IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ISOLATION OF COPPERRESISTANT BACTERIA FROM RAMGANGA WATER

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Abstract:

Because industrial effluents include large concentrations of heavy metals, it is possible that bacteria living in these environments have become resistant to these metals. The goal of the current experiment was to isolate and identify heavy metal-resistant bacteria, specifically Cu-resistant bacteria, from industrial effluent-contaminated sites in Uttar Pradesh. *Bacillus cereus* isolates have been isolated and described. Significant tolerance to elevated Cu dosages is shown by each isolate. The growth kinetic analysis showed that the isolates' growth rate was not significantly impacted by the presence of heavy metals.

Keywords: Heavy metals, Copper resistant, *Bacillus cereus,* Industrial effluent.

Introduction:

Utilizing microorganisms, bioremediation breaks down organic pollutants found in soil, groundwater, sludge, and solids. The micro organism degrade pollutants by using them as an energy source or cometabolizing them with an energy source. Bioremediation involves the creation of energy in a redox reaction within microbial cell [1]. Respiration and other biological processes required for cell upkeep and reproduction are included in these reactions. Different sort of microbial electron acceptor classes can be involved in bioremediation such as oxygen, nitrate, manganese, iron ,sulfate or carbon dioxide reducing and their related redox potential [2].

To increase the activity of microorganisms. Micro organism such as organic substrates or other electron acceptors , nutrients and other compounds that affect can limit treatment in their absence can be added [3]. Mourao et al., concerned about colistin-resistant bacteria in animal feed, the environment, and human environments, they recommended that the poultry industry apply colistin limitations and investigate alternative trace metals and copper feed additions. Clarifying how these strategies affect the selection and persistence of colistin-resistant Klebsiella pneumonia in the poultry production chain is essential [4].

After a long term removal of colistin resistant and copper tolerant Klebisella pneumonia in chickens maintained with inorganic and organic copper formulae from 1 day old chicks to meat [5]. Whole genome sequencing , molecular and culture methods were used to assess clonal diversity and adaptive characterstics of Klebsiella pneumonia was present in the majority of chicken flocks with a substantial decrease in meat batches and occasional contamination of water and feed. Regardless of diet , significant prevalence of colistin resistant/ negative Klebsiella pneumonia were detected in fecal samples [6].

Most of the isolates in the sample were copper tolerant, multidrug resistant, and silA and pcoD positive. A build-up of F type multireplicon plasmid was discovered by WGS, which contained mutations that led to colistin resistance as well as genes that were resistant to antibiotics and copper. The poultry business was home to multiple lineages of the polyclonal Klebsiella pneumonia population [7]. The plasmids ST15-KL19, ST15-KL146, ST392-KL27, and lncF were comparable to those from clinical isolates of human worldwide , indicating that chicken production serves as a source for clinically relevant Klebsiella pneumonia lineages and gene that may pose a risk to humans through exposure to the environment. In addition to providing important insights on the persistence of clinically relevant Klebsiella pneumonia in the poultry production chain, this work emphasizes the need for continuous surveillance and preventive food safety measures from a single health perspective [8].

They proposed that figuring out how bacteria resist heavy metals is essential to comprehending bioremediation of the natural environment. Pseudoxanthomonas spadix ZSY -33, a bacterium resistant to various heavy metals , was isolated and studied in this work . The mechanism behind copper resistance was discovered by looking at the physiological traits, copper distribution, and genomic and transcriptome data of strain ZSY-33 cultivated at different copper concentrations [9-12]. The growth inhibition assay, which was carried out in basic medium, revealed that the addition of 0.5Mm copper inhibited the growth of strain ZSY-33. Higher copper concentrations resulted in increased synthesis of extracellular polymeric material. By combining transcriptome and genomic research, the mechanism behind strain ZSY-33's copper resistance was elucidated [13]. The intracellular copper homeostasis was preserved at reduced copper concentrations by the Cus and Cop systems. When copper concentration increased, the Cus and Cop systems cooperated with a variety of metabolic pathways, including the metabolism of pro-energy, sulfur, and amino acids, to control copper stress. These results showed that strain ZSY-33 has a flexible copper resistance mechanism, which it

might have picked up from extended exposure to its environment [14].

