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Isolation, Identification and Role of Glyphosate-Degrading Bacteria from Soils of Baghdad

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Abstract: Glyphosate (N-phosphonomethylglycine) is the most commonly used herbicide worldwide. Due to concern regarding its toxicity for non-targeted species in soil, finding glyphosate-degrading microorganisms in soil is important as these bacteria can grow by utilizing glyphosate as a source of carbon. Two bacteria species were isolated from soils of Baghdad. Morphological characteristic and biochemical reactions indicated these species were identified as Bacillus and Serratia marcescens. These bacterial isolates showed an ability to consume glyphosate as energy and sole carbon source at 20 mM. The growth of bacteria in the media containing glyphosate was determined after two days of incubation at 30 C by measuring turbidity (O.D) at 680 nm. The maximum growth of Bacillus in halogenated compound containing media was found to be (O.D=0.3836) after two days of incubation as compared to control media (O.D=0.0170) without halogenated compound. In comparison to *Bacillus*, *Serratia marcescens* showed less growth activity (O.D=0.06) in halogenated compound containing media after two days of incubation as compared to its growth in control media (O.D= 0.03). The maximum chloride ion released due to dehalogenase enzyme activity was higher for *Bacillus* (O.D=1.3199) as compared to *S. marcescens* (O.D= 0.3) with respect to their control media (O.D =0.0491 and 0.04) respectively. This result gives hint regarding the role of dehalogenase present in the bacteria for their affinity to substrate and utilizing it for their growth. For a better understanding of dehalogenase enzyme produced by these two bacterial species, more research has to be explored for their possible use as bioremediation tools in the natural environment.

Keywords: Glyphosate, Soil bacteria, Bio-degredation

Introduction

Glyphosate is a non-selective herbicide having potential to kill both broadleaf plants and grasses. The sodium salt form of glyphosate regulates plants growth and help in ripening fruit instead of inhibiting plant growth (Eker et al., 2006). Glyphosate prevents the plant growth by inhibiting certain enzymes of shikimic acid pathway which is an important pathway necessary for plants and some microorganisms (Tohge et al., 2013). Due to its tight binding with soil, it is not likely to get into groundwater and will remain in soil for up to 6 months depending on the type of climate and soil. It is well established that the half-life of glyphosate present in dead leaves were broken down in 8 or 9 days. In addition, some of glyphosate were absorbed by carrots and lettuce grown in soil containing glyphosate (Diamand and Broon, 2001).

Recently, biological decontamination processes are preferred more as compared to conventional approaches due to the utilization of some microorganisms to degrade and detoxify many toxic xenobiotics, especially pesticides, which is an efficient tool for the decontamination of polluted sites, which in general microorganism does it without producing toxic intermediates in the environment (Ortiz-Hernandez 2013; Javaid et al., 2016).

Some strains of bacteria that are able to degrade carbamate pesticides have been isolated from soil around the world (Hamada et al., 2015). Previous studies have shown that atrazine-degrading bacteria applied as single strains or as consortia can increase degradation of atrazine in soil (Wanget al., 2014), thus, the present study was

undertaken to isolate and characterize glyphosate-degrading bacteria from different untreated soils of Baghdad. Together, the assessment of growth response of these isolates and optimization of some abiotic parameters for the cultivation of isolated strains providing maximal effective condition for the glyphosate degradation.

Method

Determination of Glyphosate Degrading Strains

Fresh samples collected from soil were inoculated into Luria-Bertani (LB) agar plates containing 1.0% tryptone, 0.5% yeast extract, 1.0% NaCl and halogenated compound and then incubated at 30 °C for 16 h. Several colonies were selected from this plate and streaked onto fresh LB agar medium to obtain a pure colony. Pure bacteria colony grown were inoculated in glyphosate liquid broth containing 20 mM concentration of carbon and incubated in shaker (150 rpm, 30 °C). At different time interval, small quantity of liquid medium were taken out to determine the growth of bacteria by measuring turbidity (absorbance value) of broth at 680 nm.

Determination of Chloride Ion Released in Growth Medium

The protocol of Bergman and Sanik (1957) was followed to measure dehalogenase enzyme activity. Bacteria sample (1mL) was added into 100 µL of 0.25 M ammonium ferric sulphate prepared in 9 M nitric acid and mixed thoroughly. After proper mixing, 100 µL of mercuric thiocyanate-saturated ethanol was added and mixed. The color was allowed to develop for 10 min and chloride ion liberation were determined by taking absorbance at 460 nm.

Results

Isolation and Identification of Bacteria

Two different bacteria were isolated and identified by morphological characteristics and biochemical reaction as *Serratia marcescens* and *Bacillus* from different soils of Baghdad. These isolate were able to grow in a culture medium in presence of herbicide glyphosate and utilized it as an energy source.

Bacterial Degradation of Glyphosate Medium

Bacteria were grown in minimal medium that supplied with 20mM Glyphosate at 30 oC. The growth was determined by measuring the optical density at 680nm, the highest growth was (OD average = 00.3836) in Bacillus after two days of incubation compared to control (OD average = 0.0170), while the lowest growth was (OD average = 0.06) in *Serratia marcescens* compared with control was (OD average = 0.03).

Chloride Ion Released into Growth Medium

Maximum amount of ion chloride was released by dehalogenase enzyme activity. The ODs averages were about 1.31 and 0.055 compared with control 0.0491and 0.03 for *Bacillus* and *Serratia marcescens*, respectively.

Discussion

In the present study, two different bacterial species were isolated from soil and identified as *Serratia marcescens* and Bacillus. These bacteria are a glyphosate degrading microorganism that could grow by utilizing glyphosate as a sole source of carbon or phosphorus, and able to produce dehalogenase enzyme therefore have potential to degrade halogenated compound. The reduction in microbial population when glyphosate was added to the medium culture can be explained by the mode of action of glyphosate which makes the organisms unable to synthesize essential aromatic amino acids (Kryzsko-Lupicka and Orlik, 1997). Allison et al reported that *Serratia* has faster growth rate (doubling time) than Rhizobium Sp and ability to consume 2,2-dichloropropionate as a source of carbon at 20 mM with cell doubling time of 5 h and maximum chloride ion

release of 38µmol/ml (Adel et al., 2012; Alison et al., 1983), however, the present work showed that the expression of dehalogenase enzyme in *Serratia* is lower as compared to *Bacillus* because of which *Serratia* releases less amount of halogens causing its slow growth on glyphosate medium. There were some other species which were reported to degrade glyphosate like Alkaligenes sp., Rhizobium sp. , Agrobacterium sp., *Bacillus megaterium*, Flavobacterium sp. (Tolbot et al., 1984) (Peng et al., 2018). In the natural environment, the use of microorganisms for glyphosate as a sole source of energy provides a substantial competitive advantage over other microorganisms in successful removal of herbicide.

Conclusion

This study is the first report on the isolation and characterisation of soil bacterial strains of Serratia marcescens and Bacillus. from untreated soil in baghdad that possesses the capacity to degrade glyphosate. In addition, this work provides vital information on the glyphosate degradation and dehalogenase enzyme production by these bacteria.

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