Effect of Heavy Metal on Staphylococcus Vitulinus 2019Y Strain Using Well Diffusion Method

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Abstract **- In the present study we have observed the effect of heavy metals Viz. Zinc, Copper, Mercury, Magnesium, Cadmium, Lead, and Cobalt on Staphylococcus vitulinus 2019 Y bacterial stain isolated from carrica papaya (red lady verity) pulp. Staphylococcus vitulinus strains 2019 Y has 99.93% similarity with the Staphylococcus vitulinus, an endophytic bacterium which helps to promote the plant growth and increases the crop yield. In this study we have found that Staphylococcus vitulinus 2019 Y stain shows tolerance to Heavy metal Zinc and magnesium where as in case of mercury and copper it shows inhibition at different concentration which indicate that heavy metal pollution has adverse effect on plant growth promoting bacteria and make soil unfit for the growth and development of plants.**

Index Terms **- Staphylocoocus vitulinus 2019Y, Heavy Metal, Inhibition, Well diffusion method. NCMR PUNE, 16s rRNA, ncbi gene bank.**

I.INTRODUCTION

Endophytic bacteria are colonize in the internal tissue of plants viz. root, stem, leaves, fruits and seed [13]. Many researcher have published in vitro data on common bacterial endophytes in plant, endophytic bacterial species are useful as they are the plant growth promoting agent, and ultimately to increases the crop yield. [6,7,8 and 12]. Staphylococcus vitulinus strains 2019 Y is endophytic, gram positive, Coagulase negative identified by the 16s rRNA gene sequence analysis from the *NCMR PUNE.* And Partial 16s rRNA gene sequence has been submitted in the ncbi gene bank websit *(Accession no-MN 640907).*Heavy metals constitute a very heterogeneous group of elements widely varied in their chemical properties and biological functions. They are the group of metals and metalloids with atomic density greater than 4g/cm3. Heavy metals due to their bio-accumulative and non-biodegradable properties constitute a major group of pollutants [4]. Heavy metals are one of the most common ubiquitous pollutants in the environment and are kept under environmental pollutant category due to their toxic effects on plants, animals and human being [2]. Knowledge about the fate of chemicals in environments is essential for proper understanding of the conditions, such as the concentrations and bioaccumulation of heavy metals, which make them harmful, and how biotoxicity occurs [15]. As Toxicity is a relative property of a chemical, which refers to its potential to have harmful effects on a living organism. It is a function of the concentration of the chemical and the duration of exposure. Heavy metal concentrations in ecosystems are usually monitored by measuring their concentrations in water, sediments and biota [3]. Which generally exist in low levels in water and attain considerable concentration in sediments and biota [10]. Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology since they are highly persistent and all have the potential to be toxic to living organisms [14]. Heavy metal pollution ultimately effect the plant growth by decreasing the soil fertility [3]. Some of the metal are essential for metabolic reaction in plant development viz. zinc, iron, magnesium and phosphorus [1]. but some of them have no known role in the biological system and they are toxic even at low concentration and affect the growth and development of plants and microbes in other words they deteriorates the quality of water and soil which ultimately affect growth and development of plants and microbes present in the soil, some act as carcinogenic and mutagenic agents in biological system [5 and 9] (EISLER, R 1986, IARC IAFROC 1990).

The main objective of this study is to find out the effect of heavy metals on Endophytic bacteria, Staphylocoocus vitulinus 2019Y strains isolated from

carrica papaya (red lady verity) pulp. Grown locally in Aurangabad as well as different region of the Maharashtra state in India. Staphylococcus vitulinus strains 2019 Y is endophytic, gram positive, coagulase negative identified by the 16s rRNA gene sequence analysis from the *NCMR PUNE*. And Partial 16s rRNA gene sequence has been submitted in the ncbi gene bank websit *(Accession no-MN 640907)*. Staphylococcus vitulinus strains 2019 Y has 99.93% similarity with the Staphylococcus vitulinus.

II.MATERIAL AND METHOD

Freshly harvested, healthy ripen carrica papaya of red lady variety (about 1-2 kg) were collected from the farmer of the Aurangabad, The fruits were taken to the laboratory immediately after harvesting. In laboratory fruit was washed thoroughly under running tap water and surface sterilized by using 70% alcohol followed by wash with the sterile distilled water (twice). The fruit was cut into two equal part using sterile knife. under aseptic condition red pulp were removed using wire loop and transferred on the nutrient agar plate which were incubated at 37 °C for 24 hours in bacteriological incubator. An isolated well-developed colonies of Staphylocoocus vitulinus 2019Y strains was identified and were passed from the subculture to the new nutrient agar plate to obtain the pure culture in aseptic condition and was incubated at 37^0C for 24 hours. In pure culture strain inoculated on the nutrient agar plate under aseptic condition a wells were prepared and filled with 40 µl of heavy metals viz. Zinc, Copper, Mercury, Magnesium, Cadmium, Lead, and Cobalt having different concentration like 100, 200, 300, 400 and 500 ppm and incubated at 37°C for 24 hours. After 24 hours the results were observed.

