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Reduction of Soil Acidity for Agriculture Activities in Malaysian Ultisols by *Rhodopseudomonas palustris*

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The potential of the phototrophic bacterium, *Rhodopseudomonas palustris (R. palustris*), to reduce the acidic soil pH of Malaysian Ultisols (Bungor Series) was investigated. The *R. palustris* was first adsorbed onto the dried pineapple leaves (DPL) before being applied. The most suitable broth condition for the bacterium that could be adsorbed onto the DPL was identified. Different conditions and amounts of *R. palustris* adsorbed onto the DPL % (w/w) were tested in soils where the soil pH was monitored. It was found that the maximum cell adsorption onto the DPL was obtained using an initial cell concentration of 1.5 x 10^7 colony-forming units (CFU) mL^{-1}, at pH 8.0 with the ionic strength of 5 $mmolL^{-1}$ ammonium nitrate ($NaNO_3$). The *R. palustris* was adsorbed onto the DPL surface area of 64.33 Å. The acidic soil was treated with *R. palustris* adsorbed onto the DPL by direct application. The calcium carbonate ($CaCO_3$) was introduced as a benchmark to reduce the soil acidity. The results showed that, with the application of *R. palustris* adsorbed on 5.0 % (w/w) of DPL, the soil pH was improved from 4.9 to more than 6.0 after 12 d of application. This improved soil pH will make the soil more applicable for agricultural activity.

1. Introduction

Managing soil acidity is critical for agricultural purposes. In Malaysia, the soil condition is naturally acidic. It is necessary to reduce the soil acidity during land preparation for agricultural activities. The current solution to reduce the soil acidity is the use of $CaCO_3$ (chemical treatment), known as a liming treatment. After a duration, the soil can become acidic again after the chemical reaction from the $CaCO_3$ is completely accomplished (Paradelo et al., 2015). This would require the addition of more $CaCO_3$ to the soil. Excess of application of $CaCO_3$ can causes to harden the soil, bad effect for plant root to survive. The treatment efficiency of $CaCO_3$ will decrease over a duration, over-liming can damage the availability of micronutrients in soil (Aye et al., 2016). The advantages of biological treatment, especially using a beneficial microbe such as the phototrophic microbes, *Rhodopseudomonas palustris (R. palustris)*, can offer potential for soil treatment. Biological method is expected to be more environmentally friendly and sustainable by improving the soil conditions, by following the nature. To enhance the potential of *R. palustris* in reducing soil acidity, the bacteria was adsorbed onto DPL as a lignocellulosic support medium to enhance the sustainability of *R. palustris* prior to application in soil (Ch'ng et al., 2014; Huck, 2014). The effectiveness of the application of *R. palustris* adsorbed on the DPL to reduce Malaysian acidic Ultisol soil (Bungor Series) was studied and compared.

2. Materials and methods

2.1 *R. palustris* adsorbed on dried pineapple leaf (DPL)

R. palustris was adsorbed on 5.0 % (w/w) DPL. The total surface area of the DPL for the adsorption of *R. palustris* was 9.57 m^2/g. The attachment of *R. palustris* onto the DPL was carried out as follows: 10 % (v/v) of

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overnight-grown *R. palustris* cells were inoculated into a 250 mL Erlenmeyer flask containing 50 mL of nutrient broth (M105443, Merck, Germany) followed by incubation at 200 rpm at 30 °C for 12 h. Fifty mg of the DPL (500 to 700 µm mesh size) were added to the culture broth and incubated for a further 36 h. To ascertain the attachment of the *R. palustris* onto the DPL, at the end of the incubation period, the nutrient broth was removed via filtration, the DPL collected were dehydrated prior to overnight drying in a desiccator. Batch experiments were conducted to measure the maximum adsorption of *R. palustris* onto the DPL as a function of the initial bacterial concentration, pH, temperature, and ionic strength. The cell concentration in the range of 1.5 to 20.0 colony-forming units (CFU) mL^{-1} of cell suspension was added to 10-mL centrifuge tubes containing 50 mg of the DPL. The distilled deionised (DDI) water was supplemented to bring the final volume to 10 mL (Dunham-Cheatham et al., 2015). The experiments were carried out at 30 °C at different pH in which 50 mg of the DPL were added to 10 mL of the cell suspension containing an initial cell concentration of $20.0\times10^{7}\pm2.02$ CFU mL^{-1}. The ionic strength of the experiments was investigated by suspending the cell in DDI water in the presence of 0.1-100 $mmolL^{-1}$ of $NaNO_3$ (S5506, Sigma-Aldrich, USA). In each experiment, the *R. palustris* with DPL mixture was shaken (200 rpm) for 2 h at 30 °C, except when measuring the effect of the incubation temperature on the growth of *R. palustris* adsorbed onto the DPL (Pokrovsky et al., 2013). The separation of the unattached bacteria from the fraction containing the DPL and the attached bacteria was accomplished by injecting a sucrose solution (60 % w/w) into the bottom of the mineral-bacteria suspension. The percentage of the attached bacteria was determined based on Eq(1).

