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Evaluation of Toxicity of Iron, Chromium and Cadmium on *Bacillus cereus* Growth

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Abstract

Objective
High concentration of iron and other trace elements could restrict bacterial growth and modify their metabolic pattern as well. However, this study aimed to find out the influence of iron, chromium, cadmium and synergism or antagonism between these elements on the growth of a gram positive bacterium.
Materials and Methods
In a series of experiments, *Bucillus cereus* was cultured in a nutrient broth which supplemented with Fe^{+2}, Fe^{+3}, Cr^{+3}, Cd^{+2} separately, or in combination with each other, at 37° C for 5 hours. Bacterial growth was measured every half – hour, using spectrophotometer.
Results
The results indicated that bacterial growth rate reduced in the presence of 0.5 mM/L concentration of Fe^{+2} or Fe^{+3}, in comparison with control and the growth of bacteria was inhibited by 1 mM/L concentration of iron. The results also revealed that Fe (III) as well as Fe (II) was toxic for bacteria. Chromium had partial inhibitory effects on the growth of bacteria and cadmium was very toxic. Cr^{+3} and Cd^{+} had antagonistic effect with iron on the growth of bacteria.
Conclusion
Data obtained here provide a potentially interesting conceptual advance in toxic effects of trace elements on pathogenic bacteria.

Keywords: *Bacillus cereus*, Cadmium, Chromium, Iron, Toxicity

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Introduction

Microorganisms exposed to various metal ions in their environment and interact with them, which is sometimes beneficial or detrimental depending on the chemical/ physical nature and oxidation state of the metal ion (1). Iron is an essential nutrient for living agents due to its noticeable activity in electron transport reactions in biological systems, but its insolubility and reactivity lead to problems of poor availability and toxicity, respectively (2). Due to insolubility of this element at physiological pH all living agents have involved to use iron transport systems and storage proteins. Bacteria elaborate and secrete high-affinity extracellular ferric chelators (siderophores) and many of them have ferrous iron transporter, to solubilise iron prior to transport (3- 5). They may posses other iron transport systems (6, 7). Furthermore, a study was demonstrated that extracellular iron is not the only source of available iron and many bacteria deposit intracellular reserves of this nutrient within iron storage proteins. These iron stores can then be used to enhance growth when external iron supplies are restricted (8). However, a relationship between concentration of iron and microbial infection was seen by many investigators using experimental studies on human and laboratory animal. These revealed that pathogens often use low environmental iron levels as a signal for the induction of virulence genes. For example, induction of exotoxins and proteases by many bacteria such as enterohaemorrhagic E. coli (Shiga-like toxin I) which affect the bacterial virulence (9, 10). On the other hand, high concentration of iron is extremely toxic and may implicate to enhance bactericide effects of antimicrobial agent or noxious substances (11, 12). Also, another study indicated anti bacterial effects of INPs (compounds belonging to acylated hydrazones of salicylaldehydes) is directly or indirectly related to iron (13).

Cadmium is a heavy metal pollutant, widely distributed in the environment. Like other heavy metals, it can induce multiple toxic effects on tissues and it affects immune response to bacterial pathogen (14). In other view, some microorganisms are able to accommodate to growth inhibiting-concentration of cadmium (15). Also, it may play an important role in iron metabolism in bacteria.

Chromium is a unique transition metal ion, which has been established to be biologically significant at all the levels of living organisms. Out of the two stable oxidation states of chromium, -VI and -III, trivalent chromium has been shown to play positive role in controlling carbohydrate and lipid metabolism (1, 16, 17). Recently, bacterial growth inhibition and decrease of pathogencity induced by chromium suggested by Yamini Shrivastava et al. (1). Also, role of various essential and trace elements such as iron, cadmium and chromium on antibacterial activity of various antibiotics are new subject to investigate effects of different metal complex of antibiotics against pathogenic bacteria in order to have new antimicrobial agents (18, 19).

However, to date very little progress has been made in combating the toxic potential of the microbe through chemical route (1). This work aimed to evaluate toxic effects of iron (Ferric and Ferrus), chromium and cadmium on *B. cereus* growth. It also focused on synergic or antagonistic effects of these elements on the bacterial growth and providing conceptual progress in toxic effects of trace elements against pathogenic bacteria.

