

Assessment of multi-metal resistant bacteria from Periyar river, Southern India

S. Prasanth^{1*} and V. Mahesh²

¹Department of Microbiology, Maruthu Pandiyar College, Thanjavur, Tamil Nadu – 613 403, India

²Department of Botany, MR Govt Arts College, Mannargudi, Tamil Nadu - 614 001 India.

*Correspondence Info:

S. Prasanth
Department of Microbiology,
Maruthu Pandiyar College, Thanjavur,
Tamil Nadu – 613 403, India
E-mail: prasanthkannadi@gmail.com

Abstract

A total of thirty bacterial strains were isolated from the water and sediment samples of Periyar River during premonsoon 2015 for multi-metal resistant study. In this study, the bacterial isolates were challenged against three different concentrations (10, 100 and 200 mM) of copper and chromium metal salt solution by using of plate diffusion method. Most of the isolates from Periyar River were resistant to minimum concentration of the both metal solutions. Nearly, 93.3 % were resistant to copper solution whereas 90.0 % were resistant to chromium solution. In 200 mM of Cu, 46.7 % of the populations showed growth rates between 71-80%, whereas 3.3 % of the populations were observed with a 51-60 % growth rate. At 200 mM Cr, 6.7 % of the populations showed 51-60 % growth rate. A growth rate between 61-70 % was observed for 13.3 % of the bacterial populations at 200 mM of Cr, whereas no population was growing at a growth rate of 0–50 % with 200 mM of Cr. The results indicated that the river received various pollution sources throughout the river-line. The sensitivity nature of the bacterial isolates against metal solutions were varied depend on the concentrations of the metal solutions. Hence, the river needed throughout impoundment for maintaining sanitation.

Keywords: Periyar river, Multi-metal resistance, Copper, Plate diffusion method.

1. Introduction

Heavy metal pollution has drawn increasing attention worldwide owing to a dramatic increase in anthropogenic heavy metals in ecosystems through air, water and soil [1]. Especially, heavy metals comprise the major elemental composition of sea. In estuaries, river water velocity decreases, relative to the river channel areas, as fresh water mixes with sea water. This process results in the deposition of sediments with associated heavy metals [2]. Moreover, the historical inputs of these metals are still a primary problem for fragile ecosystems and coastal areas, particularly in economically-challenged countries [3]. One of the effects is an increase in heavy metal solubility, which results in the accumulation of these toxic elements in the environment.

As a result of increasing industrialization, water pollution due to heavy metals has posed serious problems in many aquatic systems since the bacteria can acquire resistance after exposure to these agents [4]. Marine micro and macro organisms contains numerous bioactive compounds [5][6] and those organisms were involved in the bioaccumulation

process. Marine bacteria develop its resistance behavior to adopt themselves to extreme environments including toxic heavy metals. It has been suggested that the metal resistance may not be a fortuitous phenomenon and bacterial resistance against heavy metals appears to be directly related to the presence of these elements as environmental pollutants [7]. As a consequence, heavy metal contaminated sites become inhospitable and only those microorganisms which are able to tolerate the high concentration of heavy metals can survive. Also they need some heavy metals for their metabolic activities which are received from the marine environments through their specially designed heavy metals transporter proteins/ channels. Their sustainable growth in heavy metal environment depends on their tolerance to heavy metal. The optimum concentration of heavy metal is a critical factor that influences the survival of microorganisms.

Bacteria have developed a variety of resistance mechanisms to counteract heavy metal stress. These mechanisms include the formation and sequestration of heavy metals in complexes, the reduction of a metal to a less toxic species and direct

efflux of a metal out of the cell [8][9]. Resistance to toxic heavy metals and their accumulation by bacteria is a widespread phenomenon that can be exploited for the improvement of the environment. Metal-resistant bacteria have developed very efficient and varying mechanisms for tolerating high levels of toxic metals and thus hold potential for controlling heavy metal pollution [10]. The aim of this study was to determine the level of pollution indicators and physiochemical parameters from tropical perennial river water samples and also find their sources of pollutions.

