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RESEARCH ARTICLE

ROLE OF*Pseudomonasaeruginosa* AS A BIOREMEDIATING ORGANISMIN SOME AQUA PONDS OF WEST GODAVARI DISTRICT, ANDHRA PRADESH, INDIA

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Abstract

Pseudomonasaeruginosa well known for its bioremediation property is found in various ecosystems.If*Pseudomonasaeruginosa* is introduced in the aquatic environment in a scientific manner during the production of fish and prawn the trace element contamination can be easily addressed by a way of bioremediation.West Godavari district in Andhra Pradesh is well established for aqua culture ponds, as the land and climatic conditions support for it. Commercial culturing of fish and prawn in this region has been practiced from decades which helped in contribution of nutritious food in the form of aqua products. But production of these aquatic produce in a controlled condition is a big challenge for the farmers which is the area focused in the present study. The role of microorganisms in maintaining balance of some parameters for better yield was studied which revealed that heavy metals such as Cd, Cr, Pb and Cu are under bioremediation process by*Pseudomonas aeruginosa* in some aqua ponds of West Godavari district, Andhra Pradesh. Using MIC technique, it is revealed that this microorganism isolated from those aqua ponds have shown bioremediation as Cu<Pb<Cd<Cr.

Introduction:-

Bacterial heavy metal remediation was reported decades back (Brown 1985; Foster 1987; Misra et al., 1984; Silver, 1981; Mergaey, 1985). Removal of heavy metals by bacteria is mainly by processes such as biosorption or bioaccumulation in aquatic environment (Gadd, 2000; Xaing, 2000; Badaret al., 1999). The metals such as Cadmium, Chromium, Lead, Copper, Zinc, Arsenic, Silver and also some other metals which are found at seven grams per cubic millimetre density in aquatic environment are regarded as heavy metal contaminants in general (Shahid et al., 2021).

Since the surface of microorganism's cell is negatively charged , it is readily accepting metal cations (Punamiya et. al.,2010). The microorganisms shows resistance to various metals by variety of mechanisms such as destruction of metals by enzymes, removal of metals, destruction by metal ligand bonding , metal chelation within or outside the

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cell, oxidation, demethylation, organometallic damage, inhibition of metals permanently by metallothioneinformation, bioactive compounds production and by methylation. (Iravani and Varma , 2020).

Pseudomonas species of bacteria have special mechanisms which help them get rid of toxic shock while accumulating heavy metals that is by accumulating the heavy metal in its periplasmic space. This mechanism prevents the cytoplasmic entry of excess heavy metal making it a suitable microbe for bioremediation (Cooksey, 1990).Bioaccumulation and resistance to heavy metals such as Copper, Cadmium, Nickel and Chromium in their ionic forms in waste waters is better exhibited by*Pseudomonas* species (Hussein, 2004).

The fact that the microorganisms can bioaccumulate heavy metals by various biological mechanisms has gained much attention in recent years, exploration in that area which made the present study to understand the capability of *Pseudomonas aeruginosa* in bioremediation of certain heavy metals such as Cadmium, Chromium, Copper and Lead in pond waters of West Godavari district, Andhra Pradesh.

Materials and Methods:-

Isolation, biochemical and molecular characterization:

The microorganism was isolated from the pond waters of five study stations of West Godavari district Andhra Pradesh such as Komarada, Undi, Kumudavalli, Vissakoderu and Eduru villages. Though other microbes such as *Staphylococcus aureus, Streptococcus pyogenes, E.coli* and *Pseudomonas putida* found in these study stations but *Pseudomonas aeruginosa* was found in all stations. So *Pseudomonas aeruginosa* was isolated and confirmed biochemically as well as by molecular studies using standard protocols.

The DNA was isolated and 16S rRNA gene was sequenced using standard protocols then by comparing the phylogenetic tree the species was established.

Heavy metal bioremediation:

Onto Muller Hinton media, *P. aeruginosa*was streaked and heavy metals such as Cadmium, Chromium, Copper and Lead were placed at a concentration of 0.05 µg/ml. And kept for incubation at 29^0C. After 12 hours of incubation, the zones of inhibition formed were measured using a ruler.

Results:-

Isolation and biochemical characterization:

Unipolar, non sporing,β- haemolytic and gram negative bacteria which showed Citrate positive, Indole positive and Methyl red positive biochemically confirming it as *Pseudomonas* species as shown in figure 1.

Figure 1:- *Pseudomonas aeruginosa*.

Molecular characterization:

By targeting 16S rRNA gene, the *Pseudomonas aeruginosa* species have been confirmed which also revealed the evolutionary relationship of the organism in the form of phylogenetic tree as shown in figure 2.