$$\%\ \text{of bacterial attachment} = \frac{(\text{Initial bacterial quantity} - \text{quantify of unattached bacterial})\ \text{x}\ 100\ \%}{\text{Initial bacterial quantity}} \tag{1}$$

All experiments were carried out in triplicate. For the control experiment, $CaCO_3$ was applied to the acidic soil in place of the *R. palustris* absorbed on DPL.

2.2 Effectiveness of *R. palustris* compared to $CaCO_3$

R. palustris adsorbed on different concentrations of DPL (1.0 %, 2.5 %, and 5.0 % (w/w)) were separately mixed with Bungor Series soil (250 g with 42.0 ± 0.75 % humidity) in each conical flask. The mixtures were incubated for 14 d. Five g of the mixture were sampled and determined (soil : water, 1:5 (w/v)) for soil pH (DELTA 320, Mettler Toledo, USA). To compare the effectiveness of *R. palustris* in amending soil pH, liming using $CaCO_3$ (M102067, Merck, Germany) was introduced as a benchmark for the acidic soil treatment. The DPL was also used as control for soil pH amendment; the amount used was the same as $CaCO_3$ (% w/w).

3. Results and discussions

3.1 Optimum condition of *R. palustris* adsorbed onto the DPL

Figure 1 shows the highest adsorption of *R. palustris* onto the DPL (95.7 ± 1.98 %) with an initial bacterial count of 1.5 x 10^{7} CFU mL^{-1}. The lower the number of CFU (1.5 x $10^{7}\pm1.98$ CFU mL^{-1}) introduced, the higher the percentage (95 %) of *R. palustris* adsorbed on the DPL surface. At low CFU mL^{-1}, the binding sites on the surface of the DPL were saturated by *R. palustris*. This is evidenced by the image of the saturation of *R. palustris* on the DPL surface as shown in Figure 4(c). The natural formation of *Pseudomonas putida* adsorbed onto the DPL was also reported by Jiang et al. (2007) and Rong et al. (2008).

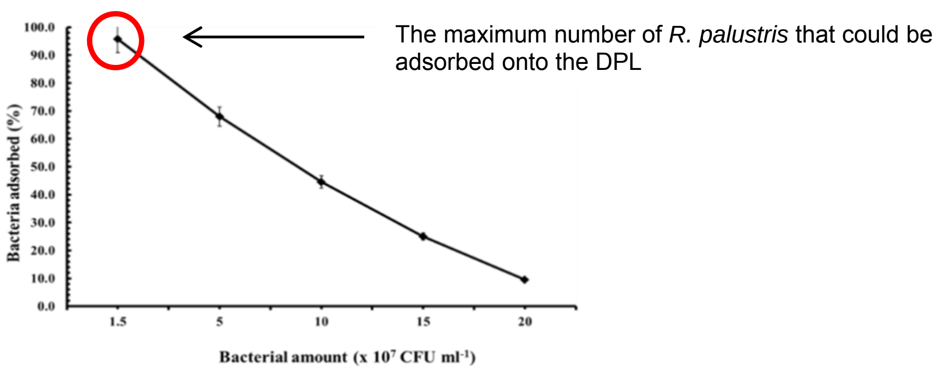


Figure 1: The effect of the initial number of bacteria on the adsorption (%) of *R. palustris* on *DPL.*

Figure 2 shows that the adsorption of *R. palustris* onto the DPL was highly dependent on the pH of the solution. Low adsorption was observed at a high acidic pH (14.4 ± 3.34 % at pH 4). The % of adsorption gradually increased to 19.2±3.48 % at pH 6 and reached a maximum of 95.2±2.66 % at pH 8. The % of adsorption dropped slightly to 75.0±2.83 % at pH 9, followed by a reduction to 14.4±2.25 % at pH 10. As the pH increased, the surface of the DPL was progressively ionised, which would increase the overall net negative (-ve) surface charge of the DPL. This would increase the repulsive electrostatic interactions between the DPL and *R. palustris* hence decreased the adsorption of *R. palustris* (Guo et al., 2011). Similar results have been supported by other studies. The phototropic bacteria adsorbed well onto the lignocellulosic substrate during the hydrolysis process at pH 6 to 8; while Pandey (2015) reported that *Streptomyces lividans* adsorb and hydrolyse cellulose at the best condition of pH 7 to 8.

