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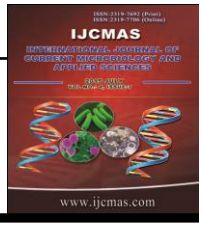

Original Research Article

Isolation and Characterization of Sulfosulfuron Utilizing Bacteria from Wheat Cultivated Soil

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Keywords

Bacillus sp., Bioremediation, Enrichment, Optimization, Sulfosulfuron

A B S T R A C T

Two sulfosulfuron degrading bacteria were isolated using serial dilution technique followed by selective enrichment on minimal medium with sulfosulfuron as the sole carbon source, from soil samples collected from a wheat field in Baidauli Mahua Dih, a village approximately 6 km from District Kushinagar, Uttar Pradesh, India. The isolates were characterized by staining and different biochemical tests. The strains B1 and B2 were identified to be *Bacillus sp*.. For maximum degradation, the cultures were optimized on different parameters such as incubation time, pesticide concentration, temperature and pH. Microbial growth during the study was monitored by measuring the optical density at 620nm. Both the isolates showed growth at temperatures ranging from 27°C to 47°C and pH 4.0 to 9.0. The best result for growth of both the isolates was on minimal medium enriched with 0.5% sulfosulfuron at pH 6.0 and temperature 37°C, incubated for 72 hrs at 150 rpm. These results show that both the isolates may possess potential to be used in bioremediation of sulfosulfuron contaminated environment.

Introduction

Pesticides are defined as "Any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies.

The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport" (FAO, 2002). The term pesticide encompasses a variety of different types of chemicals including herbicides, insecticides, fungicides, and rodenticides, among others. Pesticides are necessarily poisonous but they play an

important role in increasing the crop yield and for the consistent supply of food to the ever growing human population (Akthar & Ahmed, 2002). The excessive use of pesticides lead to an accumulation of a huge amount of pesticide residues in the food chain and drinking water environment that further leads to a substantial health hazard for the current and future generations due to uptake and accumulation of these toxic compounds. This problem needs to be solved to prevent it from becoming worse. Degradation of pesticides is usually a combination of a number of processes, including chemical hydrolysis and microbial degradation, and is also influenced by some physico-chemical properties such as pH, organic carbon and moisture content (Gunther, 1974).

However, biodegradation is the primary mechanism of pesticide breakdown and detoxification in many soils. Bioremediation constitutes an attractive alternative to physico-chemical methods of remediation, as it is less expensive and can selectively achieve complete destruction of organic pollutants (Alexander, 1999). For a successful bioremediation technique an efficient bacterial strain that can degrade largest pollutant to minimum level is required. Soil microorganisms can carry out pesticide degradation and can use the xenobiotic as a source of carbon, energy and other nutrients to promote microbial growth (Durkin, 2003). A considerable number of bacterial strains have been isolated that possess the ability to degrade xenobiotic compounds (Kumar & Philip, 2006; Malghani et al., 2009) and microbial process plays an important role in biological transformation of pesticides (McGuinness & Dowling, 2009; Sarkar et al., 2008). Many microorganisms degrade pesticides, because of their comprehensive enzyme systems, which have the capacity to hydrolyze, reduce and oxidize these compounds (Audus, 1960). Sulfosulfuron is a broad-spectrum herbicide that kill plants by inhibiting the enzyme acetolactate synthase. It is a selective, systemic sulfonyl urea herbicide, absorbed through both roots and leaves. It translocates throughout the plant and acts as an inhibitor of amino acid biosynthesis, hence stopping cell division and plant growth. It is effective against grasses and broad-leaved weeds in wheat. Barley and oats are sensitive. Sulfosulfuron is the common name for the chemical 1-(4,6-dimethoxypyrimidin-2-yl) -3- (2-ethylsulfonylimidazo-(1,2-a) pyridin-3-yl) sulfonylurea. The molecular formula is $C_{16}H_{18}N_6O_7S_2$ and the Chemical Abstracts Service CAS number is 141776-32-1. The aim of the present work was to isolate and characterize sulfosulfuron utilizing bacteria from wheat cultivated soil by using enrichment culture technique. In addition some parameters affecting growth of the isolated bacterial strains were also optimized.

