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Effect of salt and pH on the biomass and phosphatase activity of *Burkholderia* sp.

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Salinity and pH stress have effect on bacterial growth, enzyme activity and cell biomass. Optimum salt concentration and pH were need for the normal growth of bacteria except for halophiles, acidophiles and alkaliphiles. Different concentration of salt (2.5, 5, 10 and 20%) and pH (5, 7, 9 and 11) were introduced on phosphate-solubilizing bacterial (*Burkholderia sp.*) culture. The result showed that with the increase in the salt concentration there was a decrease in bacterial colony size, biomass and phosphatase activity. The lowest colonial growth and phosphatase activity were observed at 20% salt concentration. It was also found that at low pH and high pH treatments, a significant drop on bacterial cell biomass and phosphatase activity were observed. pH7 showed the best bacterial colony size and phosphatase activity.

Keywords: Phosphatase activity, salinity, pH, phosphate-solubilizing bacteria, *Burkholderia* sp.

INTRODUCTION

Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, transporting of the genetic traits. Soil microorganisms play a key role in soil phosphorus dynamics and subsequent availability of phosphate to plants (Richardson, 2007). Phosphate-solubilizing bacteria (PSB) are known to improve plant growth and health, enhance hormonal level and produce various secondary metabolites detrimental to plant pathogen (Dey *et al.*, 2004).

The population, growth, survival activities and P solubilization efficiency of PSB are greatly influenced by soil physical, chemical and biological stresses. Stress can be detrimental for sensitive microorganisms and decrease the activity of surviving cells, due to the metabolic load imposed by the need for stress tolerance mechanisms (Schimel *et al.*, 2007; Yuan *et al.*, 2007, Ibekwe *et al.*, 2010; Chowdhury, 2011).

In a dry hot climate, the low humidity and soil salinity are the most stressful factors for the soil microbial flora. The composition of the microbial community may be affected by salinity (Pankhurst *et al.*, 2001; Gros *et al.*, 2003; Gennari *et al.*, 2007; Llamas *et al.*, 2008; Chowdhury *et al.*, 2011) since the microbial genotypes differ in their tolerance of a low osmotic potential (Mandeel, 2006; Llamas *et al.*, 2008). In phosphate-solubilizing bacteria, a low osmotic potential decreases spore germination and the growth of hyphae and changes the morphology (Juniper and Abbott, 2006) and gene expression (Liang *et al.*, 2007), resulting in the formation of spores with thick walls (Mandeel, 2006).

There is a significant reduction in the total bacterial count in soils salinized with different concentrations of sodium chloride. Soil pH affects the chemical form, concentration and availability of substrates (Kemmitt *et al.*, 2006) and influence cell growth and activity. There is also strong evidence that soil pH is an important determinant of bacterial diversity and community structure on a global scale (Fierer and Jackson, 2006).

Rates of nitrification is significantly reduced in acid soils (de Boer and Kowalchuk, 2001), and significant batch growth of pure cultures of bacteria in liquid growth media does not occur below pH 7 (de Boer and Laanbroek, 1989; Allison and Prosser, 1991; Jiang and Bakken, 1999). pH strongly influences abiotic factors, such as carbon availability, nutrient availability, and the solubility of metals. In addition, pH may also control biotic factors, such as the biomass composition of bacteria and their enzyme activity.

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MATERIALS AND METHODS

Test organism

Burkholderia sp., phosphorus-solubilizing bacteria, was selected for the test organism. Stock culture was prepared in 250 ml conical flask containing 50 ml of nutrient broth (Difco Manual, 1953).

Measurement of bacterial colony diameter

Nutrient agar medium (Sunder Rao & Sinha, 1963) was prepared with different concentration of salt (2.5, 5, 10 and 20%) and pH (5, 7, 9 and 11). On solidification, a hole was made using corkborer and then *Burkholderia* sp. from stock culture was transferred to each hole. After 48 hrs of incubation the colony diameter of PSB was measured.

Measurement of bacterial biomass

Pikovskaya's broth medium (Sunder Rao and Sinha, 1963) was used to culture *Burkholderia* sp. Pikovskaya's medium without any treatment was used as control (pH 6.37) for the study. The test cultures were divided into triplicates each containing 50 ml of the medium broth treated with different concentration of salt (2.5, 5, 10 and 20%) and pH (5, 7, 9 and 11) values. To each flask, 1ml of bacteria from the stock culture was added. After 48 hours of incubation, the inoculated flasks were filtered with Whatman filter paper No. I. The whole portion of the residue (filter paper) were dried and weighed and the filtrate was used for measuring the bacterial phosphatase activity.

