The Growth and Survival of Lead Solubilizing Strains of *Pseudomonas* in the Presence of Carbon and Nitrogen Sources Supplements in a Lead Culture Medium

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**Abstract**

Cell growth and survival of three strains of Pseudomonas species obtained from a battery manufacturing factory effluent were investigated in varying concentrations of lead in the presence of carbon and nitrogen sources. Glucose and fructose as well as ammonium nitrate (NH$_4$NO$_3$) and potassium nitrate (KNO$_3$) served as carbon and nitrogen sources, respectively. Turbidimetric readings of growth showed that highest growth changes in carbon sources occurred at 96 hours in 2% w/v glucose supplement medium having 0.76, 0.70, and 0.36 absorbance reading for strains B6, D4, and E4, respectively, while strains B6 and D4 had 0.31 and 0.33 at 3% w/v fructose supplement at 48 hours, and at 72 hours strain E4 had its highest growth of 0.16 at 3% w/v. Exceptional growth changes of 0.82 and 0.53 absorbance were recorded in strain E4 in 0.2% w/v on NH$_4$NO$_3$ and KNO$_3$ supplement at 96 hours while other strains did not show appreciable growth changes in both sources of nitrogen.

This investigation showed that single supplement with either carbon or nitrogen sources resulted in increased growth rate, but not a combination of the sources, except in E4 that showed growth changes of 1.1 and 0.9 in NH$_4$NO$_3$ combined with glucose and with fructose, respectively.

Understanding of the physiological and biochemical factors on growth of these isolates will expose the potentiality of such isolates for the bio-treatment of lead polluted environment.

**Keywords**: *Pseudomonas*, growth, lead, carbon, nitrogen.

**Introduction**

Heavy metals pollution in the biosphere often results into fatal interactions with living organisms and disruption of the ecosystem (Khan and Scullion 2000; Nguyen-Viet *et al.* 2007). The danger to health from lead residue in soil and dust has stimulated studies into the effect of concentration of contamination with this metal in our environment, particularly in the urban and industrialised areas (Atuanya and Oseghe 2006). Severe pollution of agricultural and urban soil by lead and some other metals has been established to result in accumulation and toxicity in plant (Shilev *et al.* 2007) as well as decrease in growth and yields (Wallace and Wallace 1994). Bisessar (1982) reported that lead pollution affects the metabolism and biochemical activities of surviving microorganisms. Growth rate of bacteria like *Bacillus cereus*, *Azothobacter chroococum* and *Arthrobacter indigocolor* have been reported to be reduced by lead (Chaudry *et al.* 1989) whereas Cole (1977) had earlier reported inhibition of amylase activity of soil microorganisms by lead sulphate, lead chloride, lead acetate and lead sulphide.

Hansson and Dostalek (1988) in their studies reported the effect of culture condition on cell growth and activities of *Mortierella rammanniana* when subjected to several carbon sources, while the toxicity of metals including Cu, Cd, and Zn to *klebsiella* was
found to be high when concentration of carbon source (glucose) was increased from 10 to 40 mg/l in the culture medium (Brynhildsen et al. 1988; Nguyen-Viet et al. 2007).

With the aim of a better understanding of the physiological and biochemical responses of microorganisms to pollutants, this study examined the effect of carbon and nitrogen sources on growth and survival of these lead solubilizing isolates and observed the combined effect of the two sources on the growth of isolates in lead culture medium.

Materials and Methods

Bacterial Strains

A total of fourteen (14) lead solubilizing strains of Pseudomonas isolated from soils of a battery manufacturing plant in Ibadan by Fagade and Adetutu (1999) were rescreened for lead solubilizing potentials. The best three isolates (B6, D4 and E4) with maximum solubilizing ability and minimum inhibitory concentration (MIC) of more than 500 mg/l of lead sulphide (PbS) were selected for the study.

Reconfirmation of Lead Solubilizing Ability

The strains were revived by repeatedly streaking on sterile nutrient agar plate after which each strain was streaked on lead sulphide - nutrient agar to reconfirm its solubilization potential. Using the method described by Cole (1979), 15 ml nutrient agar was mixed with 50 mg of sterile lead sulphide (PbS) and was allowed to solidify. Exactly 15 ml of the same medium without PbS was added to form another layer on the plates and allowed to solidify after which the plates were inoculated by streaking with pure cultures of the bacterial strains and incubated at 37°C for 48 hours. Solubilizing ability of the bacteria strains was detected by black pigmentation of the bacteria cells.

