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Effect of Cobalt on Synthesis of Extracellular Alkaline Phosphatase Production from *Bacillus* sp.

Abstract

Various species of *Bacillus* isolated from soil were characterized and tested for their ability to produce extracellular alkaline phosphatase. Six strains of *Bacillus* demonstrated significant activity as compared to *Bacillus subtilis* 6633 in optimized Bacillus medium ATCC 552. Highest levels of extracellular alkaline phosphatase activity were observed with 1% whey, in the presence 0·1mM $CoCl_2$ (10.4 U/ml/min). This trend was observed in five of the six strains tested.

Keywords: Alkaline phosphatase; *Bacillus*

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Introduction

Alkaline phosphatase, APase (EC 3.1.3.1), a metalloenzyme, catalyses the non-specific hydrolysis of phosphate monoesters [1] and has been extensively studied in various organisms ranging from bacteria to mammals [2,3,4]. In the bacteria alkaline phosphatase is found in the periplasmic space [5] and is induced under low phosphate concentrations suggesting its involvement in phosphate metabolism [6]. Phosphatases are thought to function as phosphate scavengers under condition of phosphate depletion. Further, addition of stimulants such as detergents, metal-salts, and various other compounds enhance the activity of the enzyme [7,8]. Very few studies have been carried out on extracellular phosphatases [9]. This investigation aims at determining the role of metal ions in the production of extracellular alkaline phosphatase in *Bacillus* species. The morphological properties and taxonomic characteristics of the organisms isolated from soil were studied [10] and biochemical analysis was processed by probabilistic identification software, PIBWIN [11]. Six strains of *bacillus* screened for phosphatases showed much higher activity as compared to *Bacillus subtilis* 6633 [12].

Bacillus medium ATCC 552 having the following composition was used (g/l): Peptone, 10g; Glucose, 10%; Beef extract, 3g, NaCl, 5g; K_2HPO_4, 5g; K_2HPO_4, pH adjusted to 7·6 and sterilized by autoclaving. The cultures were grown in 100ml of medium in 250ml Erlenmeyer flasks after inoculation with 5ml of overnight culture and this was incubated at ambient temperature on a rotary shaker having a throw of 2.5-3.81cms at 200rpm. Aliquots (5ml) were withdrawn at 4h intervals and centrifuged at 3000xg for 10 minute. The supernatant obtained was assayed for the enzyme.

Alkaline phosphatase activity was assayed as described by Sawhney & Singh [13] taking into consideration the optimum pH and temperature of the *bacillus* cultures. One unit of alkaline phosphatase activity was defined as the amount of enzyme that liberates 1µmol of ρ-nitrophenol/min under defined conditions. All reagents were obtained from Loba Chemie Pvt Ltd.

The medium was supplemented with (1% w/v) carbon sources such as lactose, maltose, malt extract, starch, sucrose or whey. A control flask of Bacillus medium ATCC 552+1% glucose was also maintained. Whey sample was prepared from Cow's milk (obtained locally) and the lactose content determined [14]. The final medium contained whey ≈1% lactose. Total enzyme activity (U) was analyzed at different time intervals during incubation.

Further the isolates were grown in medium prepared in double distilled water and supplemented with metal salts (0.1mM chloride form) such as Ba, Ca, Co, Cu, Fe, K, Li, Mg, Mn, Ni, Zn and extracellular alkaline phosphatase activity determined.

The APase activity varied among the *Bacillus* sp. and maximum activity was obtained with the medium supplemented with 1% whey as carbon source (Table I). Further, addition of 0.1mM $CoCl_2$ to the production medium enhanced extracellular alkaline phosphatase maximally in all the *bacillus* species studied except, *Taxon 18* (Table 2). Higher concentration of metal ions suppressed APase activity right across upto 24h.

Our results suggest that the stimulation after addition of cobalt on APase production in *Bacillus* is not restricted to *Bacillus licheniformis* [15] indicating that the regulatory mechanism among all the *Bacillus* species may be similar.

Table 1: Effect of Carbon sources on alkaline phosphatase production in *Bacillus* sp.

Carbon Source (1%)	*B. badius* (U/ml)	*B. cereus* (U/ml)	*B. firmus* (U/ml)	*B. licheniformis* (U/ml)	*B. smithii* (U/ml)	Taxon (U/ml)
Glucose*	71.2	46	69.3	32.1	25	16.9
Lactose	14.9	18.8	14.5	40	15.3	5.2
Maltose	44.4	42	63.4	31.7	51.2	45
Malt extract	7.8	2.4	1.4	7.8	10.4	6.5
Starch	3.1	3.1	6.9	6.2	5.1	1.1
Sucrose	7.3	2.9	6.4	6.3	6.6	3.8
Whey	107.9	111.1	87.2	91.6	184	161

*Control.

Table 2: Alkaline phosphatase activity in presence of metal ions. Time of incubation (h).

Organism	Metal ions (0.1mM)	Activity (U/ ml)
Bacillus badius	$CoCl_2$	252 (16h)
Bacillus cereus	$CoCl_2$	126 (16h)
Bacillus firmus	$CoCl_2$	157 (16h)
Bacillus licheniformis	$CoCl_2$	205 (12h)
Bacillus smithii	$CoCl_2$	146 (16h)
Taxon 18	$MnCl_2$	108 (20h)

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