Methodology:

Samplecollection-Sample1,2,3collectedfromthedifferentsitesofGanga river.

Isolationof copper resistant bacteriafrom watersample- 1 ml samples were serially diluted in 0.85% NaCl solution and then spread over the nutrient agar plates. The plates were incubated at 37℃ for 48 hours. The cultures were selected on the basis of their morphology. The cultures were streaked into sterilized nutrient agar plates and incubated at 37℃ for 48 hours. The cultures were streaked in nutrient agar media supplemented with copper sulfate 50 mg/l. The growth was shown as the presence of copper resistant bacteria [15].

Identification of bacteria: Identification of the copper resistant bacteria was carried out through performing biochemical tests such as, grams staining, catalase test, MRVP test etc.

Growth kinetics: The growth study was carried out by taking the absorbance of microbial growth inoculated in the sterilized nutrient broth media at different time intervals.

Optimization of the media: The optimization of the media was carried out by one factor at a time method[16].

Results & Discussions: The samples werecollectedintheplasticbottle from different sites of ganga riverforisolationofmetal resistant bacteria. Total 11 cultures were isolated from the samples. These pure plates pureisolateswerescreenedforthecopperresistanceinthepresenceofcopper supplementedmediaandfoundthatC1fromS1asthepositiveresults.

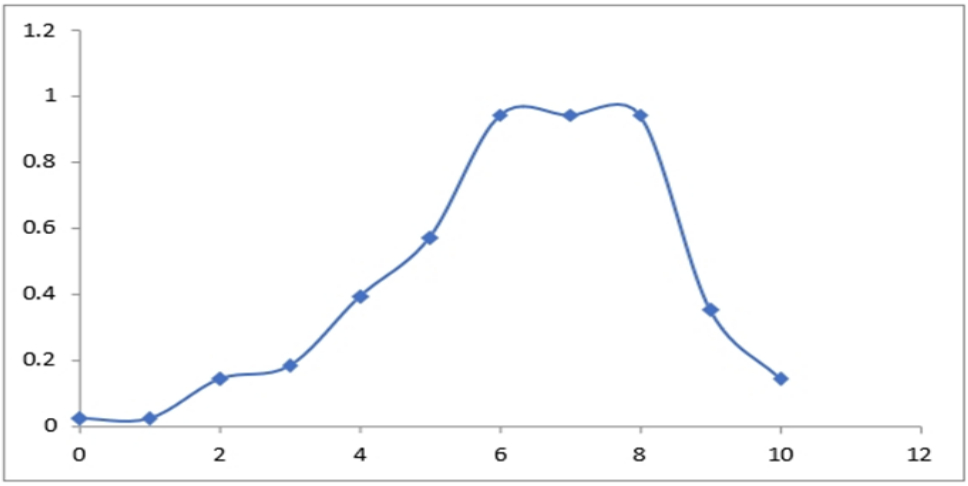

Fig. 1: growth curve study of culture.

Table 1: Selection of production media

s. no	Production media	Enzyme activity
1.	PM-1	0.129
2.	PM-2	0.129
3.	PM-3	0.086
4.	PM-4	0.120

Table2:Effects oftemperature onthe growthof culture

S no.	Temperature	Growth
1.	37°C	+++
2.	4°C	-
3.	50°C	+
4.	Room Temperature	+

Table 3: Tabular representationofeffect ofpHon culture.

