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STIMULATORY AND INHIBITORY EFFECTS OF HEAVY METAL DIVALENT IONS ON *ENTEROCOCCUS HIRAE* CELL GROWTH AND REDOX POTENTIAL CHANGES

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Enterococcus hirae grows well under anaerobic conditions at alkaline pH (pH 8.0) fermenting glucose to produce acids. In the current paper we have shown that various divalent heavy metal ions have different effects on *E. hirae* cell growth. It was revealed that copper (II) ions (within the range of 0.05 mM 1 mM) inhibit bacterial growth by decreasing specific growth rate and increasing lag phase duration while manganese (II) ions have opposite effects on bacterial growth. These effects have a concentration-dependent manner. Copper (II) and manganese (II) ions also affect the changes in E_h values during bacterial growth. Moreover, as in the case of cell growth, these ions have opposite effects on redox potential changes. At the same time cobalt (II) ions has shown no influence either on *E. hirae* growth, or on E_h changes during bacterial growth. Such results indicate that specific action mechanisms can be evaluated here. It is suggested that heavy metal ions can affect directly bacterial membrane or these effects can be mediated through oxidation-reduction potential.

Enterococcus hirae – bacterial growth – Mn^{2+} – Co^{2+} – Cu^{2+}.

Enterococcus hirae անաերոբ բակտերիաները ունակ են աճել հիմնային pH-ի պայմաններում՝ խմորելով գլյուկոզը, ինչի արդյունքում առաջանում են թթուներ: Ներկայացված աշխատանքում ցույց է տրվել, որ տարբեր ծանր մետաղների երկվալենտ իոններ ազդում են այս բակտերիաների աճման վրա: Ցույց է տրվել, որ 0.05-1 մՄ պղնձի (II) իոնների ավելացումը աճման միջավայր հանգեցնում է *E. hirae* աճման տեսակարար արագության նվազեցման և լագ փուլի տևողության երկարացման:Միևնույն կոնցենտրացիայով մանգանի (II) իոնների ավելացումն ունի հակառակ՝ խթանիչ ազդեցություն բակտերիաների աճման վրա: Պղնձի և մանգանի երկվալենտ իոններն ազդում են նաև աճման ընթացքում օքսիդավերականգնողական պոտենցիալի փոփոխության վրա, ընդ որում այս դեպքում ևս հաստատվել է, որ երկու իոններն ունեն հակառակ ազդեցություն: Միևնույն ժամանակ կոբալտի երկվալենտ իոններն ընդհանրապես չեն ազդում բակտերիաների աճման և օքսիդավերականգնողական պոտենցիալի փոփոխության վրա: Այս արդյունքները վկայում են այն մասին, որ տեղ ունեն ազդեցության յուրահատուկ մեխանիզմներ: Հնարավոր է, որ ծանր մետաղներն ազդում են անմիջապես բակտերիալ թաղանթների վրա կամ այսպիսի ազդեցությունը միջնորդված է օքսիդավերականգնողական պոտենցիալի միջոցով:

Enterococcus hirae – բակտերիալ աճ – Mn^{2+} – Co^{2+} – Cu^{2+}.

Анаэробные бактерии *Enterococcus hirae* хорошо растут в щелочной среде, в процессе чего происходит брожение глюкозы и выделение кислот. В нашей работе представлены данные о влиянии разных двухвалентных ионов тяжелых металлов на рост этих бактерий. Было выявлено, что ионы меди ингибируют рост бактерий, удлиняя продолжительность лаг фазы и уменьшая удельную скорость роста. Та же концентрация ионов марганца, наоборот, ускоряет рост. Эти ионы также имеют обратное воздействие и на изменение редокс потенциала во время роста

бактерий. В то же время двухвалентные ионы кобальта никак не влияют и на рост бактерий, и на изменение редокс потенциала. Полученные данные свидетельствуют о том, что имеют место специфические механизмы воздействия. Возможно прямое влияние этих ионов на бактериальную мембрану или такое воздействие может быть опосредовано окислительно-восстановительным потенциалом.

Enterococcus hirae – рост бактерий – Mn^{2+} – Co^{2+} – Cu^{2+}.

Almost all living organisms, including bacteria, require small concentrations of such heavy metals, as iron, cobalt, copper, manganese, zinc and iron. Such elements are called “essential” and it is found that they induce bacterial growth [1]. Such heavy metal ions are involved in many processes, such as catalysis of biochemical reactions, stabilization of proteins and regulation of gene expression [2]. At the some high concentrations of either “essential” or “non-essential” heavy metals the biological activity of the microorganisms is decreased which can lead to cells death [3]. As heavy metals are widely spread in environment due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals such as efflux or reduction of metal ions. The tolerance of bacteria to heavy metals has been proposed as an indicator of potential toxicity of heavy metals to other forms of biota [4]. Therefore, there is a dramatic increase in the interest on studying the interactions of heavy metals with microorganisms.

