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Effect of Copper and Cadmium on growth and β- amylase activity of *Bacillus megaterium*

Shobha Shrivastava

Department of Botany, Govt. Sarojini Naidu Govt. Girl's P.G. (Auto) College
Bhopal (India)
Email: dr.shobhashrivastava@rediffmail.com

Abstract

The present study was conducted to investigate the growth potential and β-amylase activity of *Bacillus megaterium* NCIM 2670 under certain defined stress environment. The chosen determinants were copper and cadmium. The observations on growth and β-amylase activity suggested that both the metals were toxic *to B. megatarium*, low concentrations of Cu although stimulated bacterial growth and enzyme activity. The bacterium was found to be more sensitive of Cd in comparison to Cu. Interaction between Cu and Cd appeared to be antagonistic as the depression of growth and enzyme activities by a mixture of the two was lesser than that caused by either of these. The study has ecological implications as the co-occurrence of Cu and Cd is common in contaminated soils.

The introduction of heavy metals into the soil environment has become a subject of global concern. Heavy metal accumulations in the soil inhibit root growth, inactivate soil enzymes and eliminate soil microorganisms[9]. Among the most toxic heavy metals, copper and cadmium are common environmental contaminants particularly in urban-industrial areas[4,11]. The biological role of copper is well established. The metal activates many physiological processes at low concentrations. Unlike copper, cadmium has no known biological function and is regarded as one of the most toxic metals[8,10].

Soil microorganisms play important role in the nutrient cycling and help maintain the soil fertility in the process of decomposition, a sizeable amount of nutrients are fixed In the plasma of living microorganisms and as a consequence nutrient are prevented from being leached out from the soil. After microorganisms die away they undergo rapid mineralization, and nutrients contained in them are released and utilized by the plants[9]. *Bacillus megaterium* is an important soil bacteria capable of synthesizing many enzymes including β-amylase. This is a starch degrading enzymes, hydrolyses alternate bonds from the non-reducing end of the substrate. The enzyme degrades amylase, amylo pectin or glycogen in an exo-stepwise fashion by hydrolyzing alternate glycosidic bonds. Thus,

the microbial enzymes in soil help to decompose organic substrates and maintain soil fertility. Kiss *et al*.[5] have suggested the significance of soil microbial enzymes in nutrient release. The present study was conducted to investigate the effects of copper and cadmium on growth and β-amylase activity of *Bacillus megaterium.*

The experimental organism *Bacillus megaterium* NCIM 2670 was obtained from National Collection of Industrial Microorganisms, NCL Pune (India). The cultures were maintained in a Bacteriological Incubator at 30 $\pm 1^0$C in the minimal defined medium (pH=7.5) as described by Acevedo and Cooney[7]. Bacterial growth was measured at regular intervals (10h) in terms of nephlo-turbidity units (NTU) through a Digital Water and Soil Analysis Kit (Electronics India, Model 191E). The observations were made in the presence or absence of Cu and Cd under single and bimetallic conditions. β-amylase activity was assayed according to Peter Bernfield[8] by measuring the reaction of reducing sugars with dinitrosalicylic acid and its reduction to nitroaminosalicylic acid.

The effect of copper and cadmium on growth of *Bacillus magaterium is* presented in Table-1. At low concentration of copper (0.5 to 1.0 µm.), growth of the bacterium improved. About 54% improvement in growth was observed at 1.0 µm concentration of copper in comparison to the control. At high concentrations, however, the growth was reduced significantly. The toxic effect of cadmium was greater in comparison to copper. The lowest concentration of cadmium considered in this study (0.5µm) was found to be toxic and only 40% growth was obtained after 10 hours of incubation. The sensitivity of *Bacillus megaterium* to such metals has also been reported by earlier workers[9]. When both the metals were taken into the medium, growth of the bacterium reduced only slightly. Even at 1.o µm of copper and cadmium each, 50% of growth was retained. This suggest that copper is antagonistic to cadmium (Table-1).

Table-1. Effect of copper and cadmium on growth of *Bacillus megaterium.*

Time	Growth measurement (NTU)*									
(h)	Control	Cu (µM)			Cd (µM)			Cu+co (µM)		
		0.5	1.0	5.0	0.5	1.0	5.0	0.5	1.0	5.0
0	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
10	1.0	1.0	1.0	0.3	0.4	0.2	0.1	1.0	1.0	0.6
20	3.5	4.0	4.2	0.3	0.4	0.2	0.1	4.0	3.0	0.3
30	6.0	6.5	8.0	0.3	0.4	0.2	0.1	5.8	3.0	0.3
40	6.0	6.5	8.0	0.3	0.4	0.2	0.1	5.8	3.0	0.3
50	6.0	6.5	8.0	0.3	0.4	0.2	0.1	5.8	3.0	0.3

*NTU: Nephlo Turbidity Unit

The enzyme β-amylase was found sensitive to copper and cadmium, although the enzyme activity was increased at low concentration of copper (Table-2).

Table-2. Effect of copper and cadmium on β-amylase activity of *Bacillus megaterium*

Time		Growth measurement (NTU)*								
(h)	Control	Cu (µM)			Cd (µM)			Cu+co (µM)		
		0.5	1.0	5.0	0.5	1.0	5.0	0.5	1.0	5.0
0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
10	5.0	6.5	7.0	2.0	4.0	nd	nd	6.5	4.0	2.5
20	10.0	15.0	16.0	1.0	2.5	nd	nd	16.5	7.0	2.0
30	40.5	50.0	50.6	nd	1.0	nd	nd	52.5	32.8	1.0
40	20.5	20.5	20.5	nd	nd	nd	nd	21.0	11.0	1.0
50	5.0	5.5	5.6	nd	nd	nd	nd	6.0	3.8	1.0

nd: not detectable

When both the metals were taken together, the toxic effect on enzyme activity was comparatively less severe. Copper, being an essential micronutrient, is required in many enzyme reactions[8]. Cadmium on the other hand has no known biological role and competes with copper. Cadmium inhibits DNA synthesis through blockin of-SH group or through inhibition on DNA polymerase activity[3,4].

The decreased cadmium toxicity in the presence of copper has ecological implications as the co-occurrence of these metals is common in contaminated soils. Bacterial strains at the soil surface promote the retention of finer particles in the soil due to presence of microbial polysaccharides and a number of extracellular enzymes[7]. Such enzymes as β-amylase help in breakdown of complex organic matter in the process of decomposition. Since microorganisms in the soil help in nutrient cycling and conservation (prevent nutrient leaching), contamination of the soil environment with increasingly high levels of toxic metals and subsequent elimination of microbial populations may have severe impact in the long-run.

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