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Biodegradation of Chicken Feather Wastes in Submerged Fermentation Containing High Concentrations of Heavy Metals by *Bacillus* sp. khayat

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ABSTRACT

The increasing use of feather in removing heavy metals from water continues to generate feather wastes contaminated with heavy metals which make it difficult for feather-degrading bacteria to degrade them. In this study, the capacity of *Bacillus* sp. khayat to grow, produce keratinase and degrade chicken feathers contaminated with various heavy metals, chemicals and surfactants was investigated. The results showed that keratinase activity, feather degradation and the bacterial growth increases with an increase in concentration up to 10 ppm for certain metals, 15 ppm for Cr and Zn and up to 20 ppm for Cu. $CaCl_2$ and Fe_2SO_4 have negative effect on growth, KA and feather degradation when placed in medium or in cell free crude keratinase except Ca ion which affect crude crude keratinase less. Feather degradation was enhanced at 5 ppm in Pb, Ni, As, Cr and Cu. However, Hg showed decreased feather degradation after 1 ppm. About 90-100 % feather were degraded in the presence of 10 ppm Ni, Ag, Cr, Zn and Co. Ni, Ag, Zn and Cu were the best tolerated metals. *Bacillus* sp. khayat, with a relatively high tolerance to heavy metals while still maintaining its feather-degrading activities, appears to be a bacterial model for toxic environmental waste.

INTRODUCTION

The use of microorganisms with capacity to efficiently degrade keratin containing wastes such as feather, is receiving a very good attention owing to its industrial importance as the source of enzyme keratinase and other valuable essential amino acids. Different bacteria, fungi and Streptomyces have been reported by scores of authors to possess such properties [1-4]. Feathers apart from being able to produce useful end products after microbial degradations also constitute environmental nuisance. The cheap source of feathers has also make a useful and cheap raw material for removing heavy metals from water, an alternative to the conventional methods of ion exchange, chemical reactions which are expensive [5]. However, tonnes of feathers used for this purpose, cannot be used for producing useful products such as additives to animal feed as a source of amino acids due to the content of the absorbed heavy metals which are toxic to animals, microorganisms and environment [6]. The disposal of such feather waste posed more hazard to human health and the environment compared to disposal of heavy metal free feathers from slaughterhouses. The former required a pre-treatment such as by using a special microorganism capable of degrading feather in high concentration of heavy metals and other chemicals. We have isolated a novel bacterium, capable of efficiently degrading different feather types at elevated concentration of heavy metals. Although heavy metals like copper, zinc, nickel and iron are essential to metabolic reactions of some organism and are therefore required in trace amount, others like mercury, silver and cadmium have no biological role and are harmful to the organisms, even at very low concentrations [7]. To the best of our knowledge, there is no report on feather-degrading bacteria that can withstand high concentrations of toxic metals such as lead, mercury etc.

The aim of this study was to screen *Bacillus sp*. khayat for ability to degrade feathers contaminated with heavy metals and

other chemicals such as metallic ions, surfactants and detergents (the form in which feathers are obtained from slaughterhouses or water treatment plants).

MATERIALS AND METHOD

Medium and cultivation conditions

The bacterial strain used in this study was *Bacillus* sp. khayat. The culture of the bacterium was maintained in 10 ml of feather meal basal medium. The medium used for the fermentation was the modified feather basal medium, where all the physico-chemical parameters were optimised by response surface methodology. The medium which contained 0.5 (w/v) NaCl, 0.7 (w/v) KH_2PO_4, 1.4 (w/v) K_2HPO_4, 0.01 $MgSO_4$, 0.1 g/l ammonium bi carbonate, 5 g/l skimmed milk and 0.05 g/l urea and 0.35 g (w/v) chicken feathers in 100 ml of distilled water [5], was inoculated with two per cent (v/v) of inoculum and incubated at 37 °C for 24 h. The changes in medium turbidity and subsequent degradation of feather indicate that bacterial growth had taken place.

Experiments with heavy metals

Experiments with heavy metals were carried out with MFBM which contains varying concentrations (ppm) of different heavy metal solutions. The metal solutions were sterilised by membrane filtration (pore size 0.22 μm). The metals used are lead, nickel, silver, mercury, tin, chromium, cadmium, zinc, cadmium and copper, all are of analytical grade. Several concentrations of each ion ranging from 1-20 ppm were prepared separately. The surfactants were prepared to different concentrations as shown in Table 2. The medium was adjusted to pH 8. We defined the bacillus to be tolerant to particular heavy metals when the bacterium produces keratinase enzyme, degrades feather and able to produce visible colonies within 24 h in the presence of the chosen metal concentrations.Flasks without inoculum or any metal were prepared as control samples. A bacterium, Escherichia coli which is not a feather-degrading was also used as control. All the experiments were conducted in triplicate and data presented are means of the triplicates. At specific time intervals, aliquots of the medium were sampled for analysis of keratinase activity and bacterial growth. Feather degradation was measured at every 24 h.