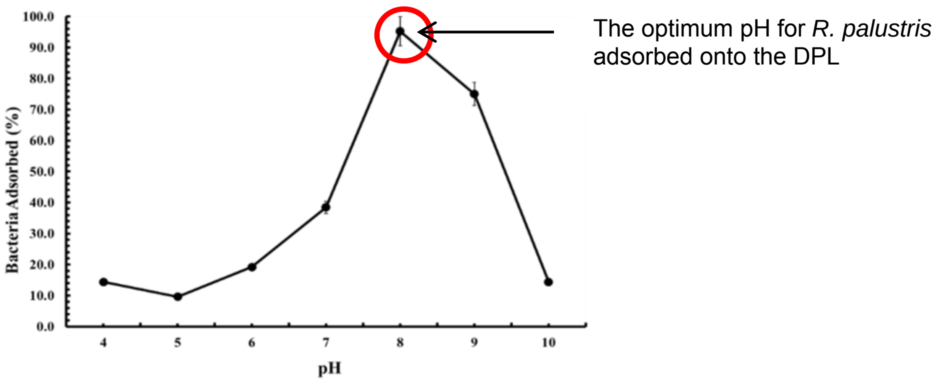


Figure 2: Adsorption (%) of R. palustris onto the DPL at different pH.

Figure 3 shows the adsorption of *R. palustris* on DPL as monitored at various ionic strength of $NaNO_3$ (0.1 to 100 mmolL^{-1}). The highest bacterial adsorption was obtained using 5 mmolL^{-1} of Na^+ (low to medium ionic strength solution). At a low to medium ionic strength (>10 mmolL^{-1} of Na^+), the double layers associated with the surfaces of both the *R. palustris* and the DPL were relatively thick, the attractive electric field extended into the solution and increased the potential for adsorption. As the ionic strength increases, the higher concentration of electrolyte ions limits the interaction between the two surfaces. Although they remain oppositely charged, the adsorption is reduced. *Burkholderia* and *Rhizobium* were used to adsorb and degrade cassava under an ionic strength solution of 5 to 10 mmolL^{-1} of $NaNO_3$. At a high ionic strength, bacterial protein is folded into a denser core, reducing the number of hydrophobic interaction site and awakening the adhesion force (Hwang et al., 2012).

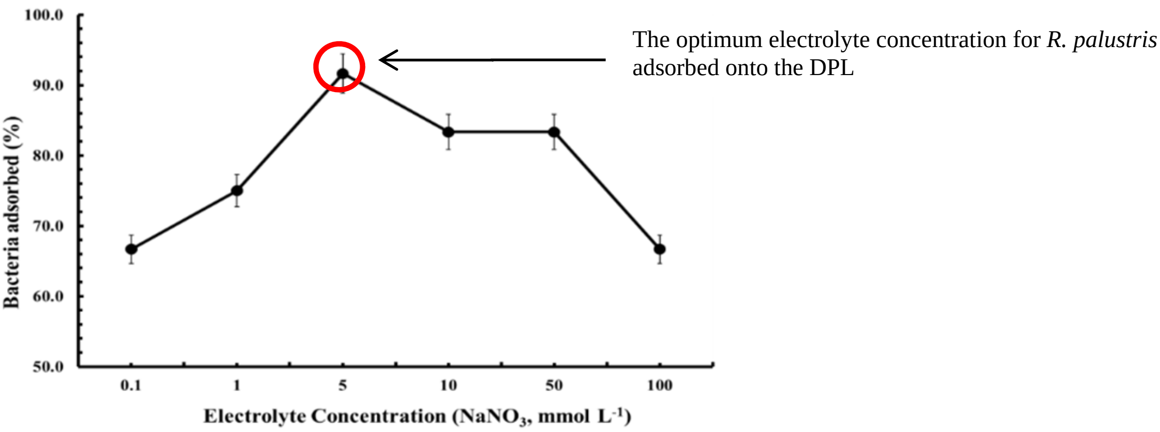


Figure 3: Trend of bacterium adsorption onto the DPL at different electrolyte concentrations.

