

Biodegradation of Glyphosate Pesticide by Bacteria isolated from Agricultural Soil

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Abstract: Three bacteria strains GDP1, GDP2 and GDA were isolated from agricultural soil heavily polluted with glyphosate which are capable of degrading glyphosate pesticide (1000 ppm). The bacteria strains were identified through cultural and biochemical characterization as *Pseudomonas putida*, *P. aeruginosa* and *Acetobacter faecalis* respectively. Degradation of glyphosate by GDP1, GDP2, GDA and a mixed consortium of the three isolates designated CGD were confirmed by solid-phase extraction SPE-LC-ESI-MS assays. Glyphosate-mineralizing populations were determined in a most probable number (MPN) technique. Cell growth levels off at approximately 72hr, which coincides with glyphosate concentration decreasing to zero level. Of three isolated bacteria GDP1 completes degradation of 50- μgml^{-1} glyphosate in 20 ml of an enrichment medium BMA at approximately 72hrs faster than GDP2 and GDA which completed at approximately 96hrs. Addition of a mixed consortium of GDP1, GDP2 and GDA did not result in significantly faster degradation processes but reduced the lag times to approximately 12hrs from 24hrs and completes degradation at approximately 72hrs. The developed consortium is potent glyphosate degrader with quick action as indicated by the shorten lag times, and it can be used to remediate soil contaminated with pesticide.

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Introduction

Pesticide is widely used in Nigeria for controlling insects, pest of cocoa, cotton, cowpea and many other crops, for example, Gamalin 20. Other pesticides such as atrazine, paraquat 2,4-D and glyphosate are used for destroying grasses and broad leaf weed in maize (Anon, 1999).

The excessive use of pesticides leads to accumulation of a huge amount of residues in the environment, thereby posing a substantial health hazard for the current and future generations due to uptake and accumulation of these toxic compounds in the food chain and drinking water.

Each year some people died as a result of insecticide poisoning. In 1956 in the United State, insecticides caused 152 death including 94 children less than 9 year old. In 1969 a family in New Mexico was reported to have been poisoned by organic mercury which was present in seed grains fed to hogs the family had slaughtered for food (Curdley *et al.*, 1991). In 2009, 2 persons in Ekiti state, and 5 people in Osun State all in Nigeria were reportedly killed by pesticide poison present in grains they used to prepare their food.

Owing to the indiscriminate use of insecticides in Nigeria, Odeyemi (1980) in assessing the pollution potential of insecticides found that arasan which is commonly used on seed crop

disappeared in samples of sewage and freshwater within 12 days of application. However, the antifungal chemicals such as phygon and spergon were found to be recalcitrant in samples of seawater, after 8 Months of application.

Contamination of soil from pesticide mixing, loading, Storage and rinsing at agricultural chemical dealership is a concern due to potential contamination of surface water and groundwater (Moormann *et al.*, 1998).

The use of microorganisms in the degradation and detoxification of many toxic xenobiotics, especially pesticides, is an efficient tool for the decontamination of polluted sites in the environment (Mohammed,2009). Biological decontamination methods are preferable to conventional approaches because in general, microorganisms degrade numerous environmental pollutants without producing toxic intermediates (Pieper and Reineke, 2000; Farukawa, 2003).

Many bacteria that are able to degrade carbamate pesticides have been isolated from soil around the World (Desaint *et al.*, 2000). Previous studies have shown that atrazine-degrading bacteria applied as single strains or as consortia can increase degradation of atrazine in soil (Mandelbaum *et al.*, 1995).

The objective of this study was to isolate several bacterial strains that have the ability to degrade high concentrations of glyphosate a common herbicide used in Nigeria.

Materials and Methods

Enrichment and Isolation

The pesticide glyphosate (in form of 480g/lit isopropylamine salt) with commercial name “weed fit” was purchased from agricultural chemical dealership shop at Osogbo Osun State Nigeria. Soil samples were collected from 5 glyphosate-treated corn fields at Farmer College along Akoda–Ede Osun State, Nigeria as source of glyphosate-degrading bacteria.

The method of Mohammed (2009) was adopted in isolation of the bacteria. 1g of each soil sample was added to 20 ml of autoclaved minimum salt medium (MSM) containing glyphosate (1000ppm) as a sole carbon and energy source. Cultures were incubated in an orbital shaker at 30⁰C and 160rpm for 7days. 1ml of each enrichment cultures was then transferred to 20ml of fresh autoclaved minimal medium and incubated for additional 7days under the same conditions. After enrichment cultivations, 0.1ml of the culture was spread on minimal salt agar plates (MSA, MM containing 1.5% Bacto Noble Agar) that contained glyphosate (1000ppm) as a sole carbon source. The plates were then incubated at 30⁰C for a week. After several transfers into fresh MSM, mixed cultures were obtained. A colony from each isolate was then used to inoculate 20ml of MSM containing glyphosate (1000ppm) and incubated for a week in an orbital shaker under the same condition in order to confirm the biodegradability. The strains that showed notable growth under this condition were selected as possible glyphosate degrader and designated as GDP1, GDP2 and GDA.