Sno.	Sample	OD@620 nm
1.	pH 4	0.119
2.	pH 7	0.117
3.	pH 9	0.105
4.	pH 11	0.122

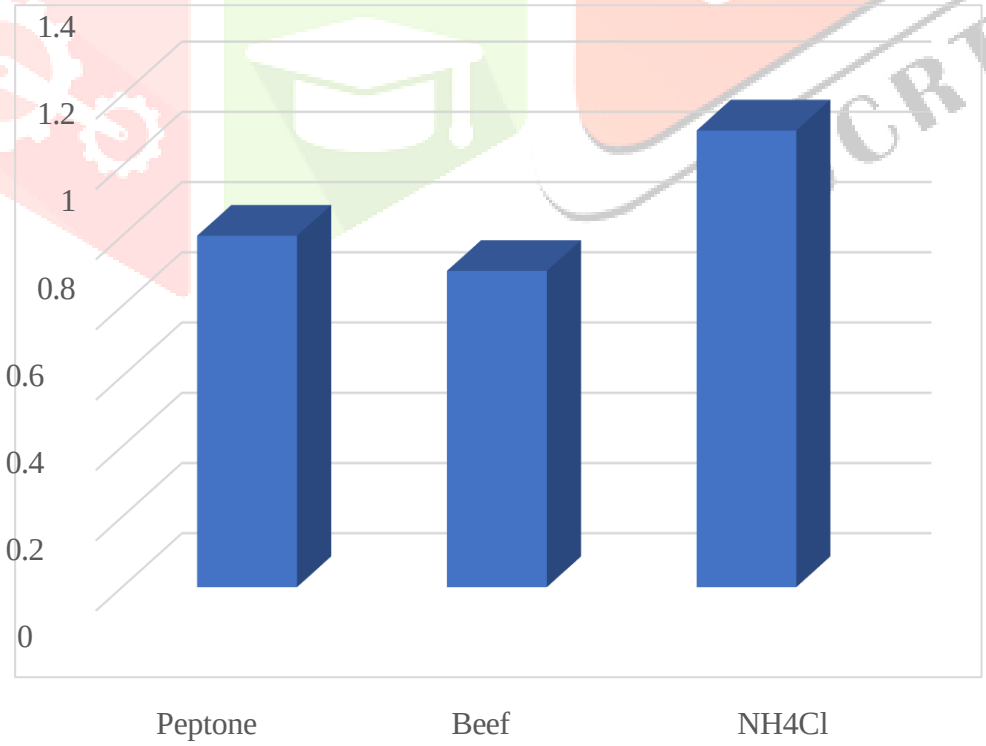

Fig. 2:graphicalrepresentationofnitrogensourceson culture.

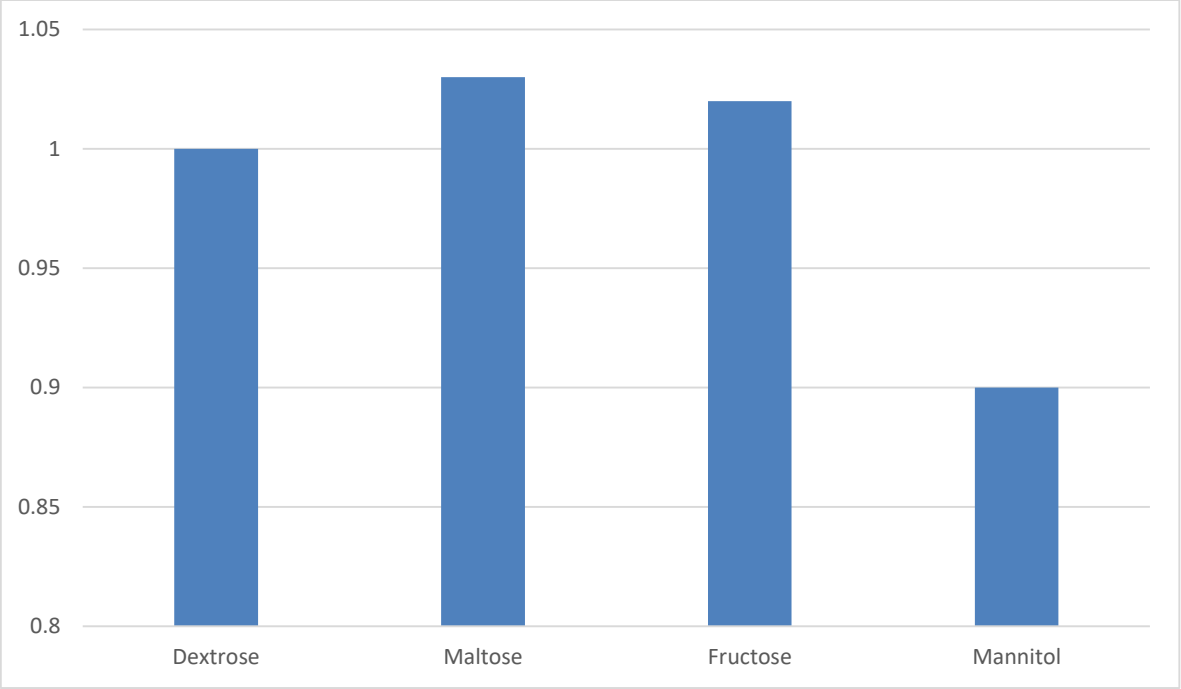

Fig. 3: Graphical representationof effectofcarbon sourceson culture.