Enterococcus hirae growth in anaerobic conditions at alkaline pH (pH 8.0) is attended by changes in pH, proton-motive force and environment oxidation-reduction potential (E_h) [5]. As the latter is a key factor for bacterial growth [6], several oxidizers and reducers that affect E_h can also regulate bacterial growth. Reducer cobalt (II) and manganese (II) and oxidizer copper (II) heavy metal ions are very abundant elements in surroundings. All these ions in small concentrations are required for cells as they are involved in various enzymes. Mn is used by bacteria as electron acceptor in respiration process [7]. It is found in nitrogenase enzyme, which participates in molecular nitrogen reducing, and in superoxide dismutase. Cobalt is an important co-factor in vitamin B12-depended enzymes and in the nitrile hydratases [8]. Cu^{2+} is a compound of superoxide dismutase and lysile oxidase. At the same time high concentrations of these heavy metals are toxic for cells [9] and it is very interesting to understand the action mechanisms.

In the current paper the effects of Co (II), Cu (II) and Mn (II) ions on *E. hirae* growth were observed. We have shown that Cu (II) and Mn (II) ions within the concentration range 0.01-1 mM have opposite effects on bacterial lag phase duration, specific growth rate and redox potential changes during bacterial growth. Meanwhile, Co (II) ions have no visible effects on *E. hirae* cell growth.

Materials and methods. This study was performed with wild-type *E. hirae strain* ATCC9790 [5], which has been kindly supplied by Prof. H. Kobayashi (Graduate school of Pharmaceutical Sciences, Chiba University, Chiba 263, Japan).

Bacteria were grown under anaerobic conditions at 37^{o}C in 0.2 % glucose containing medium (pH 8; 1 % tryptone, 0.5 % yeast extract, 1 % K_2HPO_4) as described earlier [5]. The pH of the medium was measured with pH-selective electrode (HJ1131B, Hanna Instruments, Portugal) and was adjusted by 0.1 M NaOH or HCl. Redox potential of the medium was measured by platinum electrode (EPB-1, Electrometer Equipment State Enterprise, Gomel, Belarus; GDEEE, Hanna Instruments, Portugal) as described elsewhere [10].

The rate of bacterial growth was estimated by measuring the changes in optical density (OD) of bacterial suspension using a Spectro UV-vis Auto spectrophotometer (Labomed, USA) at a wave length of 600 nm. Bacterial growth was monitored every hour during 8 h and at 24 h. Various concentrations

(0.01 mM, 0.05 mM, 0.1 and 1 mM) of $CoCl_2$, $CuCl_2$ and $MnCl_2$ were used throughout the study. The latent (lag) phase duration was determined as described previously [11]. The specific growth rate was calculated by dividing 0.693 (lg2=0.693) by the doubling time of OD in the ranges where changes in the logarithm of OD depended on time in a linear manner.

Results and Discussion. After addition of Cu^{2+} (0.1-1 mM) in the growth medium changes in optical density values were observed. As is it shown in Fig. 1 (as all effects have concentration-dependent manner, to be more distinct only the highest concentration of all heavy metals are shown in all figures), copper (II) ions notably suppressed bacterial growth as the highest values of OD are with control sample where no copper ions were present. At the same time Mn^{2+} ions within the same concentration range increase bacterial growth as after 6 hours of growth OD values with Mn^{2+} are higher than even in control sample. In the case of Co^{2+} no visible changes were observed (fig. 1). All these effects have concentration-dependent manner. These results show that all these divalent cations have different effects which indicate that specific action mechanisms can be occurred.

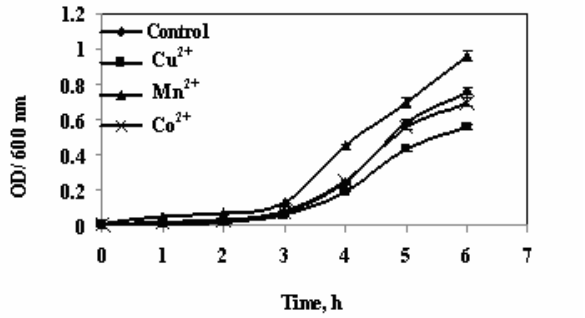

Fig.1. Changes in optical density during *E. hirae* ATCC9790 growth in the presence of 1 mM Cu^{2+}, Mn^{2+} and Co^{2+}.