Measurement of phosphatase activity

Phosphatase activity of *Burkholderia* sp. was assayed spectrophotometrically by p-nitrophenyl phosphate (PNP) method (Tabatabai and Bremner, 1969). To 3 ml of aliquot (48 hrs of PSB broth culture medium) 1 ml of modified universal buffer and 1 ml of 0.115 M of PNP were added in 50 ml volumetric flak and was incubated 1 hrs at 37°C. The reaction was stopped by adding 20 ml of 0.5 N NaOH and the volume was made up to 50 ml with dH_2O. The absorbance was taken with a spectrophotometer at 430 nm.

RESULTS

Colony diameter of PSB

In salt treatment, control plate showed maximum colony diameter followed by 2.5, 5, 10 and 20 concentration of salt (Figure 1) has the smallest colony diameter. In pH, control plate showed maximum growth followed by pH 7, 5, 9 and pH 11 has the smallest diameter (Figure 2). ANOVA showed that there was significant variation in colony diameter of *Burkholderia* sp. among different treatments (Table 1).

Biomass of PSB

Biomass of *Burkholderia* sp. was measured from different treatment of salt, *viz*. 2.5%, 5%,10% and 20% and pH viz. 5, 7, 9 and 11 and control was maintained. It was noted that Control showed maximum biomass. In salt treatment, 2.5 show the maximum biomass followed by 5, 10 and 20. There is maximum growth in pH 7 followed by pH 5, pH 9 and pH 11. ANOVA showed that there was significant variation in biomass of *Burkholderia* sp. among different treatments (Table 2).

Phosphatase Enzyme Activity

Phosphatase activity was measured from *Burkholderia* sp. treated with different salt and pH. It was noted that control show highest phosphatase activity

Table 1: One-way analysis of variance (ANOVA) of colony diameter of *Burkholderia* sp. in different concentration of salt and pH (*significant at $p \leq 0.05$).

Treatments	Sources of Variation	F-value	P-value
Salt	CTRL × 2.5 × 5 × 10 × 20	134.3984*	0.000000*
pH	CTRL × 5 × 7 × 9 × 11	75.1556*	0.000000*

Table 2: One-way analysis of variance (ANOVA) of biomass of *Burkholderia* sp. in CTRL, 2.5%, 5%, 10% and 20% of salt and pH (*significant at $p \leq 0.05$).

Treatment	Source of variation	F-value	P-value
Salt	CTRL×2.5×5×10×20	114.3230*	.000000*
pH	CTRL×5×7×9×11	159.0124*	.000000*

Figure 1: Bacteria colony diameter treated with salt after 48 hours incubation.

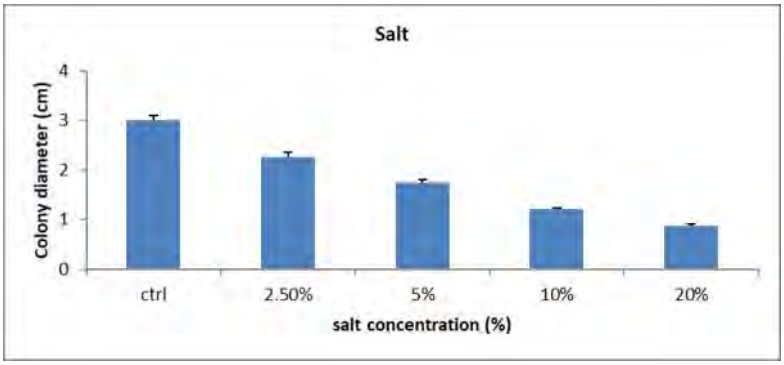


Figure 2: Bacterial colony diameter treated with different pH after 48 hours incubation.

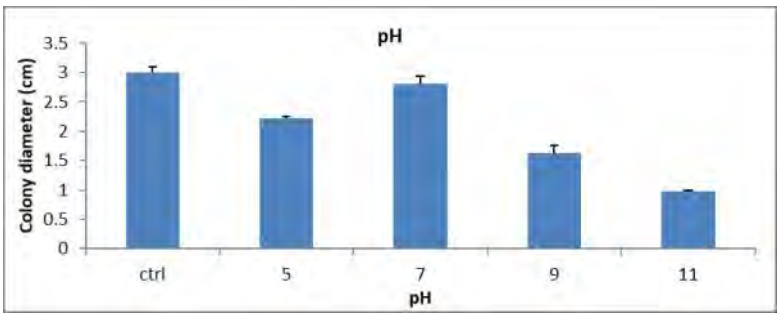


Figure 3: Biomass of *Burkholderia* sp. treated with different concentration of salt after 48 hours incubation.

