

## **Indole-3-Acetic Acid Production of Chlorpyrifos Tolerant Bacteria Isolated from Agricultural Soils**

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### **Abstract**

Chlorpyrifos, an organophosphate insecticide, has been widely used to kill a number of pests including insects and worms. The research work was carried out to isolate chlorpyrifos tolerance bacteria from agricultural soil. Forty-seven of chlorpyrifos tolerant bacterial isolates were able to grow in the medium amended with 150 mg L<sup>-1</sup> of chlorpyrifos. Investigation of indole-3-acetic acid (IAA) production of these isolates was performed. Nine isolates of chlorpyrifos tolerant bacteria, named BW1, BW3, BW7, BW9, BW11, TV11, TV24, BK27 and BK29 were capable to synthesize IAA. Out of them BW9 gave the highest amount of IAA (168.46 µg mL<sup>-1</sup>) followed by BW3 (104.66 µg mL<sup>-1</sup>) in culture media supplemented with L-tryptophan. Furthermore, IAA production of all strains was determined in a medium supplemented with 100 mg L<sup>-1</sup> of chlorpyrifos and L-tryptophan. The result revealed that chlorpyrifos had no significant effect on IAA production of all isolates except isolate BW9. Our result highlight that these bacteria would be investigated further on the chlorpyrifos biodegradation and plant growth stimulation for application in environmental and agricultural aspects.

**Keywords:** Chlorpyrifos, Organophosphate, Indole-3-acetic acid (IAA), IAA producing bacteria, Chlorpyrifos tolerant bacteria

### **Introduction**

The extensive utilization of insecticides via field application on agricultural crops are usually practice and an important factor of integrated pest management (IPM) strategy.

Organophosphate pesticides, especially chlorpyrifos, is one of the widely used for the purpose of pest control. It is one of the widely used against a broad spectrum of agricultural pests. The half-life of chlorpyrifos in soil, or the time that it takes for half of the insecticide to be broken down, is usually between 60 and 120 days, but can range from 2 weeks to over 1 year, depending on the soil type, climate and other conditions [1]. The major degradation product of chlorpyrifos is 3, 5, 6-trichloro-2-pyridinol (TCP) which has anti-microbial properties prevents the proliferation of beneficial soil microorganisms [2]. Its gave adversely affect functional diversity of the soil microbiota leading to loss of soil fertility and plant growth, which in turn put the sustainability of agricultural soil at serious risk [3].

In recent years, not only application of microorganisms for degradation pesticides from the environment has attracted several researchers but plant growth promoting bacteria have also been interested of particular attention [4] such as IAA producing bacteria. Indeed, chlorpyrifos tolerant bacteria have a potential for chlorpyrifos degradation and for mitigation of crop in contaminated soil. They also display substantial plant growth promoting traits such as phosphate solubilization, indole acetic acid production and ammonia production both in absence as well as in the presence of chlorpyrifos [1]. The objective of this study, therefore, was to isolate chlorpyrifos tolerant bacteria form agricultural soil. Additionally, the ability of production of IAA of these bacteria was also assessed.

## **Materials and methods**

### **Soil sampling**

Soil samples were collected from agricultural soil (0-10 cm depth) of Nakhon Ratchasima which were treated for few years with the pesticides and the hypothesis is that the soil bacteria are adapted to these compounds. Sampling locations were Boon Wattana, Tung Sawang and Ban Koh. Soil samples were taken in sterile bag and kept cool until they were transported to the laboratory.

### **Isolation of chlorpyrifos tolerant bacteria**

Ten grams of each soil was dissolved in 90 mL of sterile normal saline solution (0.85% w/v NaCl) in 250-mL Erlenmeyer flask. The soil suspension was shaken at 150 rpm for 30 min at 30°C. One milliliter of suspension was serially diluted to obtain a solution with  $10^{-3}$ - $10^{-5}$  dilution, and the obtained solution was subjected plated on MSM medium (1.5 g L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 0.5 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.2 g L<sup>-1</sup> MgSO<sub>4</sub>·7 H<sub>2</sub>O, 0.5 g L<sup>-1</sup> NaCl, and 1.5 g L<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub>) supplemented with 50 mg L<sup>-1</sup> of chlorpyrifos (commercially available, G.I. FOS). Plates were incubated at 30°C for 24 h, the tolerant strains were then selected for culturing in the MSM medium supplemented with 100 and 150 mg L<sup>-1</sup> of chlorpyrifos, respectively.