Analytical methods

The bacterial continue growth were estimated by plate counting method and reported as colony forming units (CFU/ml). The initial bacterial cultured in each 100 ml of medium contains approximately $3.8 \times 106\ mL{-}1$. Keratinase activity was detected using Azokeratin as substrate as described by Joshi et al. [8]. Feather degradation was estimated from the washed residual feather that remained after incubation when compared with control. Bacterial growth and keratinase activity may be affected differently by metals and others. In order to differentiate this, the effect of heavy metals, EDTA and surfactants on keratinase activity were also determined.

Various final concentrations of the metals, EDTA and surfactants were incubated with the enzyme for 30 minutes. The heavy metals which inhibited growth at lowest concentration were selected. The residual enzyme activity was determined using the keratinase assay. For control, the enzyme was incubated without heavy metal ion solutions before performing keratinase assay.

RESULTS AND DISCUSSION

It is obvious that heavy metals are toxic to microorganisms because of their role in deactivation of enzymes by reacting with their functional groups, denature them and also compete with essential cations. Despite this, microorganisms capable of resisting this toxicity to certain level are highly needed, and this has led to extensive researches on determining the inhibitory effect of heavy metals on different bacteria for application in metal reduction treatment processes [6,7]. The reported toxic concentrations of heavy metals to bacteria range from a few ppm (mg/L) to as much as 100 ppm. For instance, Cabrera et al. [9] reported inhibition of growth of sulphate-reducing bacteria by concentration of Cr, Cu above 1 ppm, Ni above 8.5 ppm, Zn 10 ppm [9,10]. Tolerance to high Zn was observed in R. mucilaginosa 1S1 and Trichosporon sp. 4S3 [11].

The results obtained showed that the response of the isolate to heavy metals depended on the metal tested and its concentration in the medium (**Fig.** 1). The results were comparable with those earlier reported in the literature [7,12,13]. However, it is known that, metals like zinc, Cu are essential to metabolism of cells at certain concentration, but at some elevated level, they tend to show toxicity. This toxicity has affected the process of biodegradation of natural products in their environments. The extent of the toxicity on both transient and indigenous microorganisms in the surrounding, are presented differently, depending on the isolate, site of isolation and other pollutants. Some isolates (tolerant) can tolerate elevated levels of metals, while others reacted negatively even at low metal ion concentration. Mechanisms like physical and physiological means have been reported to play significant role. For instance Lima de Silva [7] attributed Cr tolerance to Gram positive bacteria and Hg and Ag tolerance to Gram negative bacteria, due to structural characteristics of their cell wall. The pollution of the location of the isolation may also play role, though contrasting reports on whether microorganisms obtained from contaminated sites were more tolerant than those from natural environments are available [13].

A higher concentration of some metals promotes keratinase activity and feather degradations (**Table** 1). Metals like Pb, Ni, Ag, Cr, Cd, Zn showed lower activity in the presence of 1 ppm, but higher activity at 5 and 10 ppm, from where it decreases as the concentration increases. The mechanism for this behaviour of tolerance to many toxic heavy metals by this microorganism is unknown, however, further studies involving molecular aspects are needed to explore the mechanism. Keratinase activity and feather degradations were not seriously affected by the presence of EDTA and SDS in comparison with the control. A lower KA was recorded in the presence of Trixton 100 and Tween 20, with decrease in cell growth (**Table** 2). Inhibition of bacterial cells by Trixton 100 and Tween 20 have been reported in literature [14,15]. Some metals such as Ca, Zn, Ni only inhibited the growth of the organism, but have little effect on keratinase activity. The influence on keratinase activity varies between different type and concentration of metals. In most of the heavy metals, keratinase activities increase with increase in concentration up to 10 ppm, from where it started to decrease, except Hg and Co which decreases KA slightly right from 1 ppm upwards. Cr and Zn increase KA up to 15 ppm while Cu increases KA yield up to 20 ppm. $CaCl_2$ and Fe_2SO_4 have negative effect on growth, KA and feather degradation.

