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GROWTH CURVE ANALYSIS OF BACTERIAL ISOLATES FROM FRESH COAL MINE SPOIL

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ABSTRACT

The research examines the growth curves of two Gram-negative bacteria, one isolated from fresh coal mine debris and the other from a temperature range of 35 to 50°C. According to the results, both isolates exhibited a typical optimum development curve at 35°C. Nonetheless, as the temperature increased, the two isolates had a distorted growth curve, with the specific growth rate and exponential phase both decreasing. The results show that both isolates can withstand a higher temperature regime, indicating that they are mesophilic.

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INTRODUCTION

Due to its low pH (Blowes et al., 1995; Kristjanson and Hreggvidsson, 1995; Ledin and Pedersen, 1996; Schippers et al., 2000), high temperature profile (Bos et al., 1994; Johnson, 2002; Satyanarayana et al., 2005), and physically disturbed habitat (Juwarkar et al., 2004; Ghose, 2005; Machula et al., 2005), coal mine spoil overburden is an ideal place for soil organisms to survive (Juwarkar et al., 2004; Machula et al., 2005). Coal mine refuse is not microbiologically sterile despite these extremes; it often harbours a special set of thermoacid tolerant, chemolithotrophic, and heterotrophic bacteria (Darland et al., 1970; Belly and Brock, 1974). Our previous work on the microbiology of coal mine ruin overburdens in the Basundhara coal field region of Mahanadi coal field restricted, Orissa, found the isolation of temperature and pH (Sethy and Behera, 2009).

resistant Gram negative bacilli and cocci. In the present study, the growth performances of these two isolated group of

bacteria with respect to a temperature range of 35 to 50°C was analysed.

MATERIALS AND METHODS

Pure culture of Gram negative bacilli and cocci (isolated through our earlier sampling: Sethy and Behera, 2009) were used for the present study. A loop of bacteria from the pure culture slant of each bacterial isolate was aseptically transferred to a sterilized 250mL Erlenmeyer flask containing 100mL of nutrient broth (peptone-5g/L, Beef extract-3g/L and NaCl-5g/L) and was activated by incubating for 24hrs at 35°C.

One millilitre of active Gram-negative bacilli culture was aseptically transferred to twelve of the fifteen Erlenmeyer flasks, each of which contained fifty millilitres of sterile nutrient broth. After that, the flasks were placed in a shaker incubator and let to incubate for 285 to 300 minutes at temperatures ranging from 35 to 50°C. Concurrently, three Erlenmeyer flasks with a 150 mL capacity were used as controls, containing just nutrient broth and not infected. Spectrophotometric analysis was performed on the flask contents at 640 nm with regard to varying intervals of 15 minutes to determine bacterial growth. The Gram-negative cocci bacterial isolate's growth was also evaluated using the same method.

Specific growth rate (μ) of the bacterial isolates at different temperature was calculated as follows (Heritage *et al.*, 2002):

$$\frac{\log N_t - \log N_0}{t_t - t_0} = \frac{\mu}{2.303}$$

Where, N_0 = Absorbance at initial of the exponential phase of growth
N_t = Absorbance at end of the exponential phase of growth
$t_t - t_0$ = Time difference to achieve absorbance from N_0 to N_t

RESULTS

Figure 1 shows the temperature regime (35 to 50°C) that Gramme negative bacilli grow in. There was a lag period of 60 to 90 minutes when the germs were active. The rapid growth stage of the

