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Exploring the Role of Glucose in Optimizing *In-Vitro* Growth of Bacterial Isolates under Aluminium Stressed Conditions

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A B S T R A C T

Aluminium is one of the most abundant metals. This metal is responsible for inhibiting growth and productivity of many cultures thereby affecting the metabolism of most of the microbes. The current study is aimed at examining the effect of glucose addition on bacterial growth under aluminium stressed conditions. For this purpose, nutrient broth was supplemented with different concentration of aluminium viz. 0mM, 20mM, 60mM and 80mM. In addition to above heavy metal supplementation, the nutrient broth was additionally provided with varying concentration of glucose (0.25%, 0.5% and 1% glucose). The *Bacillus* and *Burkholderia* cultures were allowed to grow in all possible combinations of provided aluminium and glucose concentrations. In case of *Bacillus* culture, when media was supplemented with 0.5% glucose, the culture growth was increased upto 2.0-folds in 20mM aluminium toxic conditions whereas growth of *Burkholderia* strain increased upto 5.0-folds and 8.0-folds in case of 20mM and 60mM aluminium stressed conditions respectively. The results conclude that under aluminium toxic conditions, 0.5% glucose supplementations could prove to be beneficial for bacterial optimal growth.

Introduction

Aluminium is one of the most abundant metals on earth comprising approximately 7% of the earth's crust (Foy 1983).

It is a toxic metal affecting growth of microbial cultures as well as productivity of major food crops (Haug 1984; Foy *et al.*, 1978; Kochian, 2004). It has also been previously reported that aluminium toxicity effects are found in forest ecosystems where acidic precipitation is prevalent (de Vries *et al.*, 2007; Driscoll *et al.*, 2001), in aquatic plants grown in fresh water with low pH (Gensemer and Playle, 1999) and in tropical soils where aluminium is found in abundance and become a major limitation for crop cultivation (Haug and Vitorello, 1997).

In the earth's crust aluminium is mainly found in the form of oxides or silicates which are mostly insoluble and non-toxic but as the soil pH lowers, aluminium is available in more

soluble and toxic forms (Kochian, 1995). According to previous studies the aluminium toxicity is not only found to effect plants but it is equally toxic to other microbial population viz. fungi and bacteria (Date and Holliday, 1979; Foy and Gerloff, 1972; Guida *et al.,* 1991; Zel *et al.,* 1993). The studies relating to aluminium toxic effects have been made in vitro using nutrient broths as it offers an advantage of pH control during the term of studies (Blamey *et al.,* 1991). It has also been noticed that nutrient solutions always exhibits higher concentration than presented by nutrient solutions irrespective of dilutions (Callot *et al.,* 1982; Blamey *et al.,* 1991).

Almost all organisms require glucose as a primary source of energy for their growth as well as other metabolic processes but the nutrient broth which is commonly used for growth of microbes does not contain glucose but the glucose addition as a carbon source may increase the biomass production and rate of growth of bacteria gradually. However this could only be possible if optimum concentration is used when the glucose supplementation is in excess. It may prove to be inhibition for cell growth. In reference to previous studies also it has been found that high dose of glucose inhibited proteinaceous enzymes thereby limiting the bacterial growth.

The cell in the presence of high concentration of glucose, loses its ability to break and reincorporate proteinaceous entities thereby limiting the growth ability of microbial population.

The present research study is aimed at examining the optimal glucose concentration required for bacterial growth at different concentration of aluminium to determine the effect of glucose addition to nutrient broth in combating higher concentration of aluminium so that bacterial growth could be achieved in acidic conditions.

Materials and Methods

Microorganisms, growth medium, glucose supplementation and Al stress

Bacillus and *Burkholderia* strains resistant to higher doses of aluminium were isolated from north east soil (soils with acidic pH). Isolation was carried out against different concentrations of aluminium (0, 20, 60, 80mM $AlCl_3$) in media. Further the media was supplemented with 0.25%, 0.5% and 1% glucose concentration to further check the optimal glucose requirement for growth of isolates under aluminium stress conditions. The fortified medium was dispensed in tubes in triplicates. The *Bacillus* and *Burkholderia* cultures were then inoculated separately under aseptic conditions into the medium in laminar air flow. The inoculated media was put on shaker for 24-48 hrs at 37°C and then O.D. was taken at 600nm to check the effect of glucose supplementation on growth of bacterial strains under control as well as aluminium toxic conditions.

Results and Discussion

Bacterial growth was found optimal when growth media was supplemented with 0.25% glucose concentration under control conditions. But it significantly decreased on further increasing glucose concentration in media. However, when the *Bacillus* isolates were grown in media supplemented with 20mM $AlCl_3$, glucose requirement was found to be increased, the optimal growth was obtained in media supplemented with 0.5% glucose (Fig. 1). In case of 0.5% glucose supplementation in nutrient broth, the *Bacillus* growth was increased upto 2.0-folds in 20mM aluminium toxic conditions. Similarly 0.5% glucose concentration was also found optimal for growth of *Burkholderia* isolates also. The growth of the concerned strain increased upto 5.0-folds and 8.0-folds in case of 20mM and

60mM aluminium stressed conditions respectively (Fig. 2). But unfortunately, the bacterial growth drastically decreased for both the cultures in media supplemented with 1% glucose.