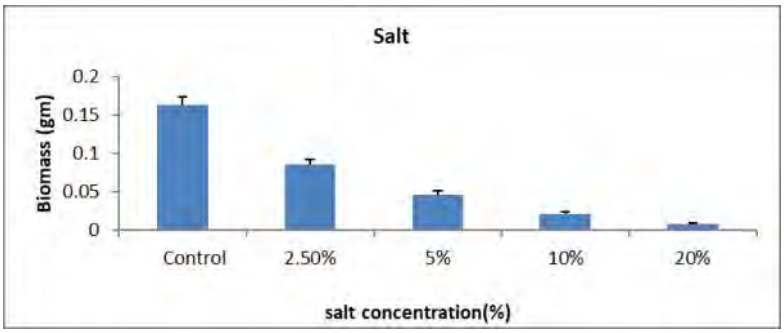


Figure 4: Biomass of *Burkholderia sp.* treated with different concentration of pH after 48 hours incubation.

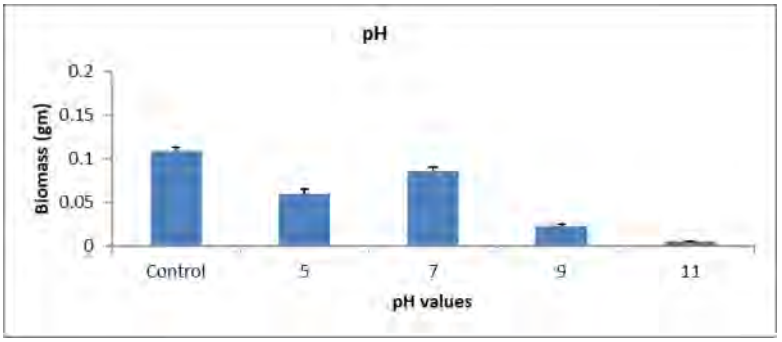
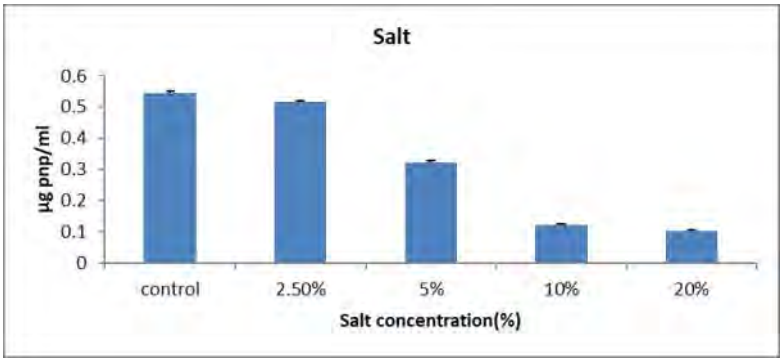


Figure 5: Phosphatase activity of *Burkholderia* sp. treated with different concentration of salt after 48 hours incubation.



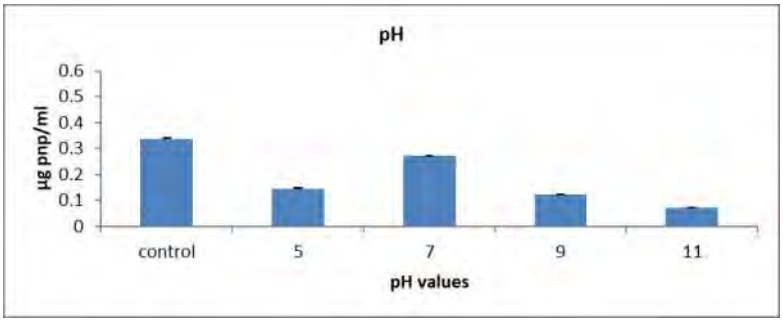


Figure 6: Phosphatase activity of *Burkholderia* sp. treated with different pH values after 48 hours incubation.

followed by 2.5, 5, 10 and 20% in case of salt treatment. In pH, control is followed by pH 5, 7, 9 and 11. ANOVA showed that there was significant variation in phosphatase activity of *Burkholderia* sp. among different treatments (Table 3).