Materials and Methods

Preparation of stock solutions

a) Fe^{+2} and Fe^{+3}: 556 mg of $FeSO_4$, $7H_2O$ and 541 mg of $FeCl_3$, $6H_2O$ were dissolved in 50 ml distilled water and then made to 100 ml to prepare 20mM/L of Fe(II) and (III), respectively.

b) Cd^{+2}: 402.8 mg of $CdCl_{2,}$ H_2O were dissolved in 50 ml distilled water and then made to 100 ml to prepare 20 mM/L of cadmium chloride.

c) Cr^{+3}: 533 mg of $CrCl_{3,}$ $6H_2O$ were dissolved in 50 ml distilled water and then made to 100 ml to prepare 20 mM/L of chromium chloride.

Preparation of bacterial culture medium

Nutrient agar (N.A.) and nutrient broth (N.B.) (Merk) were prepared as manufacturer recommendation. All culture media and stock solution of trace elements were steriled with autoclave at 121º C, 15 pound/in^2 pressure for 15 minutes. This work was carried out at pH = 7.

Bacteria and culture medium

Bacillus cereus ATCC (American Type Culture Collection) 11778, NCTC (National Collection of Type Culture) 1032 was used and cultivated on N.A. using streak plate method. The plate was incubated for 24 hours at 37ºC and then stored in the fridge.

The effect of different metal ions on the growth of B. cereus

One colony of *B. cereus* was added to 100 ml N.B. and cultivated for 14 hours at 37 ºC before performing the main experiments. 1, 5 and 10 ml of iron or chromium stock solution was added to 199, 195 and 190 ml of N.B. and mixed well in order to have 0.1, 0.5 and 1mM concentrations of these elements. 0.1, 0.5 and 1 ml of cadmium stock solution was added to 199.9, 199.5 and 199 ml of N.B and mixed well in order to have 0.01, 0.05 and 0.1 mM/l concentrations of cadmium. A sample of 14 hours bacterial culture, 5ml, was taken out and used as blank. Then, three ml of the 14 hours bacterial culture was added to each flask, mixed well and incubated at 37º C on a shaker. Bacterial growth was measured every half-hour using spectrophotometer (Bausch) at 520 nm.

The combined effect of metal ions on the growth of B. cereus

To examine the synergic or antagonistic effects of these elements various concentrations of them were used together. For example, 10 ml of iron stock solution and 0.1 ml of cadmium stock solution were added to 189.9 ml N.B. to study a combination of 1 mM/L of iron (II, III) and 0.01 mM/L of cadmium after 3.5 hours incubation at 37ºC on a shaker, using spectrophotometer (Bausch) at 520 nm.

The experiment repeated at least three times, and also the bacteria were cultured in N.B. without adding any trace elements solution, which used as control. Data were analyzed using ANOVA and post test Tukey by SPSS software.

Results

Results obtained from cultivation of *B. cereus* at the presence of various concentrations of iron are shown in Figure 1.

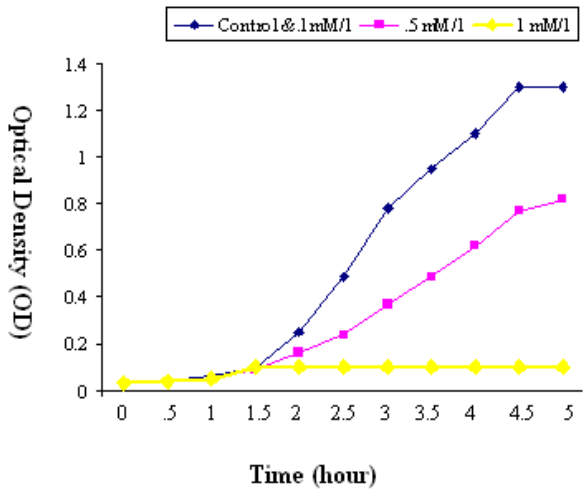

Figure 1. Study of toxic effects of iron (II) and (III) on B. cereus growth using 0.1, 0.5 and 1 mM/L of Ferro sulphate and ferric chloride. Bacterial growth was measured every half-hour.

