International Journal of Recent Innovations in Academic Research
E-ISSN: 2635-3040; P-ISSN: 2659-1561
Homepage: https://www.ijriar.com/
Volume-8, Issue-6, June-2024: 1-7

Research Article

Isolation, Identification and Characterization of Heavy Metal Bacterial Communities from Sewage

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Received: May 15, 2024 **Accepted:** June 05, 2024 **Published:** June 12, 2024

Abstract
This study explores the diversity of microbes in drainage wastewater. The present study deals with isolation, identification and characterization of heavy metal resistant bacteria was isolated from sewage water collected from boys and girls hostels in Yogi Vemana University, Kadapa, Andhra Pradesh, India. The six isolates were isolated and selected based on high level metal resistance. On the basis of morphological, biochemical, 16S rRNA gene sequencing and phylogeny analysis revealed that, the isolate was authentically identified as *Bacillus psedomycoides*-MH578628.1 with the percentage identity 98.86. The sewage isolates showed optimum growth at 30^{0}C and pH 7.0. The identified isolates were resistant to heavy metals in collected sewage water like iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb), and silver (Ag). The identified heavy metal resistant bacteria could be useful for the bioremediation of heavy metal contaminated sewage and waste water.
Keywords: Heavy Metal Resistant Bacteria, 16s rRNA, Sewage Water, Biochemical Tests.

1. Introduction

Sewage is the wastewater generated by a community, namely: a) domestic wastewater, from bathrooms, toilets, kitchens, etc., b) raw or treated industrial wastewater discharged in the sewage system, and sometimes c) rain-water and urban runoff. Domestic wastewater is the main component of sewage, and it is often taken as a synonym (Agarwal *et al.,* 2022). The sewage flow rate and composition vary considerably from place to place, depending on economic aspects, social behavior, type and number of industries in the area, climatic conditions, water consumption, type of sewers system, etc. (Yaser and Safie, 2020). The main pollutants in sewage are suspended solids, soluble organic compounds, and fecal pathogenic microorganisms, but sewage is not just made up of human excrement and water (Onu *et al.,* 2023). A variety of chemicals like heavy metals, trace elements, detergents, solvents, pesticides, and other unusual compounds like pharmaceuticals, antibiotics, and hormones can also be detected in sewage. With urban runoff come potentially toxic compounds like oil from cars and pesticides that may reach the treatment plant and, eventually, a water body (Nataraj, 2022). People are exposed to sewage by hand-to-mouth contact during eating, drinking and smoking, or by wiping the face with contaminated hands or gloves. Exposure can also occur by skin contact, through cuts, scratches, or penetrating wounds, and from discarded hypodermic needles. Certain organisms can enter the body through the surfaces of the eyes, nose and mouth and by breathing them in as dust, aerosol or mist.

Sewage and wastewater contain bacteria, fungi, parasites, and viruses that can cause intestinal, lung, and other infections. Bacteria may cause diarrhea, fever, cramps, and sometimes vomiting, headache, weakness, or loss of appetite. Some bacteria and diseases carried by sewage and wastewater are *E. coli*, shigellosis, typhoid fever, salmonella, and cholera (Kaur *et al.,* 2020). Water pollution due to mining activity is capable of rendering the water unusable as potable water source. The polluted water may have undesirable colour, odour, taste, turbidity, organic matter contents, harmful chemical contents, toxic and heavy metals, oily matters, radioactivity, high total dissolved solids (TDS), acids, alkalis etc. The organic content may be biodegradable or non-biodegradable. Pollution of surface waters (rivers, lakes, and ponds), ground waters, and sea water are all harmful for human and animal health. Pollution of the drinking water and that of food chain is by far the most worry-some aspect as elements or constituents of polluted water can act as toxins and can create severe health hazard to organisms. Irrigation of such water introduces metals in the eatables and also contaminates the water (Morseletto *et al.,* 2022; Florides *et al.,* 2024).

1.1. Sewage Water/Drainage Water

Today's rapidly growing societies generate wastes that enter water bodies. Different types of wastewater include those derived from domestic, commercial, industrial and agricultural sectors, as well as surface runoff (storm water) from urban areas. Domestic wastes are derived from human communities and contain human wastes (feces and urine) as well as water from laundry, kitchen, bathing, and other household chores (Mahesh *et al.,* 2023).

