Identification of Glyphosate Tolerance Soil-borne Bacteria From Coconut, Coffee and Oil Palm Plantations in Universiti Putra Malaysia

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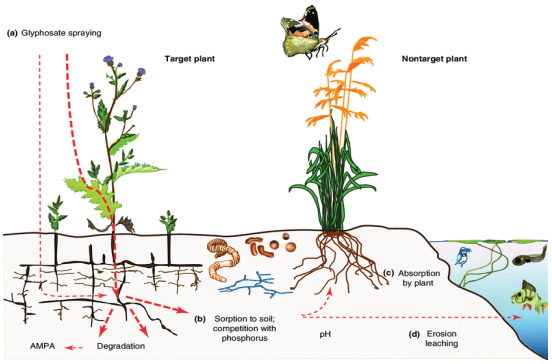

Figure 1. Glyphosate Accumulation and Transport in the field

ABSTRUCT Glyphosate is a non-selective herbicide used for killing a broad range of weeds. High level of glyphosate usage in plantations has led to soil accumulation causing various adverse effects in ecosystems. Due to the concern of its toxicity, bioremediation can be considered as an innovative way to ease its negative effect. In this study, 12 soil samples were collected from plantations in Malaysia and 28 bacterial strains were isolated by mineral salt medium (MSM) enriched with glyphosate. Glyphosate tolerance test was carried out by striking individual pure isolates onto the nutrient agar (NA) with augmenting glyphosate concentration. 3 isolates managed to survive at 30,000 ppm glyphosate. Morphological and 16SrDNA identification were investigated and two species were successfully identified as *Achromobacter xylosoxidans* and *Nguyenibacter vanlangensis*. *N. valangensis* is a glyphosate-tolerant bacterium but has yet been studied as a pesticide degrading bacterium in the past researches. This isolate was then measured its potential population growth (CFU mL/L) in MSM broth supplemented with two different concentrations of glyphosate and it showed rapid growth in higher glyphosate concentration. Further examination on *N. vanlangenesis* is needed to confirm its potential use as a bioremediation agent in the future.

INTRODUCTION

Glyphosate is a non-selective herbicide used for killing a broad range of weeds. High level of glyphosate usage for weed control in plantations has led to accumulation of the herbicide in ecosystems. Due to the concern of its toxicity, bioremediation can be considered as an innovative way to ease its negative effect, which exploits the ability of microorganisms to degrade harmful toxic substances into less toxic forms. In recent years, many scientists have isolated and screened a lot of microbial strains from soils. However, a large proportion of microorganisms and their ecological roles are still unknown. Therefore, the aims of this study are 1) Isolation and Identification of Glyphosate Tolerant Bacteria from Soil, and 2) Screening of Higher Tolerant Bacteria and Evaluation of the Cell Growth.

METHODOLOGY

From oil palm, coconut, and coffee plantation, 12 soil samples were collected from the point in where glyphosate has been applied constantly (Figure 2, A). For enrichment and isolation of glyphosate-tolerant strains, MSM with the addition of 1ppm of glyphosate as the sole phosphorous source was employed and incubated at 30°C under shaking conditions of 150 rpm for 7 days. 0.1ml of cultured broth was spread on MSM+Glyphosate agar and incubated at 37°C for 48 hours to get mixed culture(Figure 2, B). Single colonies were selected and sub-cultured on the same agar plate for purification. A total of 28 bacterial isolates have been isolated from 12 sampling points.

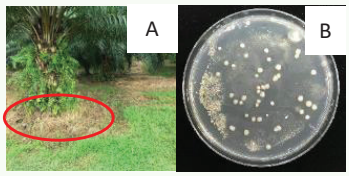

Figure 2. Sampling pint (A) and Mixed culture (B)

RESULTS 1 Identification of Selected Bacteria

Bacterial strains were identified from morphological and molecular approaches. The 16s rDNA from the cell extract obtained was amplified by PCR using 72F (5' AGA GTT TGA TCM TGG CTC AG -3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T -3') primers. 5 samples were successfully extracted their DNA and 4 samples were identified their species with the higher similarity: OP4B (Figure 3), CF1B and CF1C were identified as *Nguyenibacter vanlangensis* with the similarity rate of 98.54%, 99.08% and 99.10% respectively, and CF2Aw (Figure 4) had 99.08% of similarity with *Achromobacter xyglosoxidans*.