2. Materials and methods

2.1 Study area

Periyar river is the longest river and the river with the largest discharge potential in the Indian state of Kerala. It is one of the few perennial rivers in the region and provides drinking water for several major town. The Periyar is of utmost significance to the economy of Kerala. The Periyar basin spreads over an area of 5,398 square km (2,084 sq mi), most of it in central Kerala. It lies between latitudes 9°15'30"N and 10°21'00"N and longitudes 76°08'38"E and 77°24'32"E. The lower reaches of the Periyar are heavily polluted. Industries in the Eloor industrial zone discharge waste into the river. Greenpeace India describes the lower Periyar as "a cesspool of toxins, which have alarming levels of deadly poisons like DDT, endosulfan, hexa and trivalent chromium, lead, cyanide, BHC". Several studies have pointed out that the riverbed has deposits of heavy metals like lead, cadmium, mercury, chromium, nickel, cobalt and zinc and the ecosystem of the river has many dead zones. Some of the major recommendations are ensuring zero effluent discharge from the industrial units in the Eloor-Edayar stretch and zero emission from companies. Pollution of the river and surrounding wetlands has almost wiped out traditional occupations, including fishing and farming. Illegal sand mining from the Periyar is another major environmental issue. Studies have pointed out that the quantity of sand being mined from the Periyar is at least 30 times the actual quantity that can be taken out without causing damage to the river's environmental system. In the Periyar river basin, land sand mining is widespread in the plateau region in the highlands. Mining of sands from these areas impose severe environmental problems to the river basin environment.

2.2 Sampling

The water samples were collected from nine different places within the river during premonsoon 2015 for physiochemical and bacteriological analysis. The sampling sites were Kuttampuzha (S1), Boothathankettu (S2), Kodanad (S3), Kalady (S4), Marampally (S5), Aluva (S6), Pathalam (S7),

Varapuzha (S8) and Pizhala (S9). The sampling sites were choosed based on the massive discharges of pollutants into river. The river water samples were collected from 0 to 20 cm below the surface [11][12]. The 2000 mL of water samples were collected with a 2500 mL sterile container in each location and stored in ice box at 4 °C. The samples were transported into laboratory and processed within 12 hrs [13].

2.3 Multi-metal bacteriological analysis

The test cultures were isolated from the water and sediment sample of Periyar river, Kerala, India during July 2015. The serial dilution and pure culture techniques were used for isolation of bacterial strains and were used as test cultures [14]. The nutrient agar plate was used for both isolation and multi-metal analysis. All trials were performed in triplicate. The specific biochemical tests were performed for identification (Rapid Microbial Limit Test kits used) of bacterial [15][16]. A total of thirty (30) river bacterial isolates were challenged against three different concentrations (10, 100 and 200 mM) of copper and chromium metal salt solution for metal resistance studies by plate diffusion method. In plate diffusion assay, 500 µL of copper and chromium solution was added to a central well (1 cm in diameter and 4 mm in depth) of each nutrient agar plate separately and to allow it for metal diffusion at one day. Eight isolates were inoculated in each plate by the radial streaking method and were incubated at 37±1 °C for 48 h. All the trials were performed in triplicate. After incubation, the percentage of bacterial tolerance was calculated in terms of the ratio: length of growth in mm vs length of the total inoculated streak.

3. Result and Discussion

The development of resistant phenotypes may be related to the processes of bacterial survival and adaptation, and/or the inconsistency of selective pressures in that environment. It was stated that some toxic pollutants present in the environment can provide selective advantage to develop resistance for bacteria [17]. De Vicente *et al*[18] (1990) reported that the number of strains multi-resistant to several antibiotics and metals was higher in sea/ river water samples containing low levels of faecal indicators. In our study, the multi-metal resistant bacterial strains were analyzed against copper and chromium metals with different concentrations. Laplace *et al*[19] (2000) reported that in polluted environments some toxic pollutants (such as cadmium, mercury, lead, copper and manganese) may induce stress promoters in bacteria. The identification of resistances against different toxic metals may provide a useful tool for the simultaneous monitoring of several toxic pollutants in the environment [11].