Table I: Shows the effect of Heavy metal on Staphylocoocus vitulinus 2019Y stain.

Sr. No	Concentration of Heavy metals in PPM	Zone of inhibition (in mm)						
		Zn	Cu	Hg	Mg	Cd	Pb	Co
1	100	--	--	18	--	--	--	--
2	200	--	--	20	--	5	--	--
3	300	--	18	20	--	13	--	--
4	400	--	12	25	--	15	--	--
5	500	--	13	26	--	13	--	--

III.RERULTS AND DISCUSSION

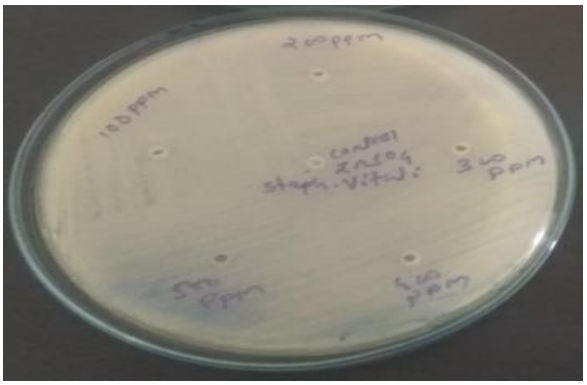


Figure I-ZnSo4

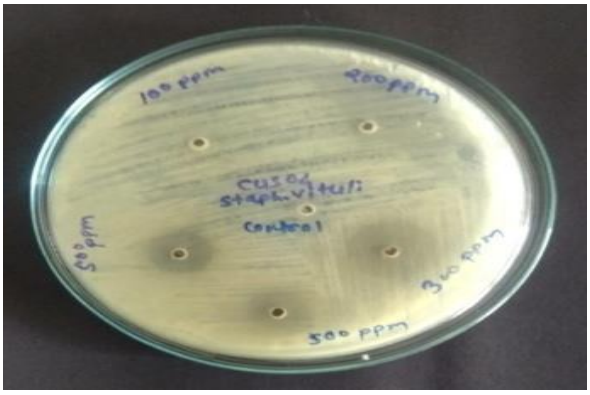


Figure II-CuSo4

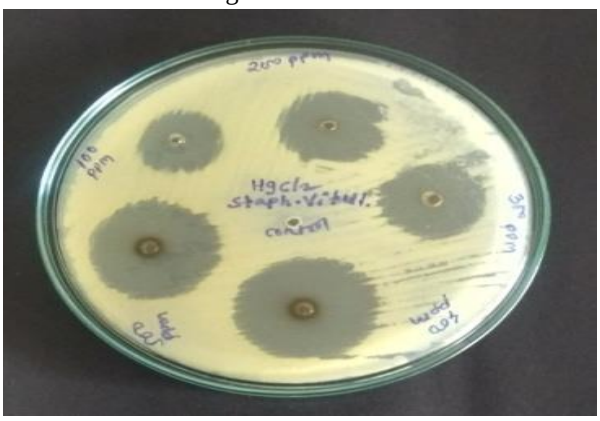


Figure III-Hgcl2

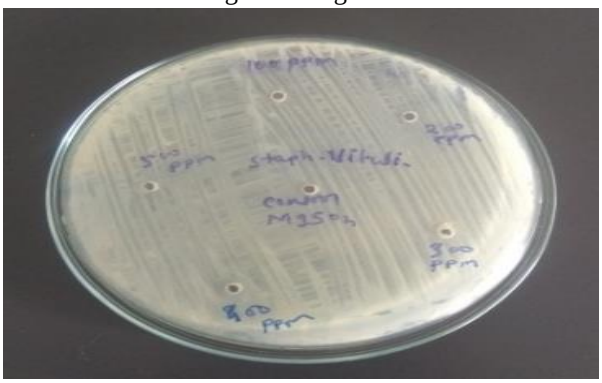


Figure IV-MgSo4

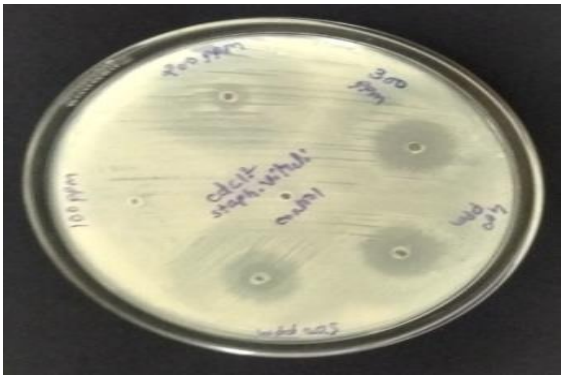
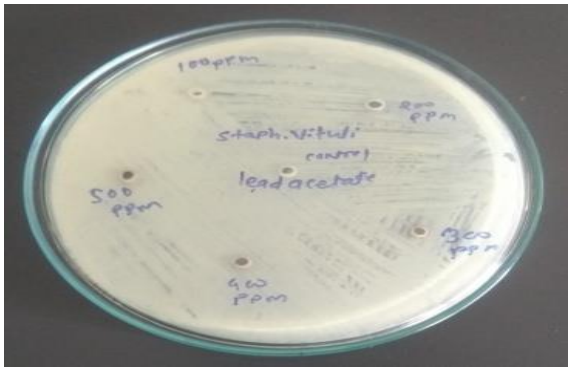


Figure V-Cdcl2



FigureVI-PbCH3COO2

FigureVII-CoCl2

Figure I- Staphylocoocus vitulinus 2019Y stains shows tolerance to Heavy metal Zinc (as Zinc sulphate -ZnSo4) at all the concentration level i.e. 100, 200, 300, 400 and 500ppm used in the study and does not show any kind of heavy metal resistance zone .

Figure II- in case of heavy metal copper (copper sulphate CuSo4) initially Staphylocoocus vitulinus 2019Y stains show tolerance at 100ppm, and 200 ppm but as we increases the concentration it shows the zone of inhibition at 300ppm, 400ppm and 500ppm which is of 18, 12 and 13 mm in size respectively

Figure III- in case of Mercury (as mercury chloride – Hgcl) Staphylocoocus vitulinus 2019Y stains shows the clear zone of inhibition at various concentration and as we increase the concentration the zone of inhibition also shows gradual increase, at 100, 200, 300, 400 and 500 ppm of concentration the size of zone of inhibition were 18, 20, 20, 25 and 26 mm respectively

Figure IV- Staphylocoocus vitulinus 2019Y shows complete tolerance to the heavy metal Magnesium (as magnesium sulphate MgSo4) at all the concentration level used in the study and does not show any kind of zone of inhibition.