### **Screening for IAA production**

Chlorpyrifos tolerant bacteria was screened for IAA production trait by plate agar assay [5]. The IAA medium consisted 10 g L<sup>-1</sup> peptone, 5 g L<sup>-1</sup> NaCl, 6 g L<sup>-1</sup> yeast extract, 15 g L<sup>-1</sup> agar, and 1 g L<sup>-1</sup> L-tryptophan, pH 7.6. The agar plate assay was prepared, plated and holes (0.7 cm, i.d.) were made in each agar plate using a sterile cork borer. A single colony of bacterial strains were cultured in 5 mL of Nutrient broth (NB) and incubated (30°C). The overnight culture (10 µL) was dropped into each hole and the plates were incubated at 30 °C for 24 h. To check for positive strains, 50 µL of Salkowski's reagent (50 mL of 35% (v/v) HClO<sub>4</sub> with 1 mL of 0.5 M FeCl<sub>3</sub>) was poured in the hole. Appearance of pink halo zone around the hole indicated the presence of IAA producers.

### **Quantitative determination for IAA production**

For quantitative analysis of IAA production by bacteria, starter culture was grown overnight on 5 mL of NB medium at 30°C, 150 rpm for 24 h. Bacterial suspension was 2% (v/v) inoculated into 5 mL of the IAA medium and inoculated at 30°C, 150 rpm for 24 h. After incubation, supernatant was harvested by centrifugation at 8000 rpm for 5 min (Hettich MIKRO 220R, Germany) and then 2 mL was mixed with 100 µL of ortho-phosphoric acid and 4 mL of Salkowski's reagent. The reaction was placed in the dark for 1 h, and its absorbance was measured at 540 nm by Evolution 600 UV-Vis Spectrophotometer, USA [5].

## Results and discussion

### Isolation of chlorpyrifos tolerant bacteria

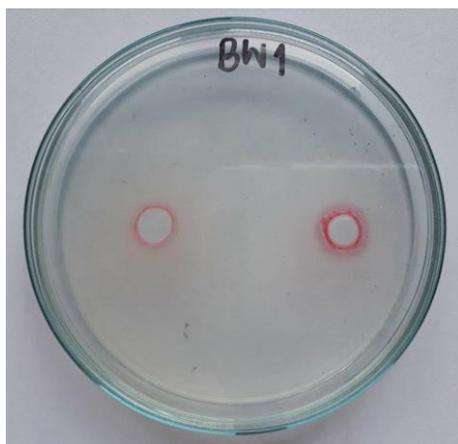
Forty-seven bacterial isolates of chlorpyrifos tolerant bacteria were successfully isolated from agricultural soil of Boon Wattana, Tung Sawang and Ban Koh (Table 1). All of chlorpyrifos tolerant bacteria were screened for in vitro production of IAA through plate agar assay.

**Table 1.** Percentage of chlorpyrifos tolerant bacteria isolated from different areas.

Areas	No. of isolates	Percentage
Boon Wattana	9	19.14
Tung Sawang	24	51.06
Ban Koh	14	29.80
Total	47	100.00

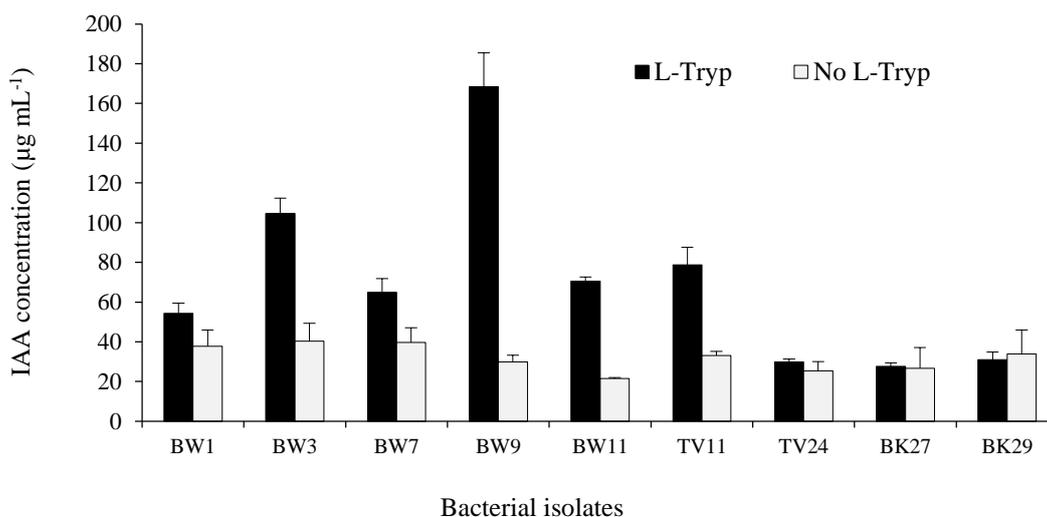
### IAA production of the chlorpyrifos tolerant bacteria