Bacteria grew in the control sample and at the presence of 0.1 mM/L concentration of Fe (II &III) (OD=0.1; p<1; 95% CI=.9999 and .9999) while the bacterial growth decreased to 48.4% using 0.5 mM/L concentration of iron ($OD_{0.5mM/lFe^{+2/3}}$ = 0.49 Vs $OD_{control}$ =.95; p=0.00; 95% CI=.3568 and .5565). The bacteria did not grow at the presence of 1mM/L concentration of Fe (II &III) (OD=0.1; p=0.00; 95% CI=.7501 and .9599).

Bacterial growth was also measured after treatment with cadmium chloride and combination of cadmium and iron. Results obtained are shown in Figure 2. The findings demonstrated that cadmium has extreme inhibitory effects on bacterial growth.

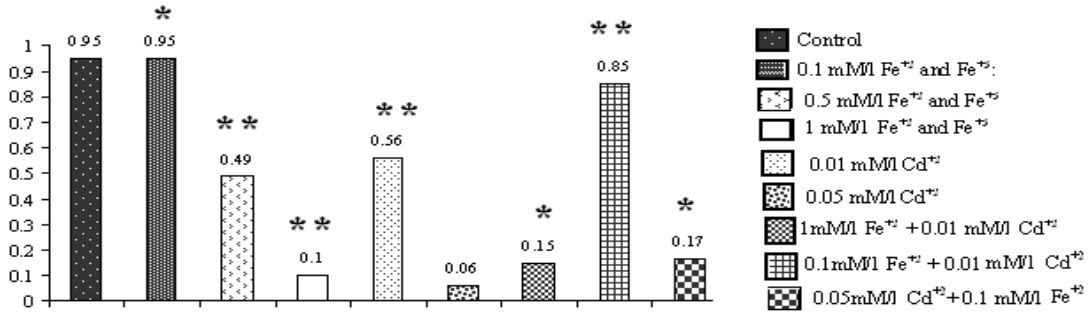

Figure 2. Effects of various concentrations of Fe^{+2}, $Fe^{+3,}$ Cd^{+2} and interaction between iron and cadmium on B. cereus growth rate after 3.5 hours incubation at 37ºC. Each data point represents the mean of three independent experiments (*, p<1 and;**, p=0.00).

Optical density was 0.56 when bacteria were cultured at the presence of 0.01 mM/L concentrations of this element while the OD of control was 0.95 (p=0.00; 95% CI=-.4710 and -.3090). The bacteria did not grow at the presence of 0.05 and 0.1mM/L concentration of cadmium (p=0.00; 95% CI=-.9676 and -.8057). It has also demonstrated that inhibitory effect of cadmium on bacterial growth was significantly removed by iron or reverse. For example, bacterial growth was increased from 41.1% to 89.5% at the presence of 0.1mM/L of Fe^{2+} & 0.01 mM/L of Cd^{2+} ($OD_{control}$=0.95, $OD_{.01mM/.lCd^{2+}}$=.56 and $OD_{0.01\ mM/L\ of\ Cd^{2+}}$ & 0.1 $mM/l\ Fe^{2+}$=0.85) (p=0.00; 95% CI=.1517 and .4083).

Inhibition of bacterial growth, which caused by cadmium was less removed by iron (III) in comparison with iron (II). Bacterial growth increased approximately 6.3% at the presence of combination of 0.01mM/L Cd^{2+} and 0.1 mM/L Fe^{3+}, compared with cadmium alone ($OD_{control}$=0.95, $OD_{.01mM/.l\ Cd^{2+}}$ & $.1mM/l\ Fe^{3+}$=.65; p<0.08; 95% CI=-.0056 and .1856).

Results obtained from supplementation of the bacterial culture medium with Cr^{3+} and in combination with Fe^{2+} or Fe^{3+} are shown in Figure 3.