DISCUSSION

Environment stresses like decrease and increase in salinity and pH greatly affect the physiological activity of bacteria like in their growth rate, enzymatic activity and even in their biomass. In *Burkholderia*, the increase in salt concentration, i.e. 2.5, 5, 10 and 20% result in decrease in the colony diameter of bacteria. (Pankhurst *et al.*, 2001; Gros *et al.*, 2003; Gennari *et al.*, 2007; Llamas *et al.*, 2008; Chowdhury *et al.*, 2011). Growth of bacteria was still observed and there was only slight different in the biomass and phosphatase activity with least concentration of salt, i.e. 2.5%. In 20 and 10% only very small clear zone can be seen and the phosphatase activity declined which was in agreement with the finding of Samiran *et al.* (2010). Vogel *et al.* (2010) state that osmotic stress brings forth by high salinity manipulates the growth of all living cells either disrupting the normal physiological activities or intracellular macromolecular structures.

The optimum pH for the growth of bacteria was about pH 6.37, i.e. the pH of the medium where the bacterium was cultured. The medium having pH 7 has shown best growth rate and the phosphatase activity was also high as compared other pH values. This was followed by pH 5, pH 9 and pH 11, it can be concluded that no growth occurs (Ahlgren and Ahlgren, 1960).

Our experimental result shows that with the increase in salt concentration and pH values, there was decline in phosphatase enzyme activity and bacterial biomass. Our findings can determine the optimum concentration of the salt and pH value at which the bacteria will show maximum growth and enzymatic activity. This result also explores the stress tolerance level of the bacteria against pH and salt concentration.

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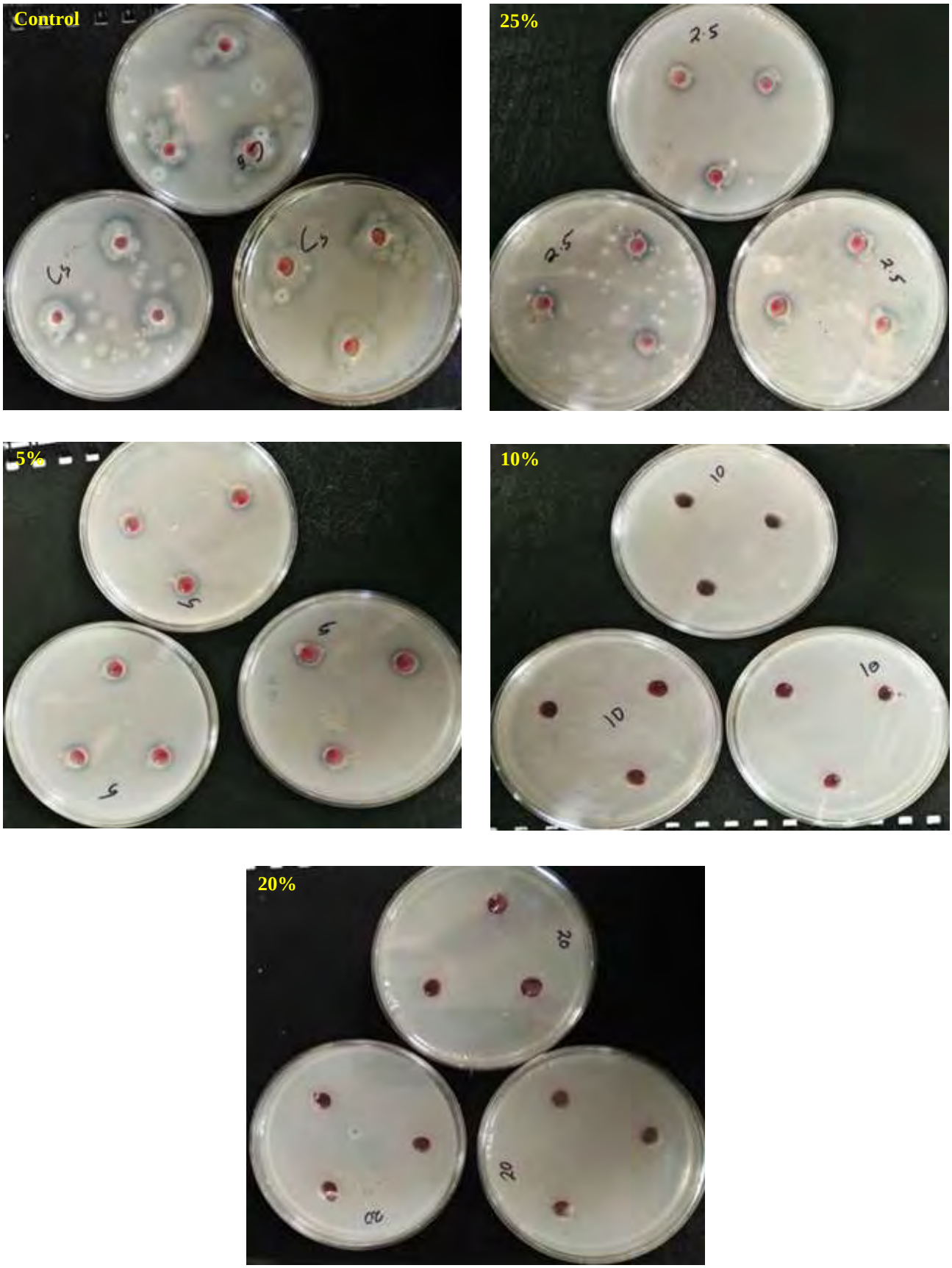

Figure 7: *Burkholderia* sp. grown on nutrient medium treated with different salt concentration.