Preparation of Inocula

Colonies from pure cultures of the screened Pseudomonas were aseptically inoculated into conical flasks containing sterilized nutrient broth and incubated for 24 hours in the environmental incubator shaker at 37°C. Precisely 5 ml each of the 24 hours active cultures served as inoculums for 100 ml of medium for each experiment.

Effect of Carbon Sources on Growth of the Strains

Glucose and fructose at varied concentration of 3% and 4% w/v were employed as the carbon sources. 2, 3, and 4 mg of glucose and fructose were weighed into separate conical flasks containing 100 ml of 500 mg/L lead sulphide - nutrient broth each. The flasks were sterilized and inoculated with the Pseudomonas strains B6, D4 and E4. Incubation was carried out at 37°C on the G24 Environmental incubator shaker (New Brunswick Scientific Co. Inc., Edison, USA) at 250 rpm for 96 hours. The absorbances of each set of concentration at 24-hour intervals for 96 hours were monitored with Bosch and Lomb Spectronic 20 at 600 nm wavelength. Un-supplemented flask served as control while uninoculated broth flask served as the blank.

Effect of Nitrogen Sources on Growth of the Strains

The influence of ammonium nitrate and potassium nitrate supplements on growth of the Pseudomonas strains in the lead sulphide medium was tested at 0.1, 0.2 and 0.3% w/v of each nitrogen sources. 0.1, 0.2 and 0.3 mg of each salt were weighed into 100 ml of lead nutrient broth in different conical flasks. The flasks were sterilized and inoculated with the Pseudomonas strains B6, D4 and E4 and incubated at 37°C on the shaker at 250 rpm for 96 hours. Un-supplemented culture served as control. The absorbance at 600 nm wavelength at 24-hour intervals for 96 hours was determined with Bosch and Lomb Spectronic 20.

Growth of the Lead Solubilizing Strains in Medium Containing both Carbon and Nitrogen at Different Concentrations

The effect of the carbon and nitrogen sources in various combinations on the growth...
of the bacteria strains was determined, 2 mg of glucose and 0.2 mg of ammonium nitrate \((C_1N_1)\), 2 mg of glucose and 0.2 mg of potassium nitrate \((C_1N_2)\), 3 mg of fructose and 0.2 mg of ammonium nitrate \((C_2N_1)\), 3 mg of fructose and 0.2 mg of potassium nitrate \((C_2N_2)\) were weighed into 100 ml of lead sulphide-nutrient broth in conical flask and sterilized. The flasks were inoculated and incubated at 37°C on the rotatory shaker at 250 rpm for 96 hours. Un-supplemented broth culture served as control while un-inoculated broth flask served as the blank. The absorbance of each set of a combination was monitored at 24-hour intervals for 96 hours with Bosch and Lomb Spectronic 20. Un-supplemented culture served as control.

Results

Confirmation of Lead Solubilizing Ability of the Strains

The results of the lead solubilization potential of screened \textit{Pseudomonas} strains are shown in Table 1. Bacteria capable of solubilizing PbS were detected by brown pigmented colour of the colonies. Out of the fourteen lead solubilizing strains tested, six showed good solubilizing ability out of which three strains were found to have highest potential based on the degree of their colouration on the PbS agar. These were \textit{Pseudomonas} strains B6, D4, and E4.

Effect of Carbon Sources on Growth of the Strains in Lead Culture Medium

Figures 1 and 2 are showing the two carbon sources (glucose and fructose) and the corresponding changes in the growth of the culture over 96 hours of incubation. Strains B6, D4 and E4 showed continuous increase in growth in 2% w/v glucose supplemented culture over the 96 hours of investigation. At this concentration, highest growth changes of 0.76, 0.7 and 0.36 absorbance were obtained in strains B6, D4 and E4, respectively. At higher concentration of glucose, the maximum growth changes occurred in strains B6 and E4 at 72 hours with absorbance changes of 0.58 and 0.24, respectively, in 3% w/v of glucose supplemented culture. The maximum growth change of 0.13 absorbance occurred at 48 hours in a culture inoculated with strain D4 at 3% w/v glucose supplementation.

