrophic strain SP1a converted propoxur to 2-isopropoxyphenol (2-IPP), which was then broken down by strain SP1b. Genomic analysis using polymerase chain reaction revealed that strain SP1a had a common ancestor with a carbaryl hydrolase gene, cehA. For the first time, researchers have shown that propoxur was entirely destroyed by two bacterial strains via syntrophic metabolism.

Agustiyani, Dwi & Dewi, Tirta & Nditasari, A & Antonius, Sarjiya. (2019). One potentially useful creature for degrading pesticides is denitrifying bacteria, which use poisons as a carbon and nitrogen substrate. The growth ability test and denitrification activity on three kinds of propoxur-containing medium were used to study the potential of denitrifying bacterial isolates TK Bali, KT, UHT, L7T4, and C.pkr in degrading propoxur. A range of propoxur concentrations (500, 1000, and 1500 ppm) was added to Mineral Salt (MS), Nutrient Broth (NB), and MS + glucose, the medium. The turbidity (OD) at 436 nm was used to analyse the growth of the bacteria. The ability to reduce nitrate was used to quantify denitrification activity. Using HPLC, the concentrations of propoxur and isopropoxyphenol were determined. The findings showed that NB media exhibited the greatest denitrification and growth activity. The growth and denitrification activities of denitrifying bacteria were shown to diminish as the concentration of propoxur increased in NB medium, while they were able to grow up to 1500 ppm. Isolates of denitrifying bacteria did not seem to be capable of utilising propoxur as a carbon and nitrogen source alone. Up to a certain point, denitrifying bacteria showed resistance

to propoxur, meaning they couldn't break it down. Alternatively, they could break it down, but at a very low capacity. After just two days of incubation, the Brevundimonas diminuta L7T4 isolate was able to convert less than 20% of the propoxur to isopropoxyphenol, a rather slow rate of degradation.

Rousidou, Konstantina & Chanika, Eleni & Georgiadou, Dafne & Soueref, Eftychia & Katsarou, Demetra & Kolovos, Panagiotis & Ntougias, Spyridon & Tourna, Maria & Tzortzakakis, Emmanuel & Karpouzas, Dimitrios. (2016) The nematicide oxamyl is mostly controlled in its environmental dissipation by microbial breakdown. Regardless, the microbes responsible for its biotransformation remain mostly unknown. We present the results of our investigation into the improved biodegradation of oxamyl by four bacterial strains isolated from agricultural soil. According to multilocus sequence analysis (MLSA), the bacteria that were isolated belonged to several subgroups of the Pseudomonas genus. The bacteria that were isolated used methylamine as a source of carbon and nitrogen, and they hydrolyzed oxamyl to oxamyl oxime, which was not further converted. Three of the four isolates tested positive for methylamine dehydrogenase, lending credence to this theory. The gene cehA, which has been found in strains that degrade carbaryl and carbofuran, is extremely similar to the gene that all oxamyl-degrading strains have. Analysis of transcription confirmed its direct role in oxamyl hydrolysis. Although they share just the carbamate moiety with oxamyl, the aryl-methyl carbamates carbofuran and carbaryl, as well as the oximino carbamates aldicarb and methomyl, which are structurally similar to oxamyl, were all converted by the selected isolates.

Ahmed, Sadiqul. (2016). Research on bacterial diversity was carried out in the soil of the Nagaon district in Assam's municipal region. By cultivating bacteria in various culture medium and using the pure culture techniques of spread plate and streak plate methods, nine species of bacteria from eight genera have been identified. Colony morphology and a battery of biochemical tests were used to identify the bacteria that were isolated.

RESEARCH METHODOLOGY

An English corporation known as British Drug House Limited supplied the chemical biphenyl. The following chemicals were purchased from the Aldrich Chemical Co., Inc. warehouse in Milwaukee, Wisconsin, USA: 2-isopropoxyphenol, catechol, 2-3-dihydroxybenzoic acid, methylamine, 4-chloroacetophenone, 4-chlorobenzophenone, phthalic acid, benzoic acid, and 4-chlorobenzoic acid.The pH was brought down to 7.0 after the medium was heated, filtered, and mixed. After that, 100 ml portions were placed into 500 ml Erlenmeyer flasks and the mixture was autoclaved at 15 psi for 20 minutes to kill any bacteria. To achieve a final concentration of 0.1%, the autoclaved medium was supplemented with substrate right before inoculation. We skipped adding NH4NO.1 to our nitrogen-free mineral-salts media. Agar in a mineral salts medium made up 1.5% of the solid media.An rise in absorbance at 260 mn, caused by the production of cis, as-muconic acid from catechol, was used as a spectrophotometric test for the enzyme. The cell-free extract, 1 pmol of catechol, and a 0.05 M phosphate buffer with a pH of 7.0 were all components of the reaction mixture, which constituted 1 ml.