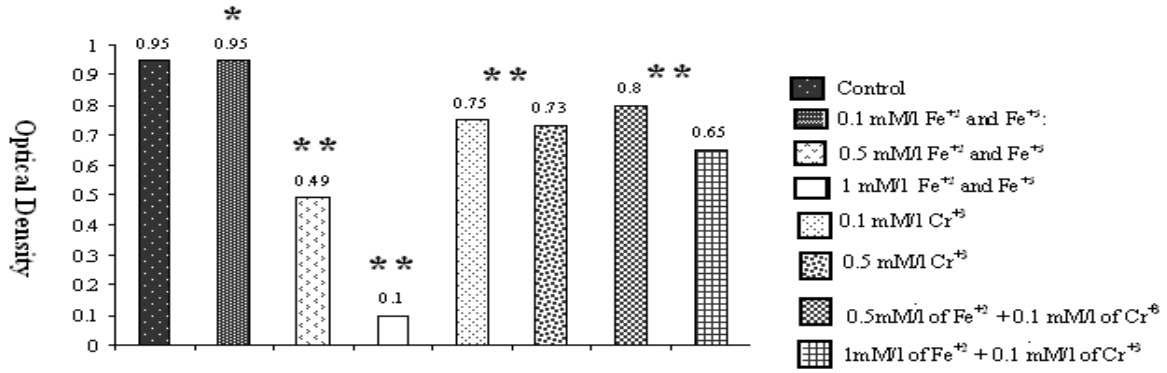

Figure 3. Effects of various concentrations of Fe^{+2}, $Fe^{+3,}$ Cr^{+3} and interaction between iron and chromium on *B. cereus* growth rate after 3.5 hours incubation at 37º C. Each data point represents the mean of three independent experiments (*, p<1 and;**, p=0.00).

These finding revealed that Cr^{3+} has partial inhibitory effects on the growth of bacteria. Bacterial growth was reduced to 79% and 76.7% using 0.1 and 0.5 mM/L concentration of chromium, respectively ($OD_{.1mM/l}$=0.75, Vs. $OD_{control}$=0.95; p=0.000; 95% CI=-.2987 and -.1347; $OD_{.5mM/l}$=0.73; p=0.000; 95% CI=-.3187 and .1547). Results from cultivation of *B. cereus* at the presence of combination of chromium and iron showed that, the inhibitory effect of iron was partially removed by chromium. For example, the growth of bacteria was significantly increased from 52% to 85% (OD= 0.8; p=0.000; 95% CI=-.3920 and -.2280) and from 10.5% to 68% (OD=0.65; p=0.000; 95% CI=-.6320 and -.4680) when 0.5 mM/L Fe^{2+} & 0.1 mM/L Cr^{3+} and 1mM/L Fe^{2+} & 0.1 mM/L Cr^{3+} were respectively used. The bacterial growth was

increased to 62% using 0.5 mM/l Fe^{3+} & 0.1 mM/l Cr^{3+} (OD= 0.62) (p<0.000; 95% CI=.2280 and 0.3920) (95% CI=-.4680 and .6320).

Figure 4 shows that results for cultivation of bacteria in the presence of combination of cadmium and chromium. These findings indicated that the inhibitory effect of cadmium on *B. cereus* growth was partially removed by chromium. Growth of the bacteria increased to 51% when 0.01 mM/L Cd^{2+} & 0.5 mM/L Cr^{3+} was used (OD=0.53; p<0.06; 95% CI=-.0316 and -.0983). The results also, showed that bacterial growth was significantly increased to 32% in the presence of 0.05mM/L Cd^{2+} & 0.1 mM/L Cr^{3+} (OD=0.3, p=0.00; 95% CI=--.3016 and -.1717), in comparison with cadmium alone (OD=0.06).