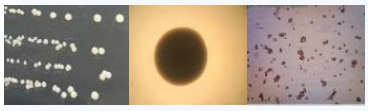

Figure 3. Nguyenibacter vanlangensis (OP4B) Colony colour (left), shape (middle), and cell shape (right, 100x magnification).

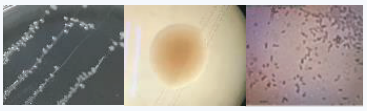

Figure 4. *Achromobacter xylosoxidans* (CF2Aw), Colony colour (left), shape (middle), and cell shape (right, 100x magnification).

RESULT 2 Glyphosate Tolerance Tests

All the 28 bacterial isolates were tested for their ability to tolerate glyphosate. Among the 28 bacterial isolates, 16 bacterial isolates were able to grow in NA supplemented with glyphosate at 9,000 ppm. These bacterial isolates were further tested with the higher concentration of glyphosate and the results showed that the cell growth of CF2Aw identified as *A. xyglosoxidans* was observed until 24,000ppm, and 3 species including OP4B identified as *N. vanlangenesis* had the ability to grow their cells in condition of 30,000ppm glyphosate (Table 1).

Table 1. Effect of different concentrations of glyphosate on bacteria

Sample Number	Species	Concentration of Glyphosate (ppm)						
		3000	6000	9000	12000	18000	24000	30000
OP4B	*Nguyenibacter sp.*	+	+	+	+	+	+	+
CF2Aw	*Achromobacter sp.*	+	+	+	+	+	+	-
OP2D	Unknown	+	+	+	+	+	+	+
CO3B	Unknown	+	+	+	+	+	+	+
CO5A	Unknown	+	+	+	+	+	-	-
CF2Ag	Unknown	+	+	+	+	+	+	-
CF2B	Unknown	+	+	+	-	-	-	-

+ : Bacteria growth is observed - : Bacteria growth is not observed

RESULT 3 Bacterial Cell Growth of Selected Isolate

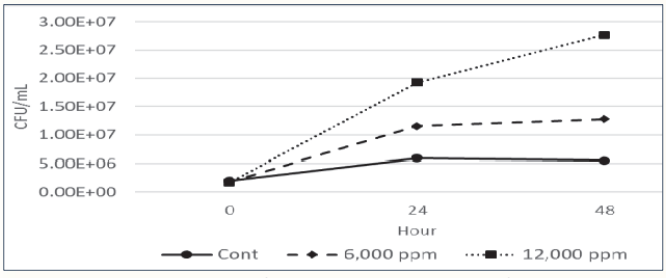

Figure. 5 Cell growth of OP4B (*Nguyenibacter vanlangensis*) in MSM media supplemented with glyphosate at 6,000 ppm and 12,000 ppm.

The growth of *N. vanlangenesis* was monitored at 0, 24 and 48 hours. 5ml of 24hours-old culture was inoculated into 50ml of MSM supplemented with different concentration of glyphosate at 6000ppm and 12000ppm, and no glyphosate was added as the control. Figure 5 showed that *N. vanlangenesis* recorded double cell growth rate at 12,000 ppm (24 h: 1.9 x 107 CFU/mL; 48 h: 2.8 × 107 CFU/mL) compared to 6,000 ppm (24 h: 1.2 × 107 CFU/mL; 48 h: 1.3 × 107 CFU/mL), whereas control showed only slight increment on cell numbers after 24 h (5.9 × 106 CFU/mL) and 48 h (5.5 × 106 CFU/mL). The result indicates that the higher the glyphosate concentration, the higher ability of *N. vanlangenesis* using components of glyphosate while withstanding its toxicity presence in the glyphosate-based pesticide.

CONCLUSION

Achromobacter xyglosoxidans has been proven having pesticide degradation activity while *Nguyenibacter vanlangensis* has been confirmed as plant growth promoting bacterium in the past researches. This study has confirmed that both *N. vanlangenesis* and *A. xyglosoxidans* strains have the abilitiy to utilise the glyphosate as the sole phosphorus source. This will be the first study to prove that *N. vanlangenesis* has a potential to be recognized as a glyphosate-degrading bacterium. Since studies regarding glyphosate degradation by microorganisms are poorly understood in tropical regions, the data reported in this study have contributed to the discovery of pesticide-degrading bacteria that could potentially be used as bioremediation in the indigenous environment.