DATA ANALYSIS

Isolation of the organism

Using a mineral-salts medium and propoxur as a growth substrate, the organism was isolated from soil using the enrichment culture process. The locations where carbamate insecticides were once disposed of were tested for their soil. After being dissolved in distilled water, about

5 g of soil samples were filtered. Five millilitres of the filtrates were added to five hundred millilitres of mineral-salts medium that was supplemented with 0.2 percent propoxur and then incubated at room temperature (about 28 degrees Celsius) on a rotary shaker set at 150 revolutions per minute. Five millilitres of the culture was transferred to new media after seven to ten days, and the procedure was repeated to monitor the growth. Some of the flasks showed promising development after three or four transfers. After plating the culture on propoxur-mineral salts agar, normal microbiological techniques were used to select and purify the various kinds of colonies that had grown. Cultures were streaked onto nutrient agar plates after being cultured on propoxur-mineral salts agar. We streaked the colonies that had formed on propoxur-mineral salts agar medium again after picking them up. Repeatedly carrying out this procedure was necessary to get pure culture.

Identification of the isolated organism

Using the procedures outlined in the commonly used manuals, researchers examined the biochemical, morphological, and cultural traits of the purified bacterial strain (Seeley and Van Dan Demark, 1972; Holding and Collee, 1971 and Coon et al. 1957). From both nutritional agar and propoxur-mineral salts agar slants, the bacterium's morphology and Gramme response were studied. According to Seeley and Van Dan Demark (1972), the hanging drop technique and Gramme staining with ammonium oxalate-crystal violet were used to examine motility. Melachite green staining allowed for the observation of spores. Both the King A and King B media showed signs of pigmentation.

The Hugh-Liefson technique (1953) was used to produce acid from carbohydrates by oxidation-fermentation. The Yamada and Komagata technique (1972) was used to study the absorption of acetate, a-ketoglutarate, lactate, oxalate, succinate, and benzoate. In a liquid media that was supplied with either 0.1% KNO3 or KNO2, the reduction of nitrate and nitrite was studied. Following the method outlined by Smibert and Krieg (1981), the addition of naphthylamine and sulphanilic acid reagents allowed for the detection of nitrite, the product of nitrate reduction. Iodine indicator was used to detect starch hydrolysis in agar medium that had 1% soluble starch supplied to it. We used nutritional broth to examine growth at a range of temperatures, pH levels, and salt concentrations. To summarise the isolated organism's cultural, morphological, and biochemical traits, see Table 1. The ideal conditions for the organism's development were determined to be 35°C and 7.0 pH, respectively.

Table1. Morphological and cultural characteristics of the isolated organism.

Characteristics	Observation
Colony morphology	Circular, entire, smooth, convex, opaque, white coloured colonies on nutrient agar plates.
Vegetative cells	Small rods, occurring singly or in short chains
Size	0.5 – 0.8 μm by 1.5 – 3.0 μm.
Motility	Motile
Flagella	Present
Gram reaction	Gram – negative
Endospores	Absent
Pigment formation	Brown pigment produced
Growth temperature	
4°C	No growth
35°C	Optimum growth
41°C	Positive

Table 2. Growth of the isolated organism on different media.

Media	Observation
Nutrient agar	Good growth, undulate, opaque.
Nutrient broth	Turbid with sediment
Anaerobic agar	No growth
Yeast extract-glucose-agar	Good growth
5% NaCl broth	No growth
7.5% NaCl broth	No growth
0.02% azide	No growth
Mineral salts medium with :	
Phenylalanine	Good growth
Tyrosine	Good growth

Table: 3. Biochemical characteristics of the isolated organism.

Characteristics	Observation
Catalase	+
Oxidase	+
Urease	+
DNase	+
Arginine dihydrolase	+
Starch hydrolysis	–
Gelatin hydrolysis	+
Indole production	–
H_2S production	–
Nitrate reduction	+
Nitrite reduction	+
Citrate utilization	+
MR –VP test	–
Oxidation or fermentation of Glucose	Oxidation
Acid production from :	
Glucose	+
Sucrose	+
Lactose	+
Ring cleavage of	
Catechol	*Ortho*
Protocatechuate	*Ortho*
Utilization of organic acids	
Acetate	+
Lactate	+
Pyruvate	+
Succinate	+
α-Ketoglutarate	+
Benzoate	+

+ Present ; – Absent;

Isolation of DNA and determination of G+C conten

Following the procedures outlined by Marmur, DNA was isolated (1961). In a 50 ml solution with 0.15 M NaCl and 0.1 M EDTA, pH 8.0, 4 ml of 25% sodium lauryl sulphate was added to the washed cells for suspension. The cells were lysed when the mixture viscosity increased, which occurred after incubation at 60°C with periodic shaking. First, the mixture was treated with 1 M sodium perchlorate to deproteinize it. Then, for about 30 minutes, it was shaken in a separating funnel with a chloroform-isoamyl alcohol combination of 24:1, v/v.