Feather degradation was enhanced in the presence of 5 ppm Pb, Ni, As, Cr and Cu. However, Hg-exposed cell showed a decrease in feather degradation after 1 ppm. About 90-100 % feather were degraded in 10 ppm in the presence of Ni, Ag, Cr, Zn and Co. Drastic reduction in FD at 20 ppm was observed in the presence of Pb, Hg, CD and AS, but only a slight decrease was seen in Ni and Co. A concentration of 10 ppm can therefore be considered maximum concentration of Pb, Ni, As and CD

tolerable by the bacterium while 15 ppm is maximum concentration for Ag, Cr and Co. This observation was supported by the growth of the bacteria, which showed that above 10 ppm and 15 ppm, the growth reduced in the presence of Pb, Ni, As, Cd and Ag, Cr, Co respectively. It is interesting to note that the following decreasing order of growth of the *Bacillus* at 1 ppm Co>As>Hg>Cr>Ag>Zn>Pd>Cd>Fe-Ca differs from that reported in other studies [13]. However, the known fact about some metals such as mercury, and to a lesser degree copper, which possess a high adsorptive and complexation capacity with media component, may be the additional reason for their inhibition of growth and keratinase activity.

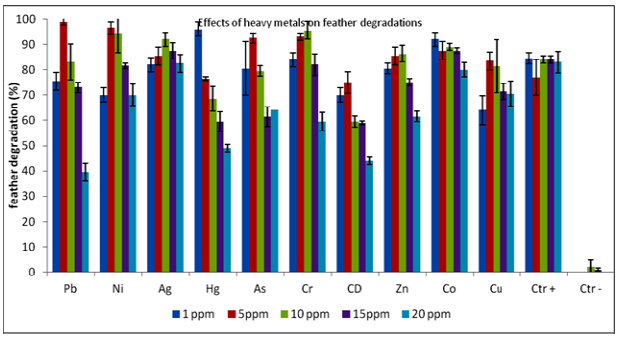

Fig. 1. Effects of heavy metals on feather degradation by strain khayat.

Table 1. Effect of metal ions on growth and keratonilytic acitivity of strain khayat.

	Concentrations of heavy metals									
	1 ppm		5 ppm		10 ppm		15 ppm		20 ppm	
Metals	KA (U/ml)	CFU/ml x 10^8	KA (U/ml)	CFU/ml 10^8	KA (U/ml)	CFU/ml x 10^8	KA (U/ml)	CFU/ml 10^8	KA (U/ml)	CFU/ml x 10^8
Pb	26.55	0.37	57.5	2.50	42.5	0.49	0.034	0.03	29.4	0.001
Ni	30.15	0.77	60.3	8.5	72.1	9.2	63.1	0.56	50.6	0.34
Ag	52.75	2.18	58.9	3.11	63.7	3.56	72.9	5.94	65.3	4.32
Hg	73.65	5.68	62.8	4.00	56.4	4.03	13.1	2.54	9.4	0.002
As	80.4	6.28	56.4	4.32	51.7	5.34	50.4	3.81	29.1	0.29
Cr	69.65	5.08	77.4	6.13	79.3	6.56	89.4	6.92	52.1	2.13
Cd	43.2	0.34	51.0	0.56	58.6	1.67	66.4	2.60	43.2	1.21
Zn	41.3	0.54	63.8	1.21	60.3	2.4	71.4	4.44	64.1	3.82
Co	89.4	8.5	82.8	6.93	66.2	5.21	56.2	4.84	44.3	2.87
Cu	43.5	0.45	55.4	0.41	69.4	3.29	72.5	2.18	83.1	2.15
$CaCl_2$ (0.1%)	0.00	0.001	0.0	0.002	0.0	0.00	0.0	0.00	0.0	0.00
Fe_2SO_4 (0.1%)	1.1	0.14	3.2	0.21	0	0.31	1.5	0.02	2.4	0.03
Ctr+	83.8	0.36	-	-	-	-	-	-	-	-
Ctr +2	0.00	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00
Ctr -	0.00	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00

Table 2. Effects of surfactants and detergents on keratinase production and feather degradations.

Substance	Final concentration	Keratinase activity (U/ml)	Bacterial growth (CFU/ml x 10^8)
Control		87.2	3.98
EDTA	10 mM	98.6	4.81
EDTA	5 mM	70	3.91
SDS	0.10%	66.0	4.11
SDS	0.50%	54.3	4.32
Triton x100	0.50%	32.25	1.61
Tween 20	0.10%	15.15	0.02
Ca	5 mM	65	
Fe	5 mM	0	
Pb	5 mM	73	
Ni	5 mM	62.9	
Cd	5 mM	43	
Zn	5 mM	72	
Cu	5 mM	48.9	

CONCLUSION

In conclusion, it was found that Bacillus sp. khayat was able to grow, produce keratinase enzymes and degrade feather in the presence of elevated concentration of different heavy metals, metallic ions, inhibitors and surfactants in a submerged fermentation. The extent of tolerance varies with type of metals, with Ni, Ag, Zn and Cu can be tolerated up to 20 ppm. In general, a concentration of 10 ppm of Pb, Ni, As and CD can be tolerated by the bacterium while 15 ppm is the maximum tolerable concentration for Ag, Cr and Co. The bacillus can therefore be used efficiently in biodegradation of wastes feather generated after use to remove heavy metals.

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