A combination of the increasing population, the flat terrain, and lack of adequate sewage and waste disposal make many localities, potential health hazard areas for their inhabitants. Sanitary and sewage systems are poor, and where they exist, poorly managed. Several of the people do not care how they dispose of their trash, and it is not surprising there are serious pollution problems in the communities. The heavy rainfall, flat terrain, poor drainage (blocked drains due to trash dumps, built up of silt, etc) or lack of drainage system could lead to serious flooding problems even with minimal precipitation. In most houses, only toilet waste is discharged through a septic system and all other household liquids are discharged directly to storm drains where they exist or into the street (Galadima *et al.,* 2011).

In addition, most houses lack indoor plumbing and adequate sources of potable water is limited. The unreliability of water supply from government-owned water board led some of the people to resort to drilling boreholes, or wells. Some buy water from water vendors in tanks. Those who could not afford these obtain their drinking water from shallow wells, less than 5m (16ft) deep. Some of these shallow well waters require treatment before meeting the WHO drinking water standard (Yusuf, 2018). During daily operations reasonable quantities of different wastes that are dumped on fresh waterways are consistently generated. Road side sellers, the major culprits, dispose various items such as cans of soft drinks, banana and orange feels, wrappers of sweets, street mechanic dusts etc. Our abattoirs are generally performing opposite (anti-sanitation) function. Blood, feces and related wastes from animal slaughter find their ways into gutters and the so called "drainage system", the final destinations are rivers, lakes, hand dug wells and reservoirs used by people as sources of household water (Sako *et al.,* 2018).

The solute of human body and also acts a medium for undergoing many metabolic processes. Therefore it is vital for all known forms of life. If it contaminated with a variety of contaminants as in sewage water or wastewater, it may become the place for the growth of different types of microorganisms which may have water plays a key role in supporting all forms of life on earth (Ahmed *et al.,* 2022). Water acts as a solvent to dissolve potential for spreading a variety of diseases. Sewage water is nothing but the unprocessed water collected from different sources such as domestic sources, hospitals and industries (Mustafa and Hassan, 2024). Depending on the location, sewage water may contain an array of substances either in solution form or in solid. The different types of waste materials in sewage water include both organic and inorganic wastes, nutrients, toxic chemicals, oils and many more components. Even though sewage contains a lot of wastes, the microorganisms will grow by utilizing the organic and inorganic wastes. These types of microorganisms will have a specific type of characteristics compared to bacteria growing in other environments. Microorganisms are well known for both positive and negative properties. They are used on a wide scale in the food industry, biotechnology, and modern genetic engineering (Ranveer *et al.,* 2024).

However, some microorganisms can cause food spoilage or serious disease. Because of these two negative effects, the identification of microorganisms in real samples represents a very important area of focus. Municipal sewage water is a location where different types of substrates from different sources were merged and hence it may act as a best medium for the proliferation of numerous microorganisms. These microorganisms which expose to a variety of substrates will develop novel mechanisms for utilizing them and hence can become a better source for studying their novel mechanisms, novel enzymes and novel bioactive compounds. Therefore it is important to know which types of bacterial genus are present in the sewage water and there after it is possible to carry out specific studies such as isolation and characterization of specific enzymes or novel bioactive molecules produced by the isolated bacteria (Maithani *et al.,* 2022). Sewage water itself is a collection of a mixture of contaminants including organic and inorganic materials, trace quantities of toxic materials, drugs, salts and many more along with several bacteria which can develop resistance to many contaminants including antibiotics (Shahi Khalaf Ansar *et al.,* 2023). Sewage water commonly includes the bacteria belonging to coliforms, clostridia, enterococcus, lacto bacilli, micrococci, proteus, pseudomonas, streptococcus and staphylococcus. These bacteria may produce certain toxins which can be responsible for causing different types of illness or it is also possible that they may produce some industrially important bioactive molecules.

The present study reveals to isolate, characterize and identification of microorganisms from drainage water obtained from our Yogi Vemana University (boys hostel and girls hostel).

2. Methodology

2.1. Collection of Samples

Yogi Vemana University is located in Pendlimarri mandal; Kadapa district of Andhra Pradesh at a distance of 20 km from Kadapa. The sewage water/drainage water samples (sample 1 (girls hostel) and sample 2 from boys hostel) were collected from around the Yogi Vemana University and were processed.

2.2. Physicochemical Properties

The pH of samples was determined. Briefly, 10g of each sewage water sample was suspended in 20 ml of deionized water and it was allowed to stand for 15 min, then the pH was measured by using digital pH meter. Conductivity of the samples was recorded with an electro-conductivity meter. The total dissolved salts are measured by following the method mentioned in Ren *et al.,* (2024).