In cultures supplemented with fructose, growth changes in response to supplementation varied among the strains as shown in Fig. 2. Strain B6 showed the highest growth in 4% w/v fructose supplementation with 0.46 absorbance change at 48 hours. Although the initial change within 24 hours was 0.38, the growth rate decreases to the minimum absorbance of 0.20 at 86 hours. In strain D4, the observed growth within 24 hours was 0.2 absorbance compared with the control. The highest growth change of 0.33 absorbance was recorded at 48 hours in 3% w/v fructose supplemented media, followed by 0.24 and 0.03 absorbance at 2% and 4% w/v, respectively, at 48 hours. Strain E4, on the other hand, showed continuous increase in growth over 96 hours of incubation. It attained the highest growth of 0.20 absorbance in 2.0% w/v, 0.16 in 3% w/v and 0.08 in 4% w/v fructose supplementations at 72 hours. After that the growth changes decreased (Fig. 2). Effect of these carbon sources showed that glucose was better utilized.

Table 1. Lead solubilization potential of screened \textit{Pseudomonas} strains.

<table>
<thead>
<tr>
<th>Strain code</th>
<th>Grades</th>
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<tbody>
<tr>
<td>E4</td>
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<tr>
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<tr>
<td>D4</td>
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Key:

+++ High solubilisation;
++ Moderate solubilisation;
+ Low solubilisation;
- No solubilisation.
Effect of Nitrogen Sources on Growth of the Strains in Lead Substrate

In cultures supplemented with ammonium nitrate, strain B6 showed increased growth rate to attain the maximum growth changes of 0.23 absolute in 0.1% w/v concentration, 0.38 and 0.58 in 0.2% and 0.3% w/v concentrations, respectively, within 24 hours of incubation (Fig. 3). After that the growth rate decreased to a minimum of zero level of absorbance in 0.1 and 0.2% w/v concentrations and 0.3 absorbance in 0.3% w/v of the salt at 96 hours. Strain D4 also showed increase in growth rate with maximum growth changes of 0.48 and 0.58 absorbance in culture medium with 0.1 and 0.3% w/v ammonium nitrate at 48 hours.
while maximum growth change of 0.6 absorbance was observed in 0.2% w/v at 24 hours. Gradual decrease in growth changes was exhibited after 48 hours.

In cultures supplemented with potassium nitrate (Fig. 4), strain B6 attained the highest growth changes of 0.18, 0.28 and 0.48 absorbance at different concentrations of 0.1, 0.2 and 0.3% w/v of the salt within 24 hours of incubation. Gradual but continuous decrease in growth changes was observed over the remaining period of 48 to 96 hours. Strain D4 showed a best growth change in medium with 0.1% w/v potassium nitrate concentration and the maximum growth change of 0.5 absorbance was attained at 96 hours.

In the case of higher concentrations (0.2 and 0.3% w/v potassium nitrate) maximum growth changes were obtained at 48 hours and were 0.52 and 0.48 absorbance, respectively. After that the growth changes decreased to reach zero absorbance in 0.3% w/v supplemented culture at 96 hours. The decrease in growth rate observed in culture with 0.2% w/v supplementation changed after 72 hours and increased to 0.5 absorbance at 96 hours. Strain E4, on the other hand, showed increased growth rate that lasted for the hours in culture with different concentrations of the potassium nitrate supplement. Highest growth change of 0.53 absorbance occurred in culture with 0.2% of the supplement. Maximum growth changes obtained in 0.1% and 0.3% supplementation were 0.38 and 0.5 absorbance, respectively, at 96 hours (Fig. 4).

**Effect of Carbon and Nitrogen Combination on Growth Changes of the Strains**

Figure 5 shows the effect of combination of the carbon and nitrogen sources on the growth rate of the strains. Strains B6 showed the following -0.13, -0.15, -0.24 and -0.25 absorbance changes compared to the control with 24 hours of cultivation in the various combinations. Decrease in growth changes continued until 72 hours after which it changed to an increase and did not result in positive growth changes as at 96 hours of incubation.