Characterization and Identification of Isolated Bacterial Strains

The selected isolated bacteria strains were characterized and identified on the basis of physiological microbiological and biochemical characterization using standard method described by Olutiola *et al.* (1991) and Fawole and Oso (2001).

Degradation Test

Degradation of glyphosate by GDP1, GDP2 and GDA strains and a mixed consortium of the 3 strains designated as CGD in liquid media were confirmed by solid-phase extraction SPE-LC-ESI-MS assays. 5 biometer flasks were prepared in triplicate containing glyphosate 50- μgml^{-1} in 20ml of an enrichment medium designated BMA, contains a basal minimal salts medium supplemented with 1g each of sodium citrate and sucrose, 20ml of vitamin solution (Moorman *et al.*, 1998). The first one (standard sample) was a non-inoculated sample, only kept as control. Four other samples were separately inoculated with 1ml (10^5cellsg^{-1}) of GDP1, GDP2, GDA and CGD respectively. All cultures were incubated in an orbital shaker for 48hrs at 150rpm at 30⁰C. Glyphosate was extracted from the liquid media by solid phase extraction method as described by Mohammed, (2009). Glyphosate-mineralizing populations were determined in a most-probable number (MPN) technique (Jayachandran *et al.*, 1998)

Results and Discussion

Three pure cultures designated GDP1, GDP2 and GDA which were able to grow in minimal media containing glyphosate as a source of carbon were generated through enrichment procedure. Biochemical and growth characterization of the three isolates were further investigated. The result showed that the three isolates were short rod Gram Negative, motile *Pseudomonas putida*, *P. aeruginosa* and *Acinetobacter faecalis*. The three isolates showed positive reactions for catalase, citrate and oxidase tests. Only *Acinetobacter* grew on MacConkey agar and its colour was translucent. *P. putida* was creamy, while *P.aeruginosa* colony was greenish. Feng and his co-workers in 1998 reported for the first time isolation of a pure culture of bacteria capable of using 3, 5, 6-trichloro-2pyridinol (TCP) as the sole source of carbon and energy under aerobic conditions (Bhagobaty *et al.*, 2007). It has been reported also that *Pseudomonas* strain ADP inoculated to soil contaminated with 1500 μg of atrazine g^{-1} resulted in mineralization of over 60% of 10 μg of the pesticide in 49 days (Yanze-Korotchou and Gschwind, 1995).

Degradation of glyphosate by the isolates

The capability of the three strains GDP1, GDP2 and GDA to degrade the pesticide (glyphosate) was confirmed. Glyphosate-mineralizing populations were determined in a most probable number (MPN) technique. Cell growth levels off at approximately 72hr, which coincides with glyphosate concentration decreasing to zero level. Of three isolated bacteria GDP1 completes degradation of 50- μgml^{-1} glyphosate in 20 ml of an enrichment medium BMA at approximately 72hrs which was faster compare to GDP2 and GDA which completed at approximately 96hrs. Addition of a mixed consortium of GDP1, GDP2 and GDA did not result in significantly faster degradation processes but reduced the lag times to approximately 12hrs from 24hrs and completes degradation at approximately 72hrs.

The rate of degradation of GDP1 was faster than the two other strains, which may be due to variation in genetic composition. It is noteworthy to

mention that the developed consortium is potent glyphosate degrader with quick action as indicated by the shorten lag times. Previous studies have shown that pesticide degrading bacteria applied as consortia can increase degradation in soil (Moorman, 1998).

Conclusion

The present study demonstrates that the isolated *Pseudomonas putida* GDP1, *P. aeruginosa* GDP2 and *Acinetobacter faecalis* GDA strains posses strong ability for glyphosate degradation. Application of bacteria consortium, improvised after isolation of potent pesticide degraders from contaminated site, can be used to remediate soil contaminated with pesticide.