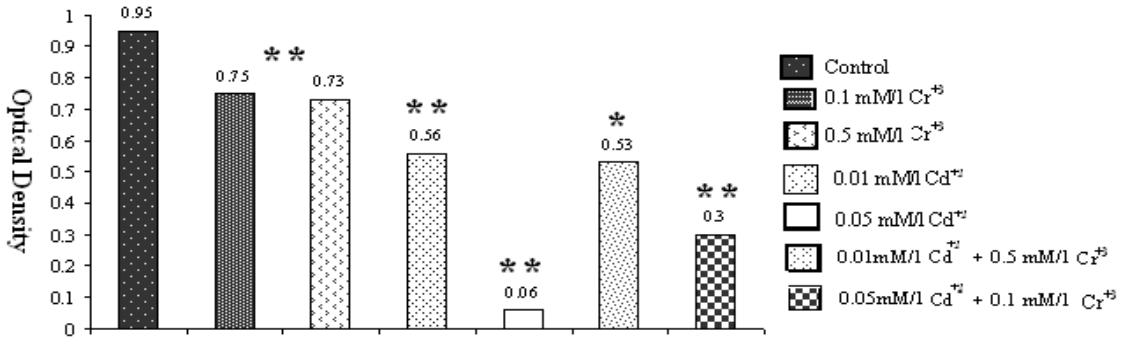

Figure 4. Effects of various concentrations of $Cr^{+3,}$ Cd^{+2} and interaction between chromium and cadmium on *B. cereus* growth rate after 3.5 hours incubation at 37ºC. Each data point represents the mean of three independent experiments (*, p<0.06;**, p=0.00).

Discussion

The present work tries to understand the effect of iron, chromium, cadmium and combination of these elements on *B. cereus* growth. Results obtained here demonstrated that iron has inhibitory effects at high concentration and Fe^{3+} as well as Fe^{2+} is toxic. These findings are in agreement with the results from other studies which indicated that Fe (III) is a toxic substance. For example, Chamnongpol and co-workers study showed that Fe (III) has toxic effects on *Salmonella enterica*, *Escherichia coli* and *Klebsiella pneumoniae*, and exerted its microbicidal activity even under anaerobic conditions (12). This study also, indicates that Fe (III) microbicidal activity is oxygen independent and different from Fe (II). Fe^{+3} acts on an extracytoplasmic target of gram negative bacteria (12) or acts like certain antimicrobial peptides as suggested by Epand, Vogel and Vaara (20, 21).

But, these results are not in line with others which have largely been considered that Fe (III) is a non- cytotoxic substance (22, 23). However, based on the best knowledge, mechanism of toxic effects of iron on gram positive bacteria is not clearly understood and seems further study is necessary .

Findings from the effects of cadmium on *B. cereus* demonstrated that this element has extreme inhibitory effects on bacterial growth. These results are on line with results obtained from many studies which indicated that cadmium is very toxic for living agents (13, 24, 25).

The results from the effect of combination of iron and cadmium on *B. cereus* growth showed that they have antagonistic effects on the growth of bacteria and the antagonistic effects of cadmium with iron (II) were more in comparison with iron (III). This finding is in agreement with results obtained from Stern and co-workers study which indicated that the minimal inhibitory concentration (MIC) of cadmium for four *Campylobacter jejuni* strains reduced significantly at the presence of iron

(II). Moreover, the numbers of colonies were greater when culture medium supplemented with Fe^{2+} (26).

Finding from the effect of chromium alone or in combination with iron and cadmium on *B. cereus* growth revealed that Cr^{3+} has partial inhibitory effects on the growth of bacteria and could partially remove the toxic effects of iron and cadmium. It seems that the chromium salts have antagonistic effects with iron and cadmium. Results from a study showed that chromium could chelate iron in the culture medium and confirm the capacity of a staphylococcus to resist the inhibiting action of transferring (27).

In conclusion, this work demonstrated that high level of iron plays an important role to inhibit the growth of the bacteria and Fe (III) as well as Fe (II) has an inhibitory effect. It also, revealed that other trace elements such as chromium and cadmium are toxic and could interact with iron metabolism in bacteria. The toxic effects of the trace elements could partially be removed in combination with other elements. The toxic effects of essential and trace elements could be another view against pathogenic microorganisms, particularly in complex with antibacterial activity of various antibiotics as suggested by Sultana et al. (18). Finally, data obtained here provided conceptual advance in toxic effects of trace elements against pathogenic bacteria and open another view to apply further studies on the mechanism of inhibition or synergy with antibiotics.

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