Materials and Methods

Pesticide used

Commercial grade Sulfosulfuron 75% WG was obtained from Saharanpur chowk near Railway Station, Dehra Dun, Uttarakhand, India and used for the experiment.

Collection of soil samples

The samples was collected about 10cm below the soil surface using a sterile spatula in sterile polythene bags from different sites of a wheat field in Baidauli Mahua Dih, a village approximately 6 km from District Kushinagar, Uttar Pradesh, India. The soil samples was collected ten days after the spraying of pesticides. The samples were stored at 4^oC till further analysis.

Isolation of bacteria

Soil suspension was prepared by mixing 5gm of sieved soil in 45ml distilled water. It was then stirred and allowed to settle. This allowed the microorganisms to come in the water phase. The suspension was then serially diluted from 10^{-1} to 10^{-5}. Minimal agar medium enriched with sulfosulfuron was used for the isolation of bacteria. Minimal agar medium has the following composition in (g/L): Dextrose, 1.0; K_2HPO_4, 7.0; KH_2PO_4, 2.0; Sodium citrate, 0.5; $MgSO_4.7H_2O$, 0.1; $NH_3(SO_4)_2$, 1.0; and Agar Agar, 15.0 with pH 7.0 ± 0.2. Minimal agar plates with different concentrations of sulfosulfuron (0%, 0.1%, 0.5%, 1.5% and 2%) were prepared and dilutions, each 100µl were spread on the surface of the plates and incubated for 24hrs at 37 C ± 1°C. The colonies so obtained on the plates were marked and numbered. They were then streaked on minimal agar supplemented with 0.5% sulfosulfuron and incubated at 37°C for 24 hours. After that, the colonies were sub cultured every 24 hours in minimal agar with 0.5% sulfosulfuron until pure cultures are obtained. Bacterial pure cultures were maintained on nutrient agar slants and stored at 4°C and sub cultured every month.

Characterization of bacterial isolates

Isolated pure strains were identified on the basis of morphological and physiological characteristics in nutrient agar plates, slants and broth and by biochemical tests. Colony size, Margins, Forms, Texture, Elevation and Colour was studied. Simple and Gram staining was carried out. Catalase test, citrate utilization test, indole production test, methyl red-voges proskeur (MR-VP) test, nitrate reduction test, TSI, H_2S production test, litmus milk test, oxidase test and urease tests were carried out for the identification (Cappuccino and Sherman, 1999).

Optimization of culture conditions

Sulfosulfuron degradation using the isolated test organisms was optimized under different conditions and parameters.

Effect of incubation period on bacterial growth

Nutrient broth enriched with 0.5% sulfosulfuron was taken in conical flasks and inoculated with the test organisms. The cultures was incubated for 120 hours at 37°C. Uninoculated control was taken as a blank. Growth was measured spectrophotometrically (620nm) at varying time intervals.

Effect of sulfosulfuron concentration on bacterial growth after 72 hours of incubation

Nutrient broth enriched with different concentrations of sulfosulfuron (0.5% to 3.0%) was inoculated with the seed culture and incubated for 72 hours. After 72 hours growth was measured at 620nm. Controls were carried out in the same conditions but without inoculum.

Effect of pH on bacterial growth after 72 hours of incubation

Nutrient broth prepared at different pH (4, 5, 6, 7, 8 and 9) was taken in conical flask with sulfosulfuron concentration 0.5% and inoculated with seed culture. Growth was measured at 620nm after 72 hours using uninoculated control as blank.