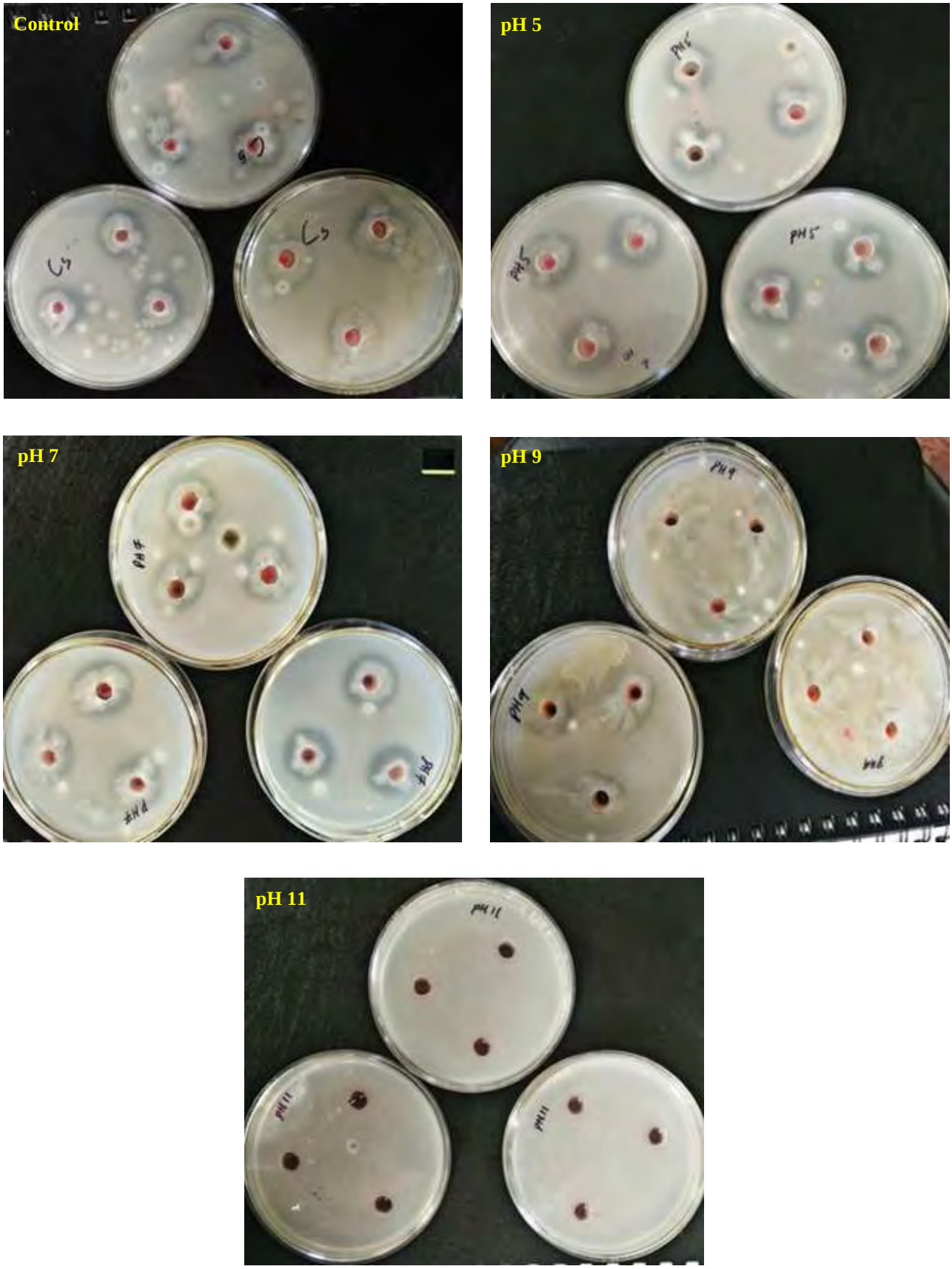

Figure 8: *Burkholderia* sp. grown on different pH nutrient medium.

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