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CHARACTERIZATION OF CARBOFURAN-DEGRADING SOIL BACTERIA

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RESEARCH HIGHLIGHTS

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranoyl-Nmethylcarbamate) was a broad-spectrum insecticide-nematicide N-methylcarbamate. It was widely used to control a variety of insect pests of different crops (1). The extensive use of carbofuran insecticides causes their build-up in soils and directly or indirectly effect the functional activities of non-target soil micro-biota. Long-term and overuse of these pesticides have serious effects on the ecology of the soil (2). Micro-organisms are among the most essential biological agents that help in the degradation of these toxic constituents or allow their recycling in the environment (3). A gram-negative bacterial strain BRC05 isolated from vegetable plantation area of Cameron highlands was found to have carbofuran-degrading ability. The isolate was characterized and investigated under various culture conditions. The strain BRC05 showed a maximum growth in mineral salts medium supplemented with 100 mg/L of carbofuran insecticide.

GRAPHICAL ABSTRAC

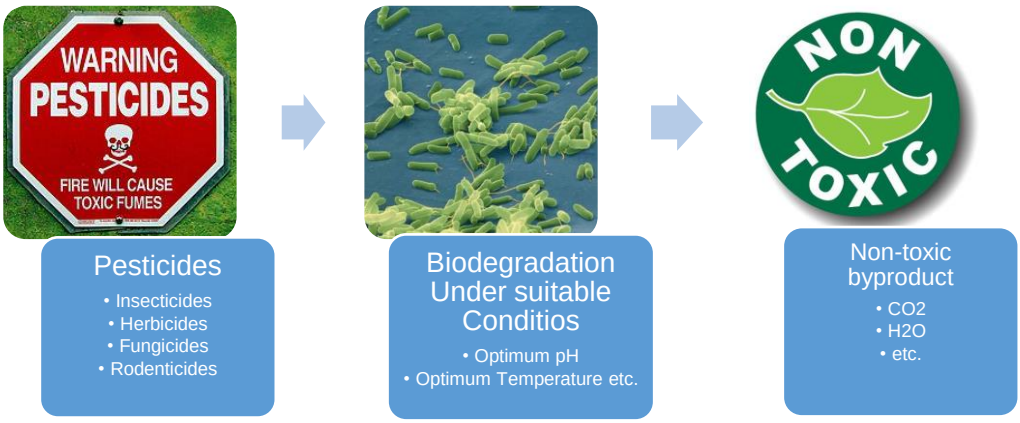

Fig. 1. Biodegradation of pesticides by micro-organism

RESEARCH OBJECTIVES

The negative effect associated with the use of pesticides such as carbofuran, requires the development of novel strategies for their management. The main objective of these research was to isolate, screen, identify and test the potential of pesticide (carbofuran) biodegradation by bacteria. Micro-organisms are highly versatile and capable of utilizing various chemicals as a supplementary or sole source of nutrients. Though many conventional treatment methods such as adsorption, volatilization, incineration, and advanced oxidation are available for the abatement of pesticide contamination from aqueous and soil phases, but micro-organisms can offer simpler, inexpensive and more environmentally friendly strategy for reducing environmental pollution from agricultural and industrial chemicals.

MATERIALS AND METHODS

Carbofuran-degrading bacteria were isolated by the enrichment culture technique. The carbofuran contaminated soils (pH 6.0) was collected from a vegetable plantation areas of Cameron highlands in Malaysia. The isolation of carbofuran degrading micro-organisms was

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done by serially diluting the enrich soils on MSM supplemented with carbofuran as the sole carbon source. Out of the many isolated bacterial strains, BRC05 was selected for further study as its shows high carbofuran degrading ability. The identification of BRC05 was conducted using biochemical tests according to Bergey's manual of determinative bacteriology (4, 5). Bacterial cell growth was determined in 250ml flasks. An aliquot of 250 µl of isolate BRC05 grown in MSM supplemented with carbofuran at an optical density of approximately 0.45 at OD600 were inoculated in MSM containing carbofuran concentration from 25, 50, 75 and 100 mg/L as the sole source of carbon and nitrogen. The inoculums were then incubated at 32°C in a rotational shaker incubator at 150 rpm (shaking) for culture aeration and 0 rpm (non-shaking) conditions for 96 hours (6)

RESULTS

The results show that physical characteristics of the isolate, are associated with gram negative rod-shaped bacteria as observed under light microscope with 100x magnification. Biochemical tests for the identification the isolate shows that BRC05 was negative for citrate utilization test and positive to starch hydrolysis test. Carbofuran degrading isolate BRC05 was also positive for H_2S production test. The gram-negative, rod-shaped strain was found to possess cytochrome oxidase enzyme as it was test positive with N'N'N'N tetramethyl-p-phenylenediamine dihydrochloride which act as an artificial electron acceptor for the enzyme oxidase. The strains also possessed catalase enzyme which was able to form hydrogen peroxide as an oxidative end product of the aerobic breakdown of sugars. MacConkey test was also conducted to differentiate the isolates as the bile salt content in this differential and selective medium inhibits the growth of mostly gram positive bacteria. The growth of bacteria isolate BRC05 was able to grow on MacConkey agar confirming further that the strain is gram negative isolate. Bacterial growth evaluation was done based on increase cell density through turbidity measurement of colony growth on agar medium determined by taking the (OD_{600}) using spectrophotometer at static and shaking conditions. Results shows that isolates BRC05 was able to show good growth on 25 and 50 mg/L carbofuran medium after 12 hours of incubation.

FINDINGS

In this study, bacteria were isolated from agricultural soils with and without pesticides application history. Soil samples with a history of carbofuran exposure were reliably positive for the existence of carbofuran-degrading isolates. Most of the isolates were obtained from farms with known history of carbofuran application. It was found that the growth of the carbofuran degrading bacteria and pesticides degrading ability were dependent on nutrient availability, pH and temperature. Many indigenous micro-organisms in water and soil are capable of degrading pesticides and other contaminants.

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