Effect of temperature on bacterial growth after 72 hours of incubation

Nutrient broth prepared at optimum pH supplemented with sulfosulfuron (0.5%) was taken in conical flask and inoculated with seed culture. The cultures were incubated

for 72 hours at different temperatures. Growth was measured at 620nm using uninoculated control as blank.

Results and Discussion

Isolation of bacterial strains

Two morphologically distinguishable bacterial colonies were observed on minimal salt agar enriched with the herbicide sulfosulfuron. Pure culture of these isolates were preserved as shown in figure 1.

Identification of the bacterial isolates

Isolated pure strains were identified by Bergey's Manual of Determinative Bacteriology, on the basis of morphological and physiological characteristics. The results obtained are shown in table 1.

Optimization of bacterial culture conditions

Pesticide degradation using the isolated test organisms was optimised under different conditions and parameters as given below.

Effect of incubation period on bacterial growth

Growth of B1 and B2 was studied by measuring the optical density at 620nm after 4, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96 and 120 hours of incubation and the results were as shown in figure 2.

Effect of sulfosulfuron concentration on bacterial growth after 72 hours of incubation

Growth of B1 and B2 was studied by measuring the optical density at 620nm at different concentrations of sulfosulfuron (0.5% to 3.0%). The results obtained are depicted in figure 3.

Effect of pH on bacterial growth after 72 hours of incubation

Growth of B1 and B2 was studied by measuring the optical density at 620nm at different pH (4, 5, 6, 7, 8, 9) and the results were as shown in figure 4.

Effect of temperature on bacterial growth after 72 hours of incubation

The degradation efficiency of B1 and B2 was studied by measuring the optical density at 620nm at different at temperatures ranging from 27 to 47^{O}C. The result obtained is shown in figure 5.

A large amount of pesticides are being used now-a-days in crop protection which has resulted in build up of such harmful compounds in the environment, proving a menace to humans, animal life as well as to soil microbes. Residues of these pesticides have been reported in soil, water and foods. Sulfosulfuron is among the most widely used pesticide for treating unwanted herbs in crops. Sulfosulfuron, a sulfonylurea herbicide may result in the development of resistant herbs displaying its carry-over effects to the next crop cultivated. These pesticides possess large half-lives and thus remain persistent in the environment which may lead to harmful consequences in near future. Besides chemical and photo-catalytic degradation of pesticides, microbial degradation has now been evolved as a much effective and safer way to eradicate these harmful compounds from the environment. However, a limited literature is available on the microbial degradation of such compounds. Sulfonylureas are a unique group of herbicides used for controlling a range of weeds and some grasses in a variety of crops and vegetables.

Table.1 Morphological and physiological characteristics of the bacterial isolates

Characteristics	***Bacillus sp. (B1)***	***Bacillus sp. (B2)***
Gram Reaction	+	+
Morphology	Rod	Rod
Arrangement	Chains	Chains
Motility	+	+
Oxidase	[+]	-
Indole	-	-
MR	-	-
VP	+	+
Citrate	[+]	[+]
Urease	[-]	-
H_2S	-	-
Nitrate Reduction	+	+
Catalase	+	+
Starch	[+]	+
Glucose	+	+
Lactose	-	[-]
Sucrose	+	+