We have shown that the addition of Mn^{2+} in bacterial growth medium within the range of 0.05 to 1 mM led to the decrease in lag phase duration and the increase in the specific growth rate (fig. 2a, b). In contrast, Cu^{2+} had opposite effects by suppressing bacterial growth (fig. 2a, b). For both $Mn^{2+}$and Cu^{2+} ions high concentration (1 mM) had more noticeable influence. Cobalt (II) ions had no visible effects on lag phase duration and specific growth rate. E_h changes were also observed during *E. hirae* growth with and without heavy metal ions. As it is shown in fig. 3, oxidation-reduction potential drops from positive values to negative ones after 8 hours of growth as culture passed to the stationary phase. The E_h drop gives evidence of many reduction processes which take place during bacterial growth [12]. After 24 hours E_h markedly increased, but did not reach the initial values (not shown). Then, changes in E_h during ATCC9790 growth were also observed in the presence of heavy metal ions (fig. 3). Mn^{2+} distinctly dropped the Eh value in comparison with control sample. Stronger effects are observed with high concentration (1 mM) when E_h dropped up to -300±10 mV compared with that of control (-200±15 mV) (fig. 3). Cu^{2+} ions had contrary effects on E_h in a concentration-dependent manner. In the presence of these ions E_h lowered down to -100±8 mV only. For comparison E_h changes during *E. hirae* growth were also observed in the presence of other divalent heavy metal ions, cobalt (II). As it is shown in fig. 3, cobalt (II) ions within the same concentration range have no significant influence on E_h value.

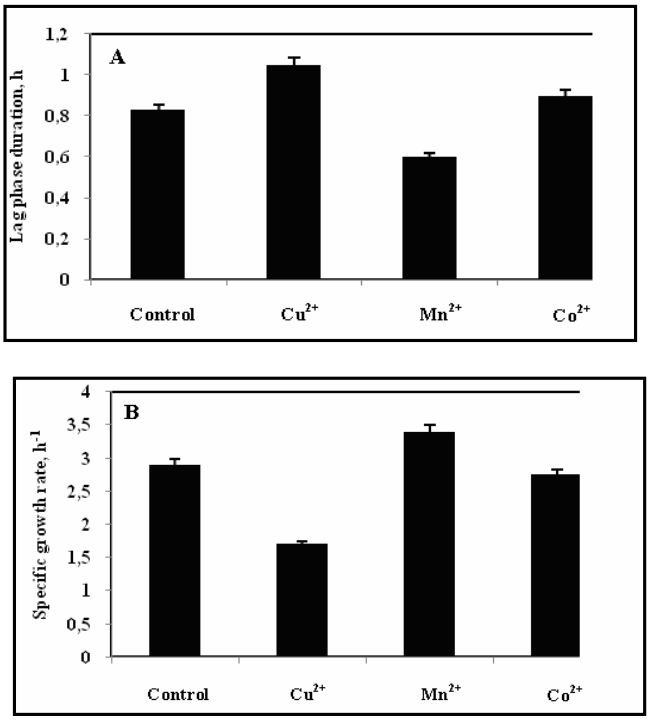

Fig.2. Effects of 1 mM Cu2+, Mn2+ and Co2+ on E. hirae ATCC9790 cell growth. A.Lag phase duration, B. Specific growth rate. Control was bacterial growth in the medium without heavy metals ions.

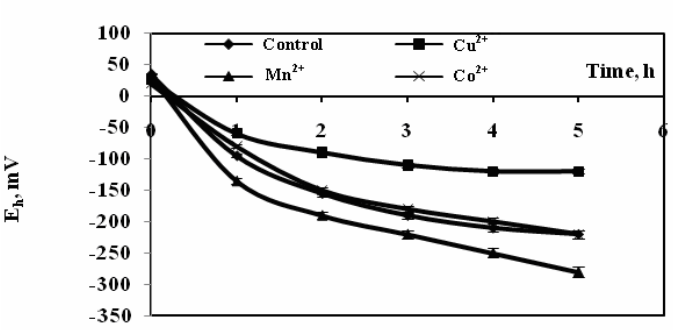

Fig.3. Changes in redox potential during *E. hirae* ATCC9790 growth in the presence of 1 mM Cu^{2+}, Mn^{2+} and Co^{2+}.

In our laboratory it was shown that a reducer of SH-groups, DL- dithiothreitol, stimulated E. hirae cell growth and enhanced acidification of the medium but an oxidant, ferricyanide, suppressed bacterial growth and decelerates oxidation of the medium [13]. Mn^{2+} and Cu^{2+} are reducers and oxidizers, respectively, so just the opposite effects of these ions could be expected. And our results are in accordance with this data. But at the same time Co^{2+} is a reducer too, but these ions have no effects on bacterial growth. This pointed out that different action mechanisms could be evaluated.

We have shown that even low concentrations of Cu^{2+} and Mn^{2+} affect *E. hirae* growth. This also causes changes in E_h drop during the growth. These effects have a concentration-dependent manner and are quite different for these ions. Such influence can be explained by direct effects of these ions on bacterial membrane or can be mediated through E_h. The findings are novel and have interest to understand the mechanisms of these effects and to regulate bacterial growth and activity during oxidative stress or in the environment which contains heavy metals.

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