Heavymetalsaretheprincipalwastewatercontaminantsthatarenon-iodegradableandso accumulated in the environment, heavy metal sequestration from waste water is a challengingprocessthatrequiresconsecutiveengrossmentaswellasmonitoring.Because presentprocedureswillnolongerbeabletoaccommodatetheescalatingdemandformuch lower levels of heavy metal in drinking water and waste water, imposing technologically superior alternative water treatment systems will become progressively challenging. Nanoparticlesperformtheirpredeterminedmajorrolebyprovidingnumerousactivesites aswellasbyenhancingthesurfaceadsorptionhencehighlyapplicableforthetreatmentof wastewater contaminated with different heavy metals As, Hg, Cd, Pb, Cr, Ni, Zn and Cu [18].

Studies favors our research which suggested that application of some microbes, may becomemorefavorableinbioremediationbyimprovingtheirmetabolicactivitieswiththe help of genetic engineering in bioremediation process. *Stereumhirsutum, Nocardia, Methanogens, Aspergilusniger, Pleurotusostreatus, Rhizopusarrhizus, Azotobacter, Alcali-genes, Phormidiumvalderium, Ganodermaapplantus*are some microbial species that assist in bioremediation of heavy metals [17].

To safeguard living things and protect the ecosystem, heavy metal contamination in soil must be remedied. Traditional restoration procedures include chemical, physical, and engineering restoration; these treatments are quick but expensive and ineffectivebecausetheyalsodamagesoilqualitiesandgeneratesecondarypollution [20]. Incontrast,biologicalmethodsarethoughttobeasuccessfulapproach for heavy metal remediation.Inthisstudythebacteriawereisolatedfromthecollectedsamplesusingtheserialdilution and spread plate method. In this procedure, the samples were diluted in a 0.85% [19] NaCl solution and then

distributed onto sterilized nutritional agar media. Following a 24-hour incubation period at a temperature of 37°C. The isolates were tested for heavy metal presence using NB agar media enriched with 100 ppm of CuSO4. The proliferation of germs and the absence of any visible zone shows a positive outcome.

From the three samples that were chosen, 11 strains in total was produced due to their unique morphological features. S1C1 was selected for additional research among these bacterial isolates.

The bacterial culture that exhibited the most favourable outcomes in the presence of CuSO4, was determined using biochemical and molecular characterizations methods. Grammestaining,catalasetest,glucosefermentationtest,andMRVPtestwereconducted forbiochemicalcharacterization.

Whileidentifyingthebacteriathroughbiochemicalcharacterizationwefoundthat the strain is gram positive and Bacillus in shape, endospore positive, catalase positive, Indole negative, MR positive and VP negative. The strains show the properties of hydrolyzing the starch, casein & cellulose.

Conclusion:

The high concentration of heavy metals present in industrial effluents, it is quitelikelythatbacteriainhabitingsuchenvironmentshavedevelopedresistancetoheavy metals. The present experiment was initiated with the aim of isolating and identifying bacteria that are resistant to heavy metals, specifically Pb, Cu, Cr, Cd, and Ni, etc from areasinUttarPradeshthatarecontaminatedwithindustrialeffluent.Theisolatesthatwere identified and defined are *Bacillus cereus*. All of the isolates demonstrate significant tolerance to high doses of Cu. The growth kinetic research revealed that the presence of heavymetaldidnothaveasignificantimpactonthegrowthrateoftheisolates.Theprotein expression pattern in the presence of several heavy metals was really intriguing. It was discovered that specific proteins were excessively produced in response to heavy metal exposure

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