Fig.1 Master plates of bacterial isolates

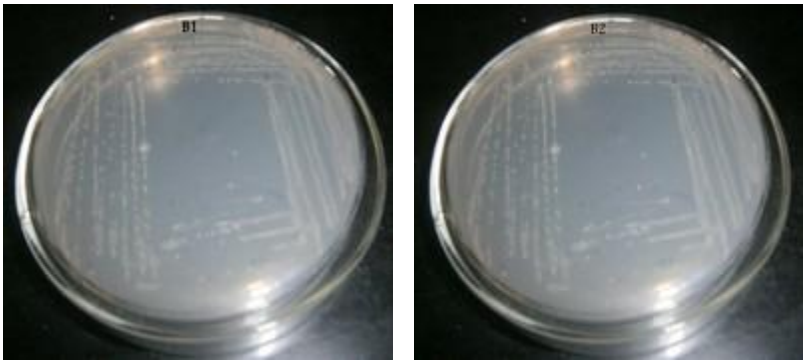


Fig.2 Effect of incubation period on bacterial growth

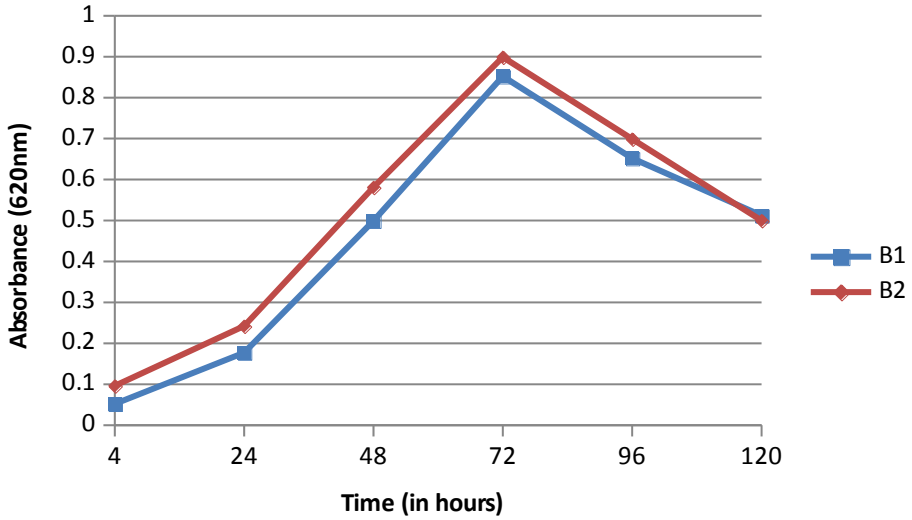


Fig.3 Effect of sulfosulfuron concentration on bacterial growth after 72 hours of incubation