The importance of soil bacteria in chlorpyrifos tolerance and their ability to synthesize plant growth regulators such as IAA make them the preferred choice for applications [4]. In this work, IAA production of the chlorpyrifos tolerant bacteria was investigated by qualitative and quantitative determination by using of Salkowski's reagent. For qualitative analysis, occurrence of pink color around the hole indicate the ability to produce IAA of the isolates. The result revealed that 9 isolates growing in medium added with L-tryptophan were able to synthesize IAA (Fig. 1). These isolates were coded BW1, BW3, BW7, BW9, BW11, TV11, TV24, BK27 and BK29 and, selected for further investigation and quantitative analysis of IAA production with and without L-tryptophan as a precursor.



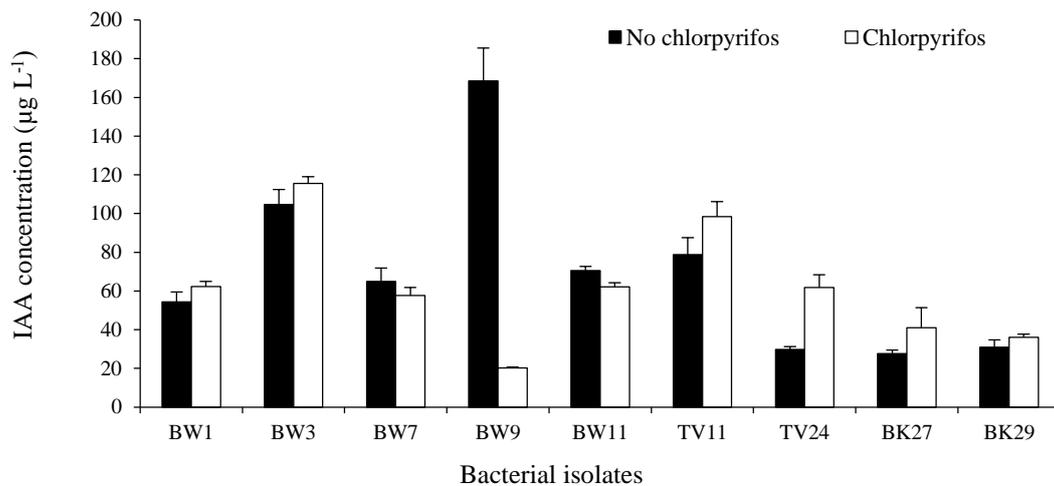
**Fig. 1** Plate assays for the screening IAA production of chlorpyrifos tolerant bacteria

For quantitative analysis, IAA production of the isolates was performed in the medium with and without L-tryptophan ( $1 \text{ g L}^{-1}$ ). Production medium supplemented with L-tryptophan was more preferable for IAA production when compared with medium without L-tryptophan (Fig. 2). Highest level of IAA concentration was recorded in isolate BW9 ( $168.46 \mu\text{g mL}^{-1}$ ) followed by BW3 ( $104.66 \mu\text{g mL}^{-1}$ ) as compared to other isolates (Fig.3). The lowest level of IAA production was observed in isolate BK27 ( $27.6 \mu\text{g mL}^{-1}$ ). Some bacteria such as *Enterobacter* sp. NRRU-N13, NRRU-N20, NRRU-N21, NRRU-D47, and *Bacillus* sp. NRRU-D40 were reported to have the ability to produce IAA [5]. IAA synthesis by bacteria plays a crucial role in plant growth and development as a regulator of numerous biological processes [6].



**Fig. 2** IAA production of chlorpyrifos tolerant bacteria in presence and absence of L-tryptophan

The production of IAA by the isolates was compared between the presence and absence of chlorpyrifos. It was revealed that chlorpyrifos had no significant effect on IAA production of all isolates except isolate BW9. There was reduction by 17% when chlorpyrifos was present at a concentration of 100 mg L<sup>-1</sup>. Previously, Fang et al [3] reported that chlorpyrifos exert an inhibitory effect on soil microbial activities and decrease in plant growth promoting traits of bacteria in presence of pesticides [1].



**Fig. 3** IAA production of chlorpyrifos tolerant bacteria in presence and absence of chlorpyrifos

## Conclusions

Chlorpyrifos tolerant bacteria isolated from agricultural soil that obtained in this work exhibited ability to growth in the medium supplemented with chlorpyrifos at high concentration and also possess IAA production. Our result highlight that these bacteria would be developed into valuable candidates for development of bioremediation and agriculture strategies.

## Acknowledgements

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