Bacillus and *Burkholderial* isolates were found to exhibit lower growth rates when they were grown in media supplemented with high glucose concentration, implying that high concentration of glucose is inhibitory for growth of bacterial population. 0.25% glucose supplementation was found optimal for bacterial growth under control trials. However in the media supplemented with $AlCl_3$, addition of 0.5% glucose produced optimal growth but it further decreased in media supplemented with 1% glucose.

Fig.1 *Bacillus* growth at different aluminium and glucose concentration

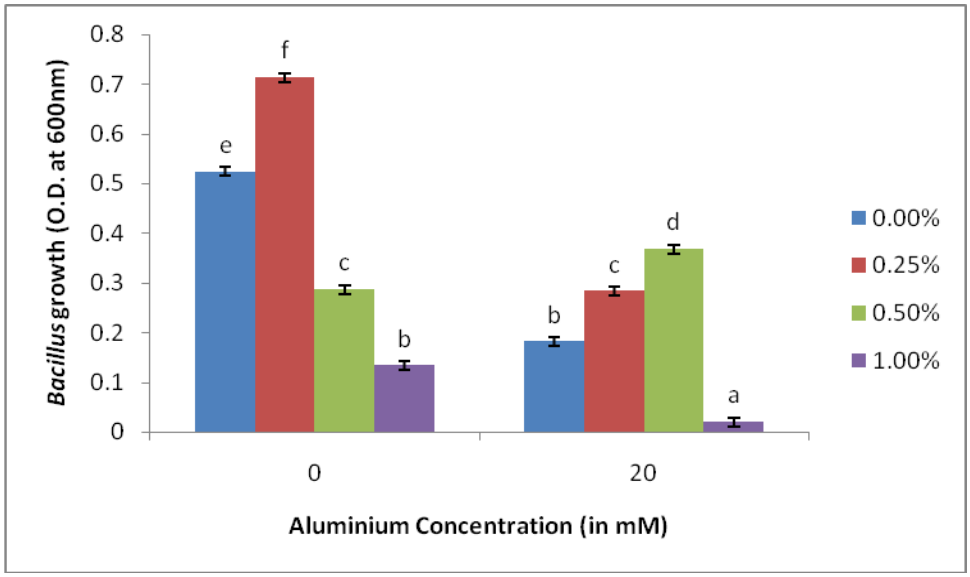
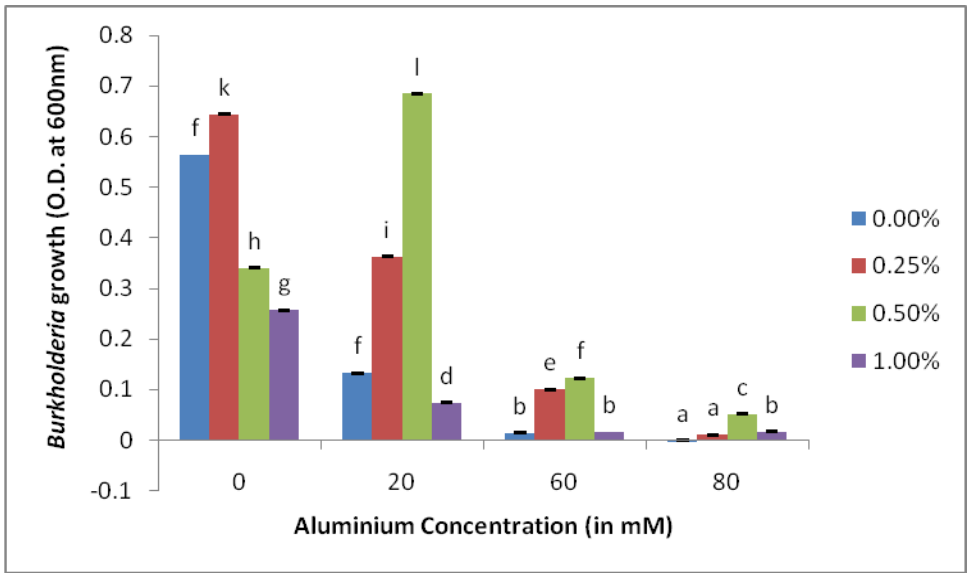


Fig.2 *Burkholderia* growth at different aluminium and glucose concentration



So, a possible explanation for observation is that excess of sugar concentration lead to accumulation of by-product such as acetic acid which decreased the growth (Boyd *et al.*, 1951).

Also, the high sugar in the environment leads to water loss from bacterial cells which ultimately lead to death (Introduction to bacteria, 1999). For example, high concentration of sugar and salt is used for preserving foods. Inhibitory effects of glucose have been found to be reported previously also (Boyd *et al.*, 1951; Kendall *et al.*, 1912; Chirife *et al.*, 1983).

Under aluminium toxic conditions, supplementing media with 0.5% glucose concentration was found favorable suggesting that bacteria under toxicity stress have required some more glucose to survive but ultimately further addition of high concentration of glucose, the growth drastically decreased suggesting that high glucose concentration further imposed a stress on bacterial growth.

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