In the present study, thirty river heterotrophic bacterial isolates were challenged against metal solutions for metal resistant study. The sensitivity nature of the bacterial isolates against metal solutions were varied depend on the concentrations which indicated that concentration depended manner play on important role in the resistant study. This study also witnessed that the aquatic bacteria were faced some of the special stress against toxic and other chemical materials. Most of the isolates from Periyar River were resistant to minimum concentration of the both metal solutions. Nearly, 93.3 % were resistant to copper solution whereas 90.0 % were resistant to chromium solution. Normally in this study, the chromium metals were highly sensitive to bacterial isolates than the copper metal. Interestingly, more than 85 % of the strains were resistant to minimum level (10 mM) of metal concentration while very few bacteria such as 6.7 % and 13.3 % strains were resistant to copper and chromium metals, respectively (Table 1). This study indicated that the increasing concentration of the metal solution affects the bacterial growth at considerable level. When the concentration of metal solution

increased the microbial growth could be gradually reduced.

A growth rate between 90-100% was observed for 93.3 % of the bacterial populations at 10 mM of Cu, whereas no population was growing at a growth rate of 0-70 % with 10 mM of Cu. At 100 mM of Cu, 20 % of the populations showed a growth rate of 81-90 %. In 200 mM of Cu, 46.7 % of the populations showed growth rates between 71-80%, whereas 3.3 % of the populations were observed with a 51-60 % growth rate. At 200 mM Cr, 6.7 % of the populations showed 51-60 % growth rate. A growth rate between 61-70 % was observed for 13.3 % of the bacterial populations at 200 mM of Cr, whereas no population was growing at a growth rate of 0-50 % with 200 mM of Cr. At 100 mM of Cr, 26.7 % of the populations showed a growth rate of 81-90 % whereas 10.0 % of the populations showed a growth rate of 81-90 % in 10 mM concentrations. The results indicated that the river received various pollution sources throughout the river-line. According to this study, some of the strains could be used to biosorption studies. The continuous monitoring is needed to avoid pollutions in the aquatic environment.

Table 1: Percentage of isolated copper and chromium resistance strains from Periyar River, Kerala

Percentage of growth	Periyar rive bacterial isolates (n = 30)											
	Copper (Cu) solution						Chromium (Cr) solution					
	10 mM		100 mM		200 mM		10 mM		100 mM		200 mM	
	N	%	N	%	N	%	N	%	N	%	N	%
0-10 percentage of growth												
11-20 percentage of growth												
21-30 percentage of growth												
31-40 percentage of growth												
41-50 percentage of growth												
51-60 percentage of growth					01	3.3			02	6.7		
61-70 percentage of growth					05	16.7			04	13.3		
71-80 percentage of growth			03	10	14	46.7		02	6.7	13	43.3	
81-90 percentage of growth	02	6.7	06	20	08	26.7	03	10	08	26.7	07	23.3
91-100 percentage of growth	28	93.3	21	70	02	6.7	27	90	20	66.7	04	13.3

4. Conclusion

The present study concluded that in Periyar River the heavy metal accumulation has resulted in adaptation of bacterial distribution, diversity and their growth is proportionate to the extent of pollution. Therefore, the heavy metal pollution and emergence of resistance strains in Periyar River is an environmental problem which demands immediate attention as it could have long-term influence on estuarine as well as human health. In this study, certain test cultures were sensitive to lowest (10 mM) concentrations whereas very few strains were resistant to high (200 mM) concentration. Furthermore, more studies should be designed at the molecular level to evaluate the presence of plasmid-encoded resistant genes, and to

identify the mechanisms involved in the association between antibiotic and metal resistance.

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