The best combination of parameters for the optimum *R. palustris* adsorbed onto the DPL can be achieved using the CFU/mL of 1.5 x 10^7±1.98 at pH 8 (slightly alkaline) and 5 mmolL^{-1} of $NaNO_3$ (low ionic strength solution). To confirm the survival of the bacterium adhesion, an image of the bacteria was taken using the field emission scanning electron microscopy (FESEM) technique, as illustrated in Figure 4.

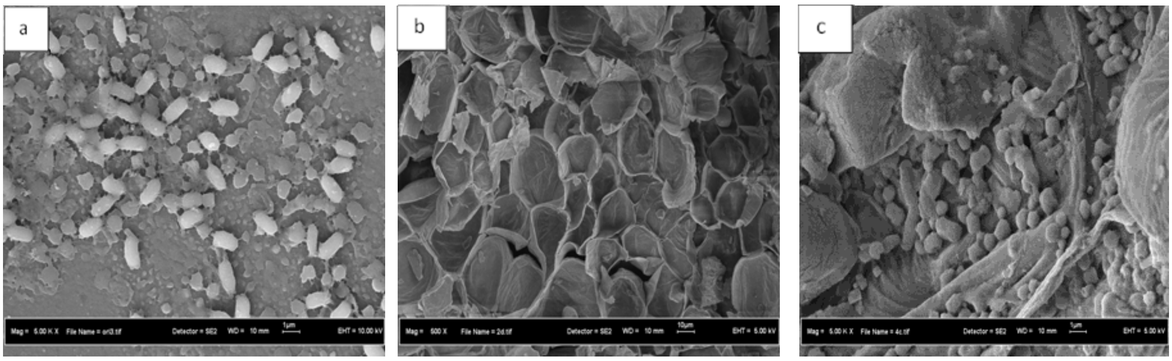


Figure 4: FESEM image of (a) attachment of R. palustris onto glass surface with magnification view of surface 5.00 K, (b) surface of the DPL with magnification view of surface 5.00 K, and (c) the formation of a thin multi-layer of bacterial cells on the surface of the DPL with magnification view of surface 5.00 K.

3.2 Effect of *R. palustris* adsorbed onto DPL to reduce the soil acidity

Figure 5 shows the effect of *R. palustris* absorbed on the DPL applied to the acidic soil for 14 d. The applications using three concentrations of DPL at 1.0 %, 2.5 %, and 5.0 % (w/w) absorbed on *R. palustris* resulted in a similar pattern in reducing the soil acidity. The highest soil pH (6.34±0.02) was achieved by the application of *R. palustris* absorbed on 5 % (w/w) of DPL in the presence of light on day 13. The microbial preparation using 1.5 x 10^7±1.98 CFU/mL could significantly reduce soil acidity to the more neutral pH suitable for agricultural activity. It was likely that the *R. palustris* was effective in converting the H^+ from the organic acid to hydrogen gases, hence resulting in the reduction of soil acidity (resulted in high soil pH reading). Malik et al. (2015) reported that, under photoheterotrophic growth, *R. palustris* can utilise organic acid compounds (in this case the organic acid sources from the acidic soil) to satisfy the carbon requirement for the bacterium. The empirical carbon turnover with time for the microbial biomass activity in soil was estimated to be 14 d.

