



BIODEGRADATION AND BIOREMEDIATION OF GLYPHOSATE HERBICIDE BY *NOCARDIA MEDITERRANIE* THS 1

Science

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ABSTRACT

Glyphosate herbicide is one of the main compounds used as a control /killing agent of a wide variety of wild grasses and other herbaceous plants. The bioremediation of these glyphosate affected soil depends upon the capability to isolate bacteria which has the capability to degrade these xenobiotic compounds. The capability of *Nocardia mediterranie* THS 1 strain to produce biosurfactants for degrading the herbicide glyphosate was studied by growing the organism in MS medium with glyphosate, the organisms capability to utilize the glyphosate as further proved by growing these organisms in MS medium incorporated with 3 different concentrations of glyphosate which is normally applied to the field. The study also proved that *Nocardia mediterranie* THS 1 had a high capability to degrade glyphosate at a higher rate as the OD values had a significant increase as the concentration increased from 10ppm to 30ppm. The GC-MS result also showed the degraded product of glyphosate that is AMPA in various fragmented peaks thus concluding that this strain could be used as a great bio controlling agents in various contaminated agricultural fields.

KEYWORDS

Glyphosate, Xenobiotics, Actinomycetes Sp, *Nocardia mediterranie* Ths 1, Ampa.

INTRODUCTION

Pesticides are considered to be one of the most widely used weed controllers and a wide variety of plant species (Mohsen Nourouzi *et al.*, 2011). The intensive use of these herbicides have helped in increasing the crop yield but reducing the natural ecological balance, this heavy use of these herbicides have lead to the pollution of streams, lakes, rivers, ponds through runoffs. The most commonly and frequently used herbicide is 2, 4-dichlorophenoxyacetic acid (2,4-D) and Roundup® (isopropylamine salt of glyphosate) (Quided Benslama and Abderrahmane Boulahrouf, 2011).

Bacteria are perhaps the most versatile and diversified organisms with regards to their nutritional requirements (Mohsen Nourouzi *et al.*, 2011). Microorganisms are key for bioremediation but these microorganisms need time to obtain the ability to degrade the newsynthetic and heavy compounds that are released to the environment (Olawale *et al.*, 2011). The aim of our study was to study the capability of our microorganism *Nocardia mediterranie* THS 1 to utilize the glyphosate as an energy source and degrade the glyphosate to simpler nontoxic product by the action of biosurfactants. The study proved the ability of *Nocardia mediterranie* THS 1 to degrade glyphosate of various concentrations and showed its potential to be used as one of the key tool for the bioremediation of xenobiotics and other heavy polluting compounds accumulated in the environment.

MATERIALS AND METHODS

SAMPLE COLLECTION

SOURCE OF GLYPHOSATE

The isopropylamine salt of glyphosate known as Roundup® was purchased from a local store supplier of agricultural products in Palakkad, Kerala.

USAGE OF MICROORGANISM

The organism used for the degradation of glyphosate *Nocardia mediterranie* THS 1 was obtained from Dr. T.H SUKIRTHA, Asst. professor, Dept. of biotechnology Nehru Arts and Science College, Coimbatore.

Identification of *Nocardia mediterranie* THS 1

The organism was identified by the biochemical test (Bergie's Manual 1981).

MINERAL SALT MEDIUM

Screening of the microorganism was carried out using the mineral salt medium (MSM). The composition of the medium in gram per liter of distilled water, (Ph-7.2) was : KH₂PO₄ (0.2), MgSO₄ (0.2), CaCl₂ .6H₂O (0.1), (NH₄)₂SO₄ (0.2), yeast extract (0.2), peptone (0.5), was used for the growth and screening of the isolate.

SCREENING OF BIOSURFACTANTS

The biosurfactants containing degradation sample medium (5ml) were

taken in a centrifuge tube and centrifuged at 6000rpm for 10mins at 4°C in a refrigerated centrifuge and the supernatants were collected and used for the various biosurfactant screening tests using the following tests:

DROP COLLAPSE TEST

The supernatants from the bacterial culture were taken in a glass slide and added with 0.1 µl of oil. The flattening property of oil was observed for a period of 1 minute (Mahalingam *et al.*, 2014).

OIL SPREADING TEST

10ml of glyphosate was taken in the petriplate and 1 ml of coconut oil was added to the centre of the plates containing glyphosate. Now 20µl of the centrifuged supernatant of the cultures added to the centre of the petriplate. The biosurfactant producing organism can displace the oil and spread in the water a control was also maintained by using water instead of glyphosate (Sukirtha and Usharani., 2013).

EMULSIFICATION INDEX (E24)

The emulsifying capacity was evaluated by an emulsification index (E24). The E24 of the culture resample was determined by adding 2 ml of glyphosate and 2 ml of the cell-free broth in test tube, vortexed at high speed for 2 min and allowed to stand for 24h. The E24 index is given as percentage of the height of emulsified layer (cm) divided by the total height of the liquid column (cm). The percentage of emulsification index is calculated by using the following equation (Mahalingam *et al.*, 2014).

$$E24 = \text{Height of emulsion formed} \times 100$$

Total height of solution

DEGRADATION OF GLYPHOSATE (ROUNDUP®) BY USING OF THE *NOCARDIA MEDITERRANIE* THS 1

1.0 ml of the culture was inoculated into 6 different 500 ml conical flask containing 250 ml of the screening medium with 3 different concentrations of glyphosate (10ppm, 20ppm, 30ppm), along with that 3 other conical flasks containing 250 ml of screened medium with already mentioned 3 different concentrations of glyphosate was maintained as a control. The flasks were incubated on a rotary shaker at 120rpm for 15 days at 27°C. The ability of the isolate to utilize glyphosate of 3 different concentrations was measured based on the turbidity of the medium at 660nm using a spectrophotometer.