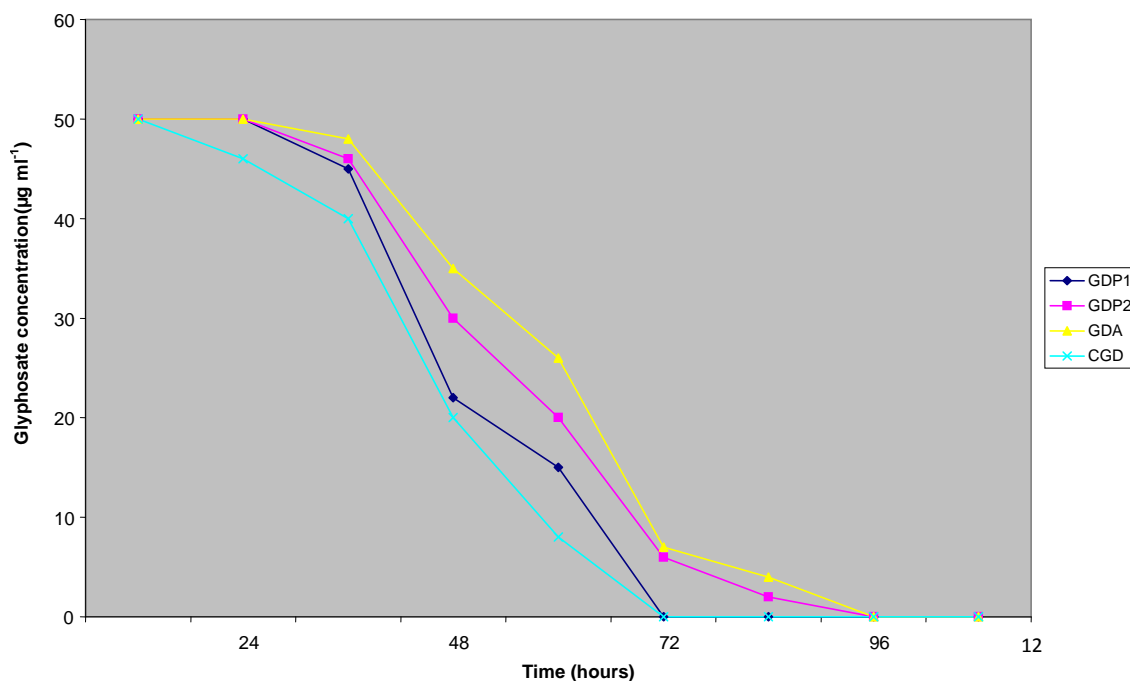


Fig 1a: Glyphosate degradation with concentration decreasing.

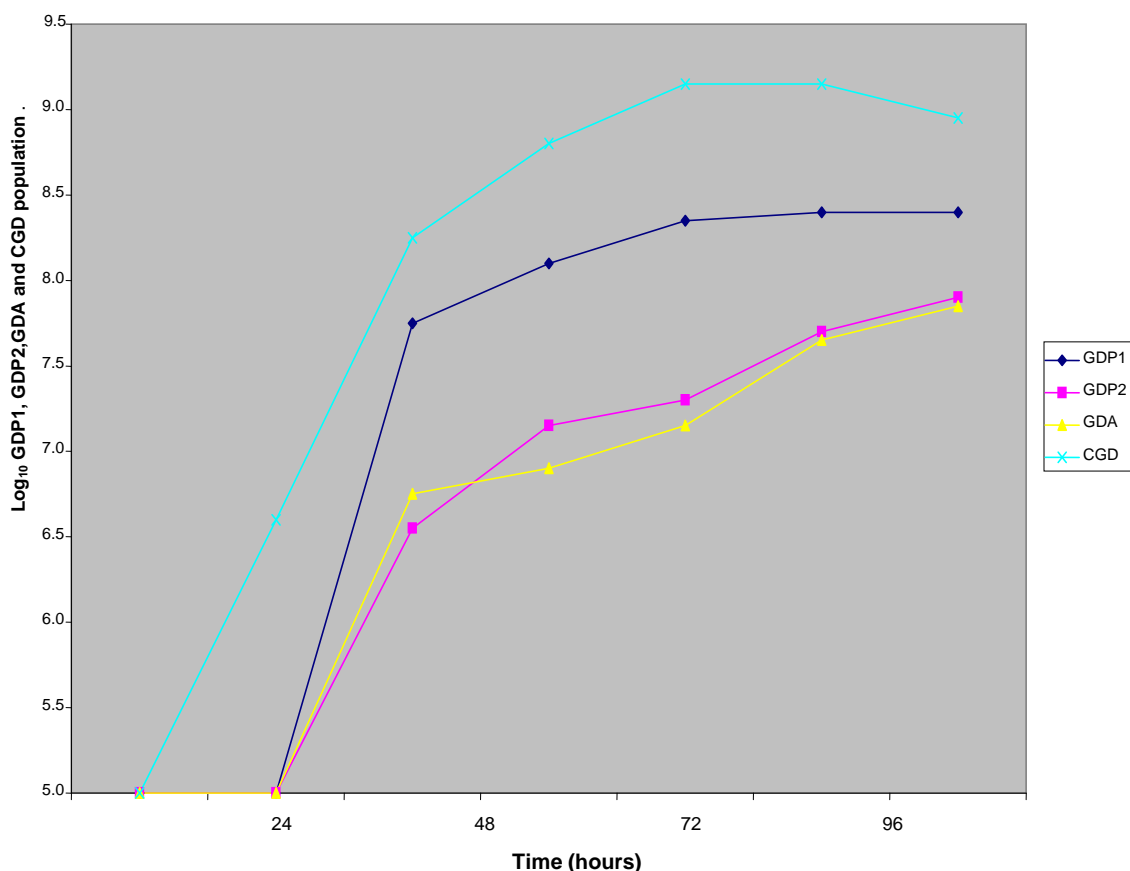


Fig.1b Cell Density of Bacterial isolates during degradation.

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