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Effect of cobalt and nickel on growth and carboxymethyl cellulase activity of *Cellulomonas* spp

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A *Cellulomonas* spp isolated from soil produced carboxymethyl cellulase (CMCase) and xylanase enzymes. Cobalt (0.1 mM) and nickel (0.1 mM) decreased the growth rate of *Cellulomonas* spp. These metal ions activated CMCase activity but not xylanase activity; cobalt being the greater stimulatory ion than nickel. A predominant long lag phase was observed in adapted cells when compared with the non-adapted cells. However, the growth level of control cells was never obtained by the adapted cells.

Keywords: carboxymethyl cellulose, carboxymethyl cellulase, *Cellulomonas* spp, growth

Introduction

The rate of cellulosis for a given organism is limited by several factors. One important factor is the effect of various metal ions present in the soil in the vicinity of the microbial community. However, the effects of heavy metals like nickel and cobalt on substrate decomposition have received little attention. Information regarding the effect of heavy metals on microbial growth and carboxymethyl cellulase (CMCase) and xylanase activity is scarce. In *Bacillus* spp different metal ions inhibited endoglucanase activity *in vitro* (Fukumori *et al.* 1985). Cobalt at a concentration of 0.1–0.5 mM enhanced the avicelase activity in *Thermomonospora fusca* (Ferchak & Pye 1983) and cobalt (5 mM) stimulated the CMCase activity 1.3-fold in alkalophilic *Bacillus* spp (Yoshimatsu *et al.* 1990). In contrast, colbalt at a concentration of 10 ppm resulted in increased cellulase yield in *Trichodema viridi*, where a 1.5 ppm concentration of colbalt affected the growth of the organism (Mandels & Reese 1957). Cellulase enzymes are generally influenced by cellobiose, sophorose, xylan, pectin, lactose and numerous other substances (Mandels *et al.* 1975, Sternberg & Mandels 1979, Mandels 1981). This study deals with the effect of cobalt and nickel on growth and enzyme activity of *Cellulomonas*.

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Materials and methods

Organism and growth

Cellulomonas was isolated from termite infested soil mounds from northern arid and semi-arid region of India, and grown according to the method of Saxena *et al.* (1992). Stock solutions of $CoCl_2$, $NiCl_2$, $PbCl_2$, $HgCl_2$, $CdCl_2$ and $ZnCl_2$ were prepared separately and sterlized by autoclaving before introduction into the culture medium or reaction mixture. The organism was grown at 30 °C with constant shaking at 200 r.p.m. Growth was determined turbidometrically at 750 nm. Media containing cobalt and nickel were inoculated with a fresh culture grown in the presence of the respective heavy metals for four successive subcultures and the cells termed 'adapted cells'.

Minimum inhibitory concentration (MIC)

The MIC was determined by adding nickel and cobalt to 50 ml medium and inoculated with 1% (v/v) mid-log-phase control culture and incubated at 30 °C for 48 h. The MIC was calculated as the lowest concentration at which no growth occurred.

Enzyme assay

CMCase and xylanase activity were assayed by the procedure of Saxena *et al.* (1991). Separate blanks were

taken for control and adapted cells. The required concentrations of nickel and cobalt were added wherever stated. The 100% activity is defined as 100 μg of reducing sugar (expressed as glucose/xylose equivalent) liberated per millitre per 20 min under the above conditions. All assays were done in triplicate. Polyacrylamide gel electrophoresis was done following the procedure of Laemmli (1970).

Results and discussion

The influence of different metal ions (0.1–1 mM) on CMCase and xylanase activity was tested in the reaction mixture. A stimulation of CMCase activity was observed with cobalt (0.15–0.25 mM); however, the increase was less with nickel when added at the same concentrations. The activity was considerably inhibited by mercury, cadmium and lead, and was inhibited to a lesser extent by zinc (Table 1). There was no increase in xylanase activity with any of these metal ions. Further experiments were performed with cobalt and nickel. The MIC values for cobalt and nickel on the growth of *Cellulomonas* sp. were 0.8 and 1.4 mM, respectively. Growth was very poor when different concentrations of these metals were added to the medium. A predominant long lag phase (24 h) was observed which indicated that the cells are dying even at low concentration.

Inhibition of growth by heavy metals was attributed to the formation of unionized molecular complexes between the metal ions and ionogenic groups on the surface of the cell membranes (Somers 1961). This inhibition may result in altered membrane permeability allowing metal ions (free or combined) to break away from the cell surface into the inner membrane. These ions interact with the proteins of the cell membrane causing conformational changes, thus resulting in the death of the cells.