A vessel was used to transmit the water-containing nucleic acid phase after centrifugation. Following the addition of two litres of ethyl alcohol to the water phase, thread-like precipitates resembling nucleic acids were seen. After removing the precipitate with a glass rod, it was dissolved in 10 ml of 0.015 M NaCl and 0.0015 M trisodium citrate (pH 7.0). To remove as much protein as possible from the interface, the solution was extracted many times using chloroform-isoamyl alcohol. We used ethyl alcohol to precipitate the supernatant after each deproteinization procedure, and then dissolved it again in saline-citrate buffer.

The solution was deproteinized once again using a chloroform-isoamyl alcohol combination after being incubated at 37°C for about 30 minutes after the addition of ribonuclease (50 pg/ml). Redissolved in about 10 ml of saline-citrate buffer, the DNA was extracted from the supernatant after precipitation with ethyl alcohol. This solution was supplemented with about 2 millilitres of 3 M sodium acetate (pH 7.0) that contained 0.001 M EDTA. With the exception

of any capsular polysaccharides and RNA fragments, the DNA was precipitated out of the solution. We rinsed the precipitated DNA.

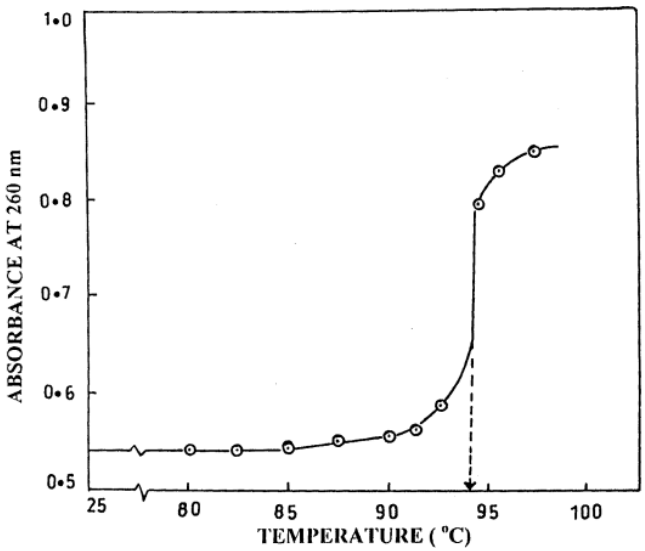

Fig. 1. Melting profile of DNA.

ethyl alcohol content ranging from 70 to 95%, without the presence of salts or butler ions. Using the Burton technique (1956), the DNA was colorimetrically measured using diphenylamine reagent. The melting points of DNA were used to determine the guanine+cytosine (G+C) content of DNA using spectrophotometry, as reported by Mandel and Marmur (1968). An initial absorbance in the range of 0.3 - 0.5 at 260 nm was achieved by adding 3 ml of a 1 mg/ml DNA solution in saline buffer (pH 7.0) to a well-stoppered spectrophotometer cuvette that was placed in a temperature-controlled cuvette holder. The DNA melting profile is given in Fig. 1. It was seen by tracking the change in absorbance as the temperature increased, until a sudden rise in absorbance occurred, which essentially stayed constant as the temperature increased even more. Taking note of Tm, the temperature at the midway of the helix-random coil transition, and using the relation (Marmur and Doty, 1962) allowed us to compute the G+C content (moles %) of DNA.

$$G+C = (T_m - 69.3) \times 2.44$$

Estimates put the G+C composition of the DNA from the isolated bacteria at 62%.

Utilization of various aromatic compounds by the organism

See Table 4 for a list of the aromatic compounds that the isolated organism may use as its only carbon and energy source. The growth media included these substances at a concentration of 1 g/1. Turbidometric measurements were taken at 660 nm every 12 hours to track the bacterial growth.

Table 4. Growth ofPseudomonas sp. on various aromatic compounds.

Compounds	Growth
Propoxur	++
2-Isopropoxyphenol	–
Carbaryl	++
Carbofuran	++
Xylylcarb	++
Metolcarb	++
Methylparathion	–
p-Aminobenzoic acid	++
Anthranilic acid	++
p-nitrophenol	–
Indole	–
Catechol	++
p-Cresol	–
Benzoic acid	+
Protocatechuic acid	++
Salicylic acid	+
Phthalic acid	++
Naphthalene	+
1-Naphthol	+

++ Good growth ; + Moderate growth ; – No growth.

CONCLUSIONS

The organisms that break down propoxur also break down other carbamate pesticides like carbaryl, carbofuran, xylylcarb, and metolcarb. They also use aromatic compounds like p-aminobenzoic acid, anthranilic acid, catechol, protocatechuate, phthalic acid, and naphthalene as their only carbon and energy sources.Metabolite extraction and identification from culture filtrates, growth and oxygen absorption experiments using the likely intermediates, and enzyme demonstration in cell-free extracts all contributed to a better understanding of the metabolic pathway that propoxur breaks down. Separation of 2-isopropoxyphenol from Pseudomonas sp. culture filtrate on propoxur was accomplished by using chromatographic, UV, IR, HPLC, NMR, and mass spectral techniques. The organism does not use 2-isopropoxyphenol for development, according to research on oxygen intake and growth, but it does use the methylamine that is generated when propoxur is hydrolyzed as a source of carbon and nitrogen.

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