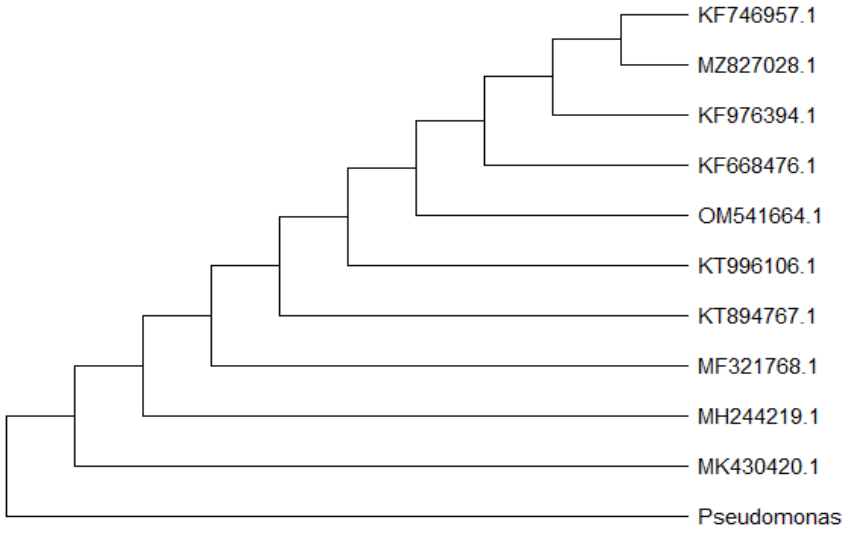

Figure 2:- Phylogenetic analysis of *Pseudomonas aeruginosa.*

Minimal Inhibitory concentration:

The minimal inhibitory concentration (MIC), test reveals how effectively a microorganism can bioremediate a metal in its presence. In the present study when the heavy metals of 0.5 µg/mL concentration ofCadmium, Chromium, Copper and Lead were tested on the Pseudomonas aeruginosa, zones of inhibition were formed of diameter of 2.3 mm, 2.8 mm, 2.1 mm and 2.2 mm respectively as shown in figure 3.

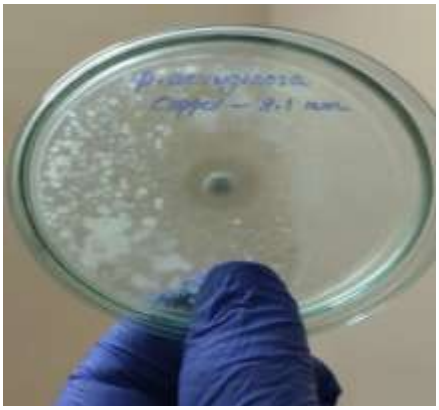

Figure 3 a:- MIC of Copper (2.1 mm).

Figure 3 b:- MIC of Lead (2.2 mm).

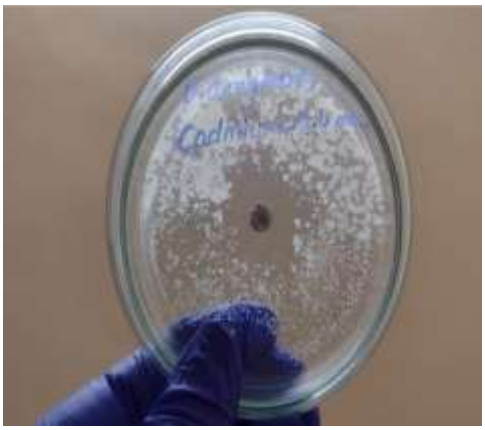

Figure 3 c:- MIC of Cadmium(2.4 mm).

Figure 3 d:-MIC of Chromium (2.8 mm).

Figure 3:- Minimal Inhibitory Concentration test with various metals on *P.aeruginosa.*