To obtain better growth, the cells were adapted to different concentrations of metals. However, maximum growth equivalent to control cells was never obtained by the adapted cells (Figure 1a and b). The

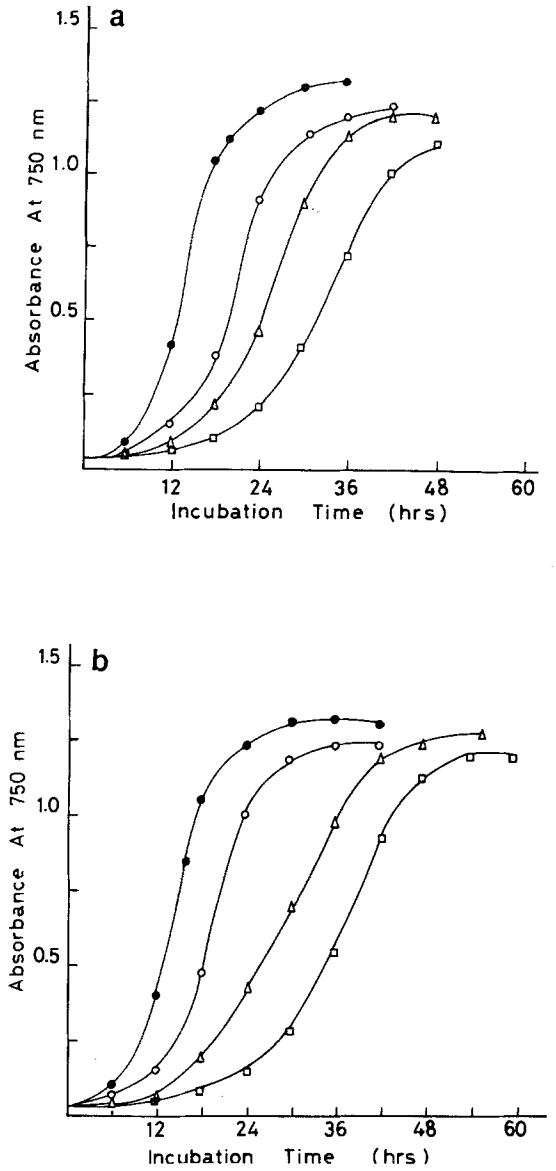

Figure 1. (a) Growth curve of control and cobalt adapted cells. ●, Control; ○, Co (0.2 mM); △, Co (0.4 mM); □, Co (0.6 mM). (b) Growth curve of control and nickel adapted cells. ●, Control; ○, Ni (0.2 mM); △, Ni (0.5 mM); □, Ni (1 mM).

Table 1. Effect of different metal ions on CMCase activity

Concentration of metal ions (mM) added to the reaction mixture	CMCase activity (percent of control)					
	Ni^{2+}	Co^{2+}	Hg^{2+}	Cd^{2+}	Pb^{2+}	Zn^{2+}
0.1	100 ± 7.43	190 ± 3.04	82 ± 3.25	72 ± 4.19	36 ± 1.69	94 ± 2.49
0.2	120 ± 2.00	220 ± 0.72	74 ± 1.88	60 ± 4.89	34 ± 3.68	90 ± 2.49
0.3	112 ± 1.53	184 ± 4.18	66 ± 2.44	42 ± 2.86	27 ± 2.85	82 ± 2.86
0.5	110 ± 1.05	184 ± 2.36	61 ± 3.26	38 ± 2.35	21 ± 1.24	80 ± 3.68
1.0	110 ± 0.15	110 ± 1.02	27 ± 2.86	27 ± 2.94	18 ± 4.10	63 ± 2.44

See material and methods. Values are means ± SD of three replicates.

specific growth rate was 0.4 h^{-1} for the control cells, and 0.12, 0.09 and 0.08 h^{-1} at 0.2, 0.4 and 0.6 mM of cobalt, respectively. For nickel adapted cells, the specific growth rates were found to be 0.13, 0.10 and 0.10 h^{-1} at 0.2, 0.5 and 1 mM of nickel, respectively. There was no effect of cobalt or nickel on the morphological characteristics or on the pigmentation of the cells, although Christine *et al.* (1984) observed that these metals inhibited the formation of pigments in *Serratia marcescens*. The intracellular protein profiles from control and adapted cells indicated no significant change in the polypeptide composition when analyzed by SDS–PAGE (Figure 2).

A 2-fold increase in CMCase activity was observed when cobalt (0.2 mM) was added in the culture medium. However, with nickel, at the same concentration, the increase was less (Figure 3a and b). The culture filtrate of cobalt and nickel adapted