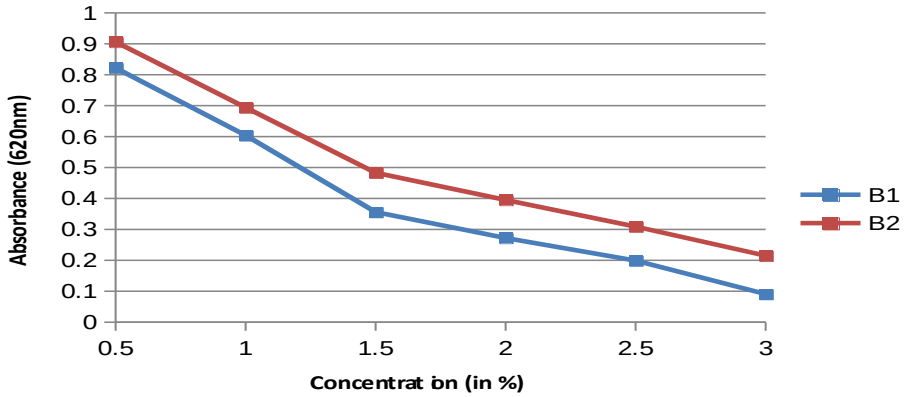


Fig.4 Effect of pH on Bacterial Growth after 72 hours of Incubation

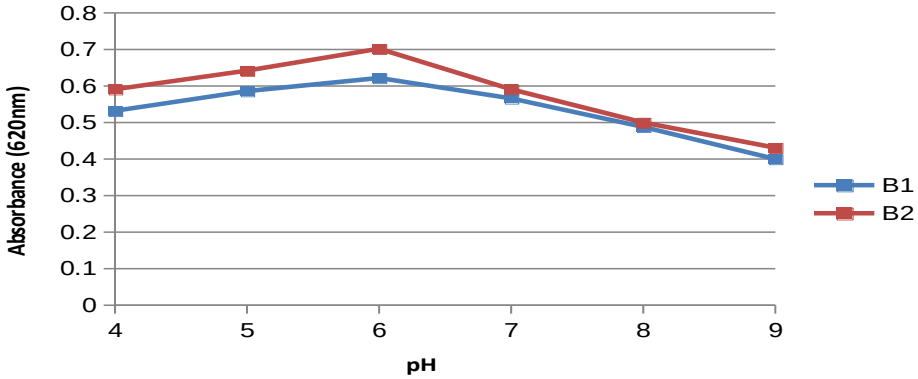
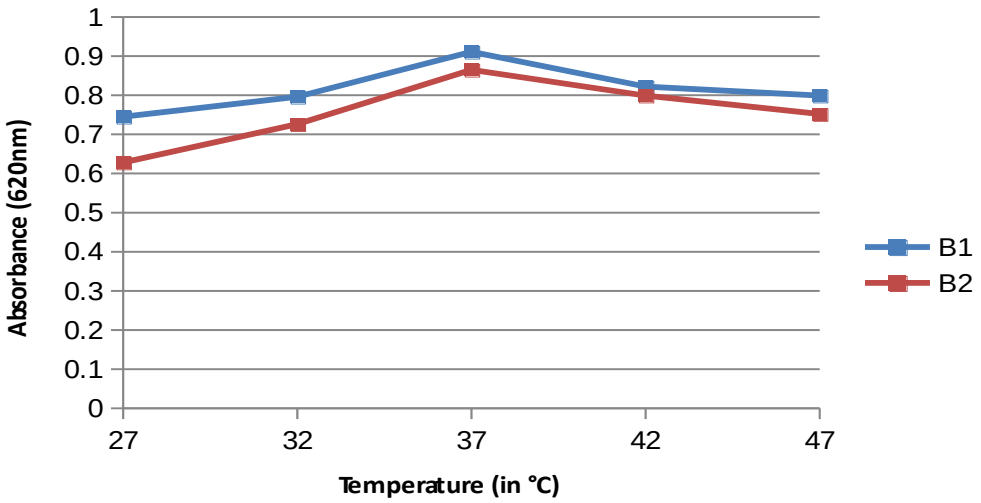


Fig.5 Effect of temperature on bacterial growth after 72 hours of incubation



They have been extremely popular worldwide because of their low mammalian toxicity, low use rate, and unprecedented herbicidal activity. Knowledge about the fate and behavior of sulfonylurea herbicides in the soil-water environment appears to be of utmost importance for agronomic systems and environmental protection. Because these herbicides are applied at a very low rate, and their mobility is greatly affected by the chemicals' anionic nature in alkaline soils, a thorough understanding of their degradation/hydrolysis processes and mechanisms under aqueous and soil systems is important.

As described by Merrill A. Ross and Thomas N. Jordan of Purdue University in 1999, sulfonylureas are primarily broken down by hydrolysis and microbes. Sulfonylurea herbicides are more tightly adsorbed to soil particles and soil organic matter at low pH. Sulfonylurea herbicides carry-over more in higher pH soils since acid hydrolysis ceases at high pH levels. The rate of hydrolysis is greatest at pH below 6.8 and as the temperature increases. Variable pH across a field can greatly affect the ability of a herbicide to persist in the soil (Merril and Thomas, 1999).

This study reports the isolation and characterization of soil-borne bacterial strains (*Bacillus sp.*) from soil samples collected from a wheat field in in Baidauli Mahua Dih, a village approximately 6 km from District Kushinagar, Uttar Pradesh, India, that possess the capacity to use sulfosulfuron. The capacity of these isolates to survive and grow in the presence of the herbicide, show that these strains may possess potential to be used in bioremediation of sulfosulfuron-contaminated environments. This study also provides important information on optimization of critical parameters to enhance sulfosulfuron degradation by the isolated bacterial strains.

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