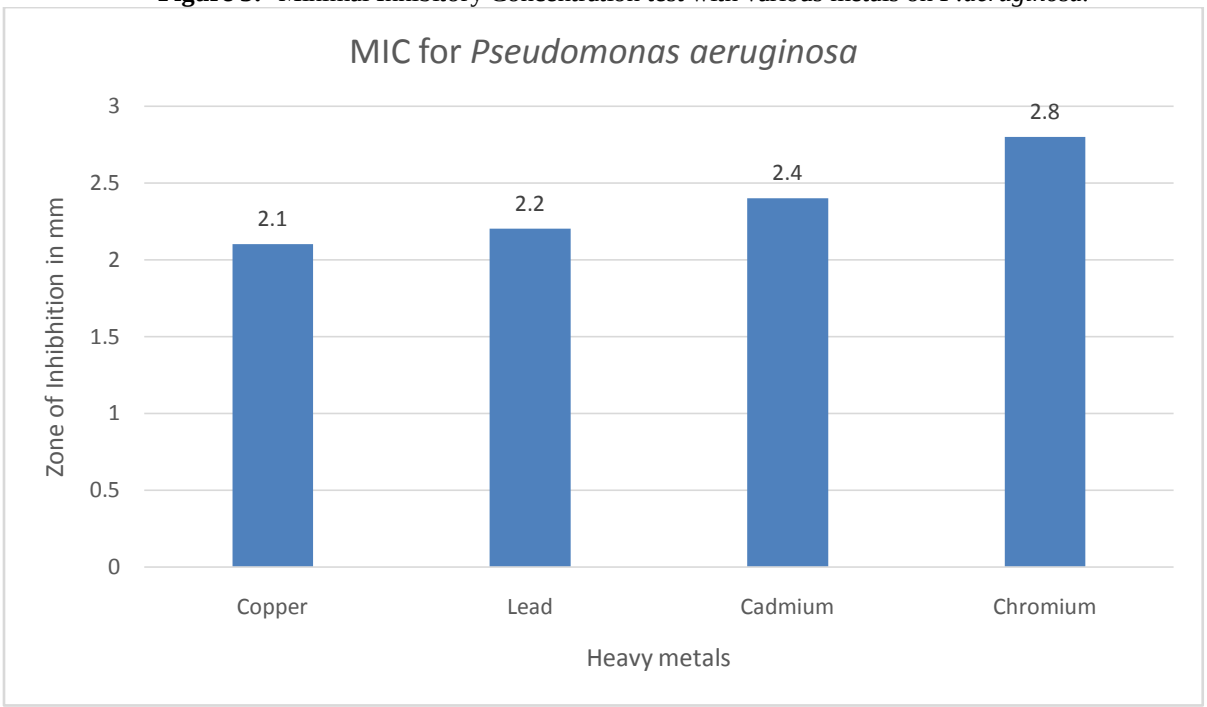

Figure 4:- Graphical representation of MIC for *Pseudomonas aeruginosa*.

Discussion:-

Aquaculture ponds are contributing in a large way for the food sector in terms of nutrition, so the demand for production of fish and prawn has increased comparatively from past decades. The role of microorganisms in maintaining the hydrographical, heavy metals and minerals availability in permissible levels in waters of the cultivated crop shows, how important these microorganisms are for quality yield of the crop.

Microorganisms have certain chemical groups in their cellular composition which help them in adsorption process of metals in their environment, those are thiol , hydroxyl, phosphoryl, carboxyl, alcohol, thioether, sulfonate, ester, amine and sulfhydryl (AyangbenroandBabalola, 2017). Commercially *Pseudomonas aeruginosa* as well as *Bacillus subtilis* are very good biosorbents(Tunalia et. al,2006 and Lin et al, 2006).

Gram negative bacteria such as *Pseudomonas* has an advantage of phosphate groups presence in their outer and inner layer of cell wall and the heavy metals tend to bind to the phosphoryl ligand of the lipopolysaccharides present in the cell wall (Moat, 2002).

In the present study, microorganism was isolated from pond waters and was confirmed through biochemical, morphological as well as by molecular evidence as *Pseudomonas aeruginosa* as shown in figure 1 & 2.The heavy metals bioremediation by *Pseudomonas aeruginosa* in the pond waters of West Godavari district, Andhra Pradesh was studied which revealed that this microorganism can remediate heavy metals like Cadmium, Chromium, Copper and Lead through its metabolic pathways. The formation of zones of inhibition when these metals were used in at a concentration 0.5 µg/mLare 2.1<2.2<2.4<2.8 respectively for the heavy metals Copper<Lead<Cadmium<Chromium which is shown in figure 3 & 4. The observation that bioremediation of Copper is less than Lead correlates with findings of (Teitzel, &Parsek, 2003).The heavy metal Cadmium has more bioremediation level than Copper and Lead as studied by (Benhalima, 2020). Though in most of the studies Cadmium is the metal that can be effectively bioremediated by *Pseudomonas* species but in the present study it was Chromium metal that showed best inhibition zone when *Pseudomonas aeruginosa* was bioremediating it. This may be due to the difference in source of isolation of themicroorganism and other parameters of the pond that differs in showing its effectiveness.

Conclusion:-

Pseudomonas aeruginosa is well known for its metal resistance property towards various metals. Its biochemical pathways allow the metals present in its environment to be biosorbed, accumulated, biorecieved, and uptake through its cell wall through phosphoryl ligand formation. Since it can take up heavy metals in its environment and help to maintain balance in its habitat, the presence or growth of this organism suggest it as a good bioindicator towards sustainable management of waters especially aqua culture ponds. The present study helped to understand the vital role of this microbe in the pond waters of fish and prawn crops cultivation at West Godavari District, Andhra Pradesh, India.Delivering this aquaculture produce in a scientific manner is need of the hour and this is to be addressed, since the production of fish and prawn is on large scale in this area andremdiation of trace element component through other methods is of cost effective process. So the aim of the study in this line is to introduce an ecofriendly solution to mitigate the trace metal accumulation and other toxicants if any. This helps to produce a quality and international standard food to the nation. This helps the farmers to increase their marginal profits by producing a quality food product.

As this microbe is also useful in commercial purpose as biosorbent, its production can be further explored industrially.

Conflict of Interest:

Authors have no conflict of interest.

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