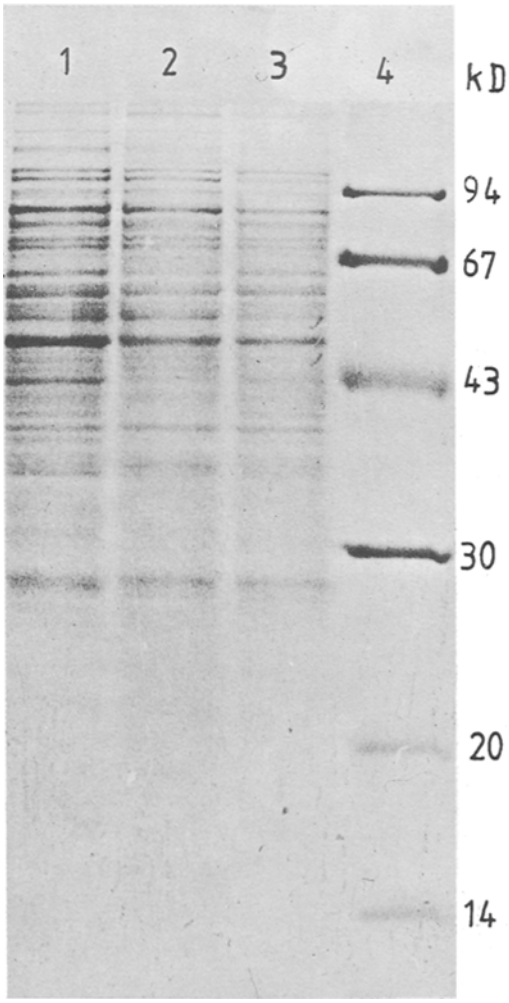

Figure 2. SDS–PAGE of intercellular milieu of control and adapted cells. Lane 1, Control; lane 2, 0.6 mM $CoCl_2$ adapted cells; lane 3, 1.0 mM $NiCl_2$ adapted cells; lane 4, marker. Protein concentrations were maintained at 1 mg ml^{-1} in all samples.

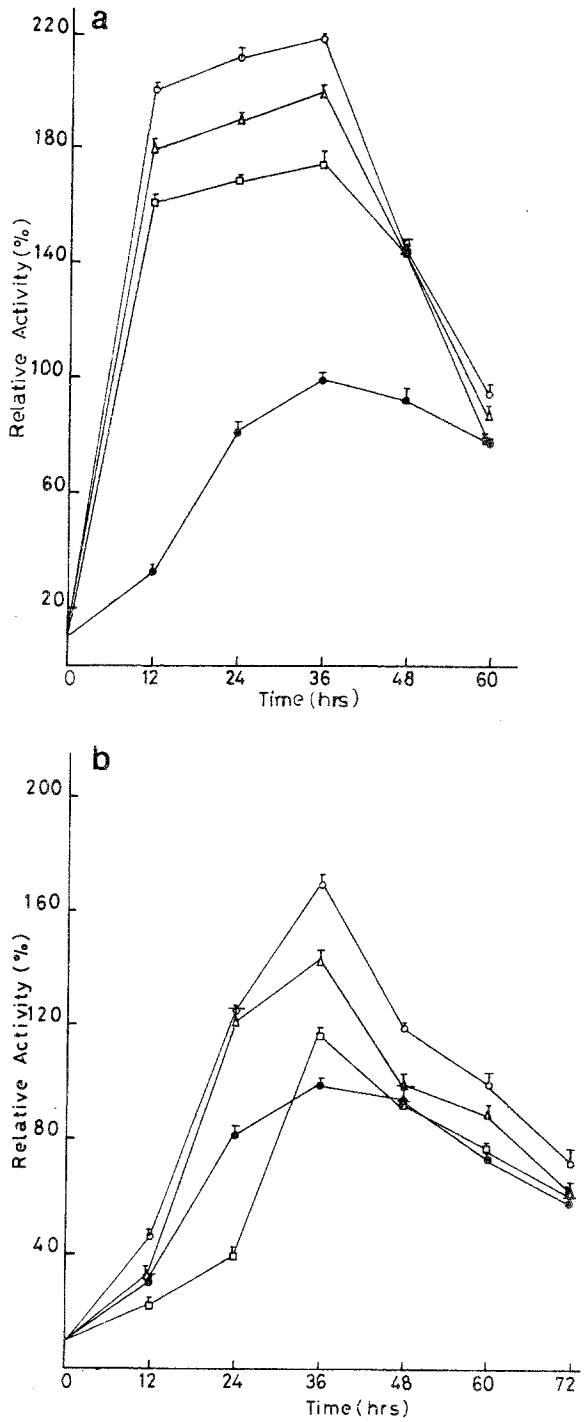

Figure 3. (**a**) Effect of cobalt adapted cells on CMCase activity. ●, Control; ○, Co (0.2 mM); △, Co (0.4 mM); □, Co (0.6 mM). (**b**) Effect of nickel adapted cells on CMCase activity. ●, Control; ○, Ni (0.2 mM); △, Ni (0.5 mM); □, Ni (1 mM).

cells showed no further enhancement in enzyme activity when the metal ions were added at the same concentration. These observations indicate that cobalt and nickel probably stimulate the activity *in vitro* but do not affect enzyme production *per se*. These results are more pronounced than those reported with *Thermomonospora fusca* where endoglucanase activity increased by only 34% in the presence of cobalt (Ferchak & Pye 1983). Activation of industrially important enzymes by metal ions has also been observed with the cellulase complex of *Thermomonospora fusca*.

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