The growth changes of strain D4 when compared with control at 24 hours of incubation were -0.04, -0.27, -0.14, and -0.22 absorbance in the different carbon and nitrogen combinations.
Fig. 4. Effect of potassium nitrate supplement on growth changes of the three strains of *Pseudomonas*.

These changes were approximately constant till 48 hours after which a sharp decrease in growth changes compared to the control occurred at 72 hours. This resulted in minimum growth changes of -0.6, -0.68, 0.68 and -0.74 absorbance compared with the control at 72 hours.

Fig. 5. Combined effect of Carbon and Nitrogen sources supplements on growth changes of the three strains of *Pseudomonas*.

In culture with strain E4, evident increase in growth changes was observed in those combinations of glucose and ammonium nitrate.
Maximum growth changes of 1.1 and 0.9 absorbance were obtained in the respective culture at 96 hours. The combination with potassium nitrate did not result in positive growth changes.

**Effect of Carbon and Nitrogen on Growth Changes with Time as [Pb] Increases**

Two percent glucose supplementation of different lead concentration media resulted in a positive growth and the change increase of the *Pseudomonas* strains in culture with lead concentration ranges between 600 mg/l to 1,000 mg/l (Fig. 6). At concentrations above 1,000 mg/l, no positive change in growth of the strains was observed.

Strain B6 attained the maximum growth changes of 0.5 and 0.44 absorbance at 600 and 1,000 mg/l, respectively, at 48 hours.
Strain D4 showed maximum growth changes of 0.4 and 0.1 absorbance while strain E4 had maximum growth change of 0.4 and 0.24 absorbance at 600 mg/l and 1, 000 mg/l, respectively, at 48 hours.

Figure 7 shows the effect of 0.2% w/v ammonium nitrate supplement on the growth changes of the strains as the lead concentration increases. Positive growth changes in strains occurred in cultures up to 2,000 mg/l concentration of PbS except for Strain B6 which showed negative growth change compared to the control in lead culture medium with concentration greater than 1,000 gl-1 pbs. Best growth changes occurred in all the Pseudomonas strains at lead concentration of 600 gl-1. Highest absorbance changes of 0.5, 0.38 and 0.3 were recorded for B6, D4, and E4, respectively, at different time of cultivation.

Discussion

Three lead solubilizing strains of Pseudomonas, B6, D4 and E4 used were found to tolerate lead at MIC values over 600 mg/l and this value is higher compared to the 0.5 mg/l recorded by Cole (1979) in lead acetate medium and 500 mg/l reported by Fagade and Adetutu (1999) in PbS medium. Glucose and fructose increased the growth changes of the Pseudomonas strains. Glucose supported the growth rate better than fructose over 72 hours in the lead substrate (Figs. 1 and 2). Brynhildsen et al. (1988) showed that metals taken up by energy driven transport system may accumulate more rapidly at high concentrations of energy sources especially carbon sources to result in toxicity as he observed in Klebsiella sp. for copper at 10-3 when carbon concentration was 10 or 40 mg/l. This was contrary to result of this study because both carbon sources (glucose and fructose) increased the growth changes of the Pseudomonas strains in the lead medium at carbon concentration of 20 to 40 mg/l. This could either be as a result of the presence of plasmid detoxification mechanisms as found in species of Pseudomonas which have been found to have high affinity for mercuric ion (Tynecka et al. 1981; Brock and Madigan 1988). It could also be that the carbon concentrations (20 mg/l - 40 mg/l) did not result in substrate inhibition in Pseudomonas sp. because most bacteria cells grow near maximum value when the carbon source concentrations fall between 10-100 mg/l (Cooney 1985). That glucose was a better carbon source agreed with Namtoothi and Pandey (1995) who showed that glucose gave best cell growth out of six carbon sources tested on Breivibacterium sp. in growth medium. Emanuilova and Kambourouva (1992) who studied the effect of five carbon sources on Bacillus storerothermophilus found that the organism showed a preferential choice of growth and activity when grown on glucose as sole carbon source.

References


