

DETERMINATION OF HEAVY METAL SUSCEPTIBILITIES OF *VIBRIO HARVEYI* STRAINS BY USING 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE (TTC)

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Abstract

In the present work, minimum inhibitory concentration of 11 heavy metals (Zn, Cu, Pb, Cd, Co, Mn, Ni, Ag, Hg, Cr and As) against *Vibrio harveyi* TEM05 and TEMS1 were determined via the dehydrogenase activity using 96-well microplates. Different concentrations of 11 heavy metals from 0.78 µg/ml to 102.400 µg/ml were studied. The growth indicator 2,3,5- tri phenyltetrazolium chloride (TTC) was utilized to indicate bacterial growth/growth inhibition. Results showed that TEMS1 had the highest MIC values for As and Pb (102.400 µg/ml) and *V. harveyi* TEM05 had the highest MIC values for Pb (>102.400 µg/ml). Therefore, these luminous bacteria are very resistant against As and Pb. Besides, according to the results of MIC susceptibility tests, heavy metals the most effective against both isolates are Cu, Cd and Hg (25 µg/ml).

Keywords : *Aegean Sea, Bacteria, Coastal Waters, Metals.*

Introduction

Heavy metals generally exert an inhibitory action on microorganisms by blocking essential functional groups, displacing essential metal ions and modifying the active conformations of biological molecules. However, at relatively low concentrations some metals are essential for microorganisms (e.g. Co, Cu, Zn, Ni) since they provide vital co-factor for metallo-proteins and enzymes [1]. Heavy metals are common marine pollutants derived from industrial and sewage treatment discharges and anti-fouling paints [2]. Luminous bacteria are the most abundant and widely distributed light emitting organisms and are found in marine, freshwater and terrestrial environments. These bacteria are all Gram-negative, motile rods and can function as facultative anaerobes. To date less than 1% of the known species have been studied in detail and most information concerns the marine bacteria of three genera: *Photobacterium*, *Vibrio* and *Shewanella*. Bioluminescent bacteria emit light when they are in an optimal environment [3]. For the first time, *Vibrio harveyi* TEM05 and TEMS1 were isolated from the gut of *Holothuria tubulosa* and coastal seawater of Izmir Bay (Turkey), respectively. The susceptibility of these isolates to heavy metals was determined using the growth indicator 2,3,5-triphenyltetrazolium chloride (TTC) and the metals zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd), cobalt (Co), manganese (Mn), nickel (Ni), silver (Ag), mercury (Hg), chromium (Cr) and arsenic (As).

Material and Methods

Microorganisms

Vibrio harveyi TEM05 and TEMS1 was isolated from the gut of *Holothuria tubulosa* and coastal seawater of Izmir Bay, Turkey. The strains were identified by morphological and biochemical characteristics and by 16S rDNA sequencing. *V. harveyi* TEM05 and TEMS1 has been deposited in the GenBank database under accession number DQ842241 and DQ842240, respectively [4]. All experiments were performed on Nutrient Broth Media (NB) supplemented with 2% (w/v) NaCl.

Determination of minimum inhibitory concentration (MIC)

All *V. harveyi* isolates were grown in 20 ml of NB (with 2% NaCl) with shaking at 150 rpm for 17 h (20°C). Minimum inhibitory concentrations of eleven heavy metals against *V. harveyi* TEM05 and TEMS1 were determined using the growth indicator TTC. Different concentrations of heavy metals from 0.78 µg/ml to 102.400 µg/ml were studied. To dilute heavy metals, appropriate amounts of stock solutions were added to steril NB with 2% NaCl in microplate wells. To each well, 125 µl of bacterial suspension with a turbidity equivalent to 0.5 McFarland standard was added and then 125 µl of different concentrations of heavy metals were transferred into a microplate well. The microplates were incubated at 20°C overnight. 20 µl of steril TTC was put in each well and then the microplates were incubated at 20°C again. After overnight incubation, the minimum inhibitory concentration (MIC) of heavy metals against the isolates was determined.

Results and Discussion

The tetrazolium salt TTC can be used as an artificial electron acceptor to detect dehydrogenase activity and thus metabolically active bacteria. The detection of activity is based on the reduction of the water-soluble and col-

orless tetrazolium salts to colored crystals of the water-insoluble formazan products. TTC is reduced to a red insoluble formazan [5]. Therefore, the wells with red color were evaluated as positive and the highest concentration of a specific heavy metal without color was considered MIC. The heavy metals which are the most effective against both isolates are Cu, Cd and Hg (25 µg/ml). *V. harveyi* TEMS1 has the highest MIC value for Pb and As (102.400 µg/ml). For *V. harveyi* TEM05, MIC of Pb and As was achieved >102.400 µg/ml and 102.400 µg/ml, respectively.

Our data showed that *V. harveyi* TEM05 and TEMS1 is very resistant against Pb and As and Pb, respectively. Otherwise the strains are very sensitive against Cu, Cd and Hg. MIC values of Mn, Ni, Zn, Pb and Cr against *V. harveyi* TEM05 were different from that of *V. harveyi* TEMS1. Thus, there are isolate-specific differences in the metal sensitivity. Rahman et al. (2004) determined antimicrobial susceptibilities of gram-negative fermentative bacteria by using redox indicator TTC in a microplate [6]. But this kind of study with bioluminescent bacteria, *V. harveyi*, is performed for the first time. With data obtained from this study, our strains can be employed to determine heavy metal contents of seawater or other material as indicator organisms.

References

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