GC-MS ANALYSIS FOR THE GLYPHOSATE DEGRADATION

The degraded samples were collected at specific intervals and 10 ml of each lyophilized samples were dissolved using ethyl acetate and analyzed for the degraded products using Gas chromatography mass spectroscopy, SITRA, Coimbatore.

RESULTS

Table.1 Identification of *Nocardia mediterranie* THS 1

ORGANISM	TEST	RESULT
<i>Nocardia mediterranie</i> THS 1	Grams staining	Gram Positive
	Acid fast staining	Partially positive
	Casein hydrolysis	Positive
	Citrate utilization	Positive
	Urease test	Positive
	Nitrate test	Positive
	Maltose fermentation	Positive
	Lactose fermentation	Positive
	Sorbitol fermentation	Positive

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$$E24 = \text{Height of emulsion formed} \times 100$$

Total height of solution

$$= \frac{1.5}{2.8} \times 100$$

$$= 53.57$$

FIG.1(A-F) IDENTIFICATION AND BIOCHEMICAL TEST

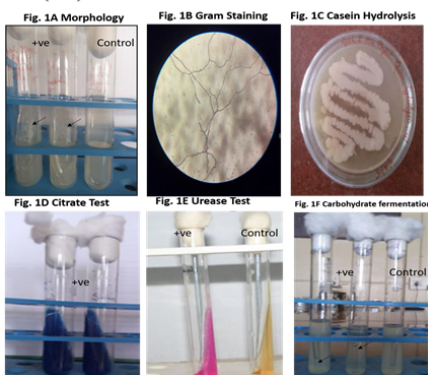
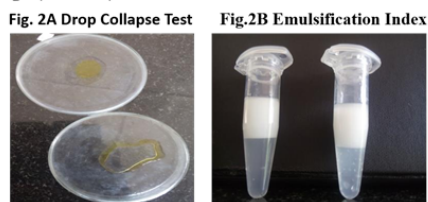
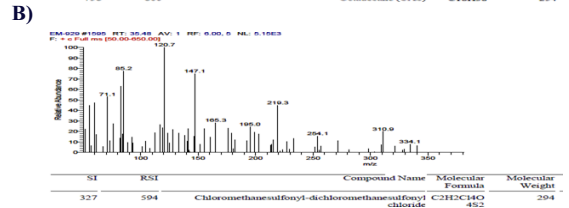
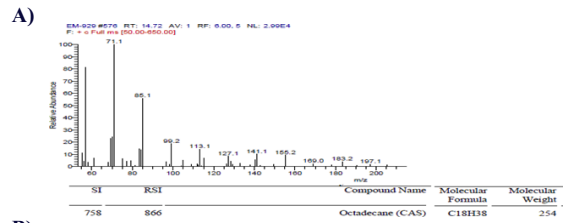


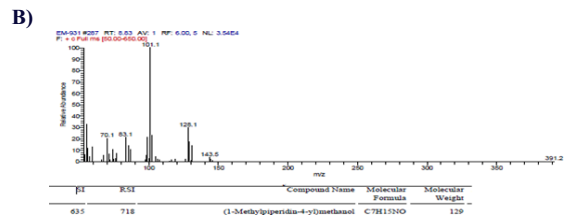
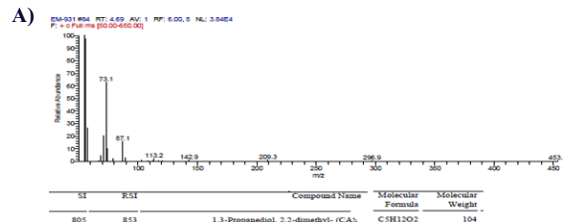
Fig.2(A and B) SCREENING OF BIOSURFACTANT



GRAPH- 1 (A – B). MASS SPECTRUM SHOWING THE FRAGMENTS OF GLYPHOSATE



GRAPH- 2 (A – B). MASS SPECTRUM SHOWING THE FRAGMENTS OF AMPA



SUMMARY AND CONCLUSION

Glyphosate is a highly toxic compound and its degradability in the soil is highly debatable and decontaminating these glyphosate affected soil is a huge concern for the future and bioremediation is considered to be one of alternative to the conventional cleanup methods that are employed. The common herbicide that is commonly in use include 2, 4- dichlorophenoxyacetic acid and Roundup®. The present study was carried out to degrade the glyphosate herbicide by using *Nocardia mediterranie* THS 1 strain. The degradation of the glyphosate herbicide was carried using three different concentrations of glyphosate were the organisms growth rate was monitored by regular checking of OD values of the medium sample at 660nm using u-v spectrophotometer and the result also showed a higher OD value as the concentration increased and also as the transmittance % also decreased as the organism was able to grow using the glyphosate as a carbon source showing that the organism has a high bio remediation capacity and could be used as a novel organism for controlling glyphosate pollution at various agricultural fields. The study also analysed on the positive potential of the organism to produce bio surfactants which could be applied to various other fields. According to the GC-MS graph as the glyphosate was degraded thus producing of AMPA as a by product it was concluded that Actinomycetes *Nocardia mediterranie* THS 1 represent a promising application in the bioremediation of glyphosate herbicide.

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