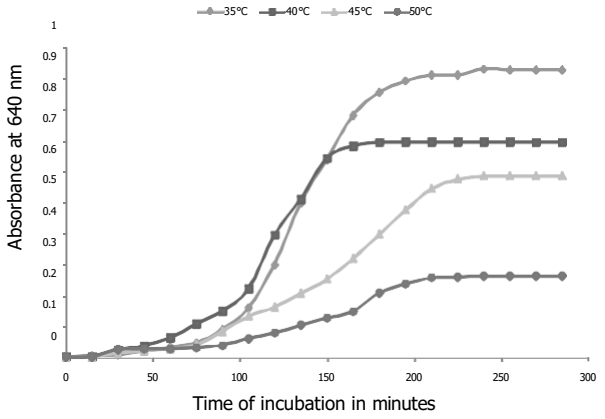

Figure 1: Growth curve of Gram negative bacilli at different temperature

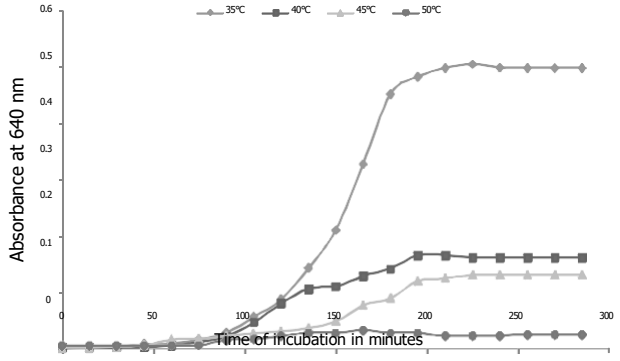

Figure 2: Growth curve of Gram negative cocci at different temperature

Figure 3: Specific growth rate of Gram negative bacilli and cocci at different temperature regime

The bacteria went into stationary phase after 195 minutes of growth at 35°C. But at 40, 45, and 50°C, the bacterial growth patterns were clearly distinct. The exponential phase peaked around 40°C, which is substantially early than other temperatures. Even though the exponential phase might last up to 225 minutes at 45°C, the phase's slope was much less steep than at 35°C or 40°C. Even during the exponential phase, the bacteria's growth was quite slow at 50°C. The temperature range of 35 to 50°C is shown in Fig. 2 as the growth curve of Gramme negative cocci. The microorganisms had a latency time of 90–135 minutes. At 35°C, the exponential phase of bacterial development continued for 195 minutes before the stationary phase began. Alternatively, the bacteria showed a somewhat earlier completion of the exponential phase when the temperature was 40°C. Nevertheless, compared to 35 and 40 °C, the slope of the phase was much lower at 45 °C, and the bacteria did not show any growth. Bacterial growth was determined to be negligible at 50°C. The bacteria's specific growth rate (μ) was found to be maximum at 35°C and to gradually drop as the temperature increased, as shown in Figure 3.

DISCUSSION

Our earlier study (Sethy and Behera, 2009) revealed Gram negative bacteria (both bacilli and cocci) to be in a major proportion of total colony forming units of bacterial population in the fresh coal mine spoil. There have been also reports according to many studies (Marsh and Norris, 1983; Ghauri and Johnson, 1991; Zhou et al., 2007) about the frequency of Gram-negative bacteria found in coal mine waste from various places. It is often believed that the exterior lipopolysaccharide layer above and above the cell wall accounts for Gramme negative bacteria's somewhat stronger tolerance to varied environment extremes (Moat et al., 2006). According to the study's findings, which corroborate those of Hallberg and Lindstrom (1994) and Zhou et al. (2007), the growth pattern of these isolates throughout a temperature range of 35°C to 50°C shows their ability to adapt to higher temperatures. Examination of several development stages in relation to varying temperatures

It showed that the isolates thrived around 35 °C, and that temperatures over that point had a significant impact on their exponential growth phase, causing them to enter stationary phase very early. Their underlying mesophilic nature with adoptability for increased temperature tolerance is abundantly shown by such observations. At a higher temperature of 50°C, the Gram-negative cocci's growth pattern revealed that there was no exponential phase, meaning that the specific growth rate was zero. Because of their lower heat adaptability compared to bacilli, cocci are characterised by this. Nonetheless, the specific growth rates of the two isolates peaked at 35°C and then decreased as the temperature increased, providing strong evidence of their mesophilic nature. The bioprospecting potential of these two isolates for industrial use may be better understood with more research into their physiological viability and cellular enzymatic characterisation in relation to the higher temperature regime.

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