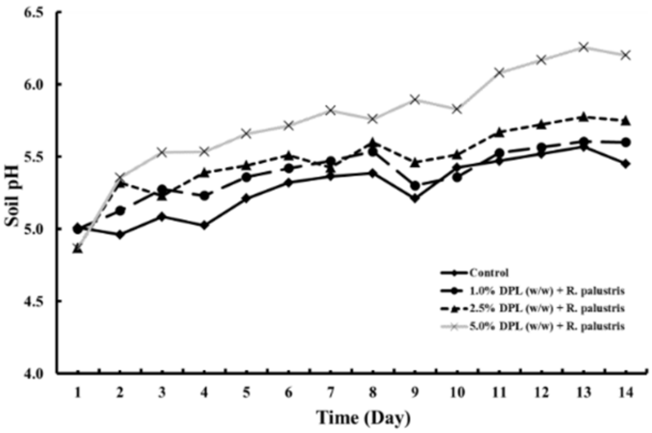
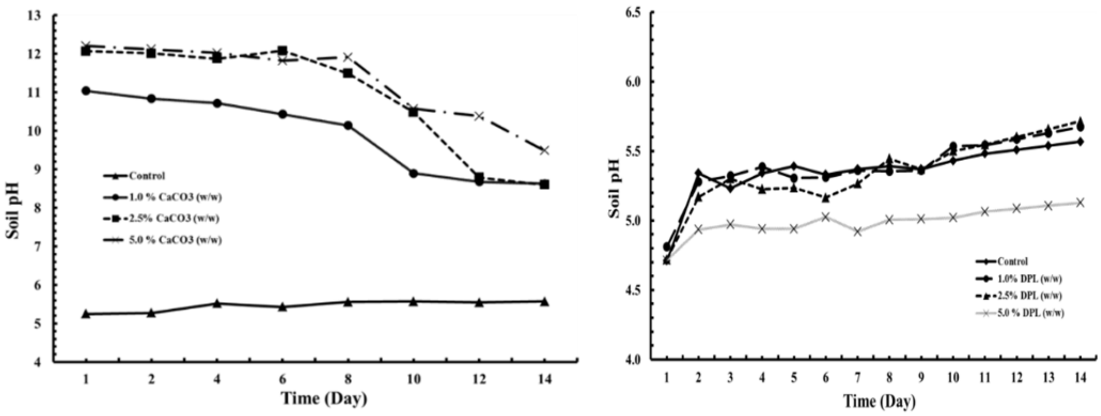


Figure 5. The changes of soil pH following application of R. palustris adsorbed on the DPL (1, 2.5 and 5% (w/w).

The conventional $CaCO_3$ was applied onto the same type of soils as benchmark for acidic soil amendment. Figure 6 shows the changes of the soil pH following the application of $CaCO_3$ at different concentrations, namely 1.0 %, 2.5 %, and 5.0 % (w/w) for 14 d. On Day 1, the soil treated with 5.0 % (w/w) of $CaCO_3$ achieved the highest pH reading (12.27±0.06), where the pH was two-fold higher than that of the controlled soil (without additives). The soil pH treated with $CaCO_3$ gradually decreased until Day 8. Between Day 6 to Day 14, for the soil treated with 5 % (w/w) of $CaCO_3$, the pH dropped drastically from 11.82±0.02 to 9.49±0.01. A similar effect was observed for other concentrations of $CaCO_3$, namely, 1.0 % (w/w) and 2.5 % (w/w). Overall, the $CaCO_3$ application to reduce the soil acidity was rapid but could not be sustained after Day 10. $CaCO_3$ produces negatively charged carbonate (CO_3^-) that would neutralize the soil acidity. As the chemical reaction ceased, the effects of the $CaCO_3$ will decrease and tend to cease in neutralizing the acidic soil. The same result was reported by Islam et al. (2014) who used $CaCO_3$ to treat acidic soil (acidic sulphate soil). The acidic soil pH reduced until Day 30 and became constant thereafter. After six months, the chemical reaction ended and the soil began to turn acidic. This study recommends the use of the biological approach using *R. palustris* adsorbed on the DPL. Although the treatment time was slower (beyond Day 10) than the chemical approach (Day 1), the acidic soil can be amended after Day 10 following application and can be well sustained up to 14 d.



(a) (b)

Figure 6. Effect of soil pH after application of (a) $CaCO_3$ or (b) DPL only.

Figure 6(b) shows the effect of only DPL applied on the acidic soil pH. This is done as a control for the application without the effect of *R. palustris*. With the application of 5.0 % of DPL (w/w), it shows that the acidic soils will be more acidic compared to the application of 1.0 % and 2.5 % of (w/w) of the DPL. From Day 2, the soil pH remained constant in the more acidic conditions with soil pH 4.92$\pm$0.03 until Day 14. With the access of DPL (organic material), it contributes to more acidic condition caused by the organic acid (Deng et al., 2014). This has been reported by Becker et al. (2015) who stated that a range of organic acid including the glycolic acid, lactic acid, succinic acid, itaconic acid can be produced from the DPL. This was due to the rapid proton (H^+) exchange between the soil particle surfaces.

4. Conclusion

The application of *R. palustris* adsorbed on 5 % (w/w) of DPL was found to be the most suitable microbial preparation to successfully reduce the soil acidity in a more sustainable manner. Although the reduction of soil acidity was fastest with $CaCO_3$ application, the effect was less sustainable. This biological treatment finding as an alternative in acid soil reduction in future for agricultural activities, especially in land preparation. These results highly recommend the biological method as a more sustainable and environmental friendly treatment technology. Effect of different weathers such as hot and dry season suggested for further study.

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