Figure V- Staphylocoocus vitulinus 2019Y shows tolerance to Cd at 100ppm concentration but as we increases the concentration it shows clear zone of inhibition at 200, 300, 400 and 500ppm having the diameter of size 5, 13, 15 and 13mm respectively.

Figure VI- Staphylocoocus vitulinus 2019Y stains shows tolerance to Heavy metal Pb (as lead acetate) at all the concentration level i.e. 100, 200, 300, 400 and 500ppm used in the study and does not show any kind of inhibition zone .

Figure VII- in case of Cobalt Staphylocoocus vitulinus 2019Y shows tolerance at all concentration level and does not show any kind of zone of inhibition.

IV.CONCLUSION

From the present study it can be concluded that the Staphylocoocus vitulinus 2019Y shows tolerance to the heavy metals like Zinc, Magnesium Lead and Cobalt at 100, 200, 300, 400 and 500ppm concentration and does not have any adverse effect on the growth of the bacteria, but on the other hand heavy metal Mercury shows the zone of inhibition which means mercury shown its adverse effect by inhibiting the growth of bacteria at all concentration level, in case of Copper the bacteria show tolerance at 100 and 200ppm of concentration but as we increases the concentration we clearly see the zone of inhibition, in case of Cadmium bacteria shows tolerance at 100ppm but as the concentration increases we found zone of inhibition at 200, 300, 400 and 500ppm, which indicate that some of the heavy metals imparts the adverse effect on growth and development of the bacteria and ultimately affect the growth and development of the plants and also reduces the crop yield, as it was reported that the endophytic bacterial species are the plant growth promoting agent, and ultimately increases the crop yield (Agostinho A.de

Lima e Silva et al 2012).or we can also conclude that heavy metal pollution can degreases the fertility of the soil, similar results has been obtained in number of studies by many scientist.

V.ACKNOWLEGEMENT

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