2.3. Isolation of Microorganisms from Sewage

1g of the sample was suspended in 9 ml of sterile distilled water and shaken vigorously 3-4 minutes. The sewage water suspension was serially diluted in sterile distilled water and the dilutions from 10^{-3} to 10^{-5} were then plated on Luria-Bertani agar medium (Peptone-2.5g, Yeast extract-1.5g, Nacl-14g, Agar-5g, Distilled water 250ml, pH-7.5) using spread plate method and incubated at $37^{0}C$ for 24 hours. Colonies giving clear zone of inhibition were isolated and re-streaked over a fresh media plate for pure cultures. Different colonies having zones of inhibition were picked and streaked on separate Luria-Bertani agar plates to get pure cultures. These colonies were sent for screening. All the strains were stored at $4^{0}C$.

2.4. Identification and Characterization of the Sewage Bacteria

Selected sewage isolates were grown on MacConkey agar media (HiMedia, India). The shape and colours of the colonies were examined under the microscope after gram staining. Isolates were biochemically analyzed for the activities of indole, methyl red, citrate utilization test, catalase, urease, V-P test, hydrolysis of gelatin test, amylase production test, hydrogen sulfide test (Seldin and Penido, 1986).

2.5. Heavy Metal Analysis

Heavy metal (iron, manganese, zinc, copper, cadmium, lead, and silver) analysis of sewage was conducted by Flame atomic absorption spectroscopy (Nazir *et al.,* 2015).

2.6. Effect of Heavy Metals on Bacterial Growth

The effects of different concentrations of selected heavy metals viz., iron, manganese, zinc, copper, cadmium, lead, and silver on bacterial populations from two samples, in triplicate, were determined. The final concentrations of each metal 1 mM, 2 mM, 3 mM, 4 mM, 5 mM and 6 mM were added to bacterial samples (1, 2, 3, 4, 5, 6) and incubated at room temperature ($28\pm4^{0}C$) for 24 and 48 hours (Madakka *et al.,* 2021).

2.7. Molecular Characterization of Heavy Metal Resistant Bacteria

Molecular identification of heavy metal resistant bacteria in sewage water samples was conducted by 16s rRNA method (Raja *et al.,* 2009).

3. Results and Discussion

3.1. Physicochemical, Nutrients and Heavy Metal Analysis of Sewage Samples

The heavy metal data generated from sewage samples of present site are shown in table 1 and patterns of concentrations of the heavy metals found from different sites are as follows. The site 1 sample exhibited the higher concentration of metals in the following order: Cu>Pb>Fe>Zn>Mn>Cd>Ag; the site 2 sample showed the higher concentration of metals in the following order: Cu>Pb>Fe>Zn>Cd>Mn>Ag. Among seven heavy metals copper found to be at higher concentration levels from two sites, which were expressed in terms of (mg/L^{-1}).

3.2. Physicochemical Properties

The physicochemical parameters of the effluents investigated are presented in Table 1.

Table 1. Physicochemical properties of sewage water samples.

Sample	pH	EC (dSm^{-1})	TDS	DO	BOD	COD
Site 1	7.62	4920	3149	1.49	2.42	366
Site 2	7.32	1920	1229	1.56	1.84	210

Table 2. Major nutrients identified in the sewage samples.

Major nutrients (mg/L^{-1})			
Sample	**Nitrate**	**Phosphorous**	**Potassium**
Site 1	0.58	3.60	7.46
Site 2	0.30	2.20	4.42

Table 3. Heavy metals identified in the sewage samples.

Sample	(mg/L^{-1})						
	Fe	**Mn**	**Zn**	**Cu**	**Cd**	**Pb**	**Ag**
Site 1	1.84	0.44	1.14	4.22	0.20	2.10	Traces
Site 2	1.12	0.10	0.84	3.24	0.11	1.64	Traces

3.3. Isolation of Microorganism from Study Site

Bacteria were isolated from the sewage water samples on LB agar media through spread plate method by taking serial dilutions. Serial dilution 10^{-4} tube was used for further culture studies as it was showing sufficient growth of bacteria without any contamination. Colonies were observed on the surface of LB agar petri plate very clear and white colonies. Single colonies from the spread plate were taken and streaking was done by streak plate method for getting individual colonies depicted in figure 1. Total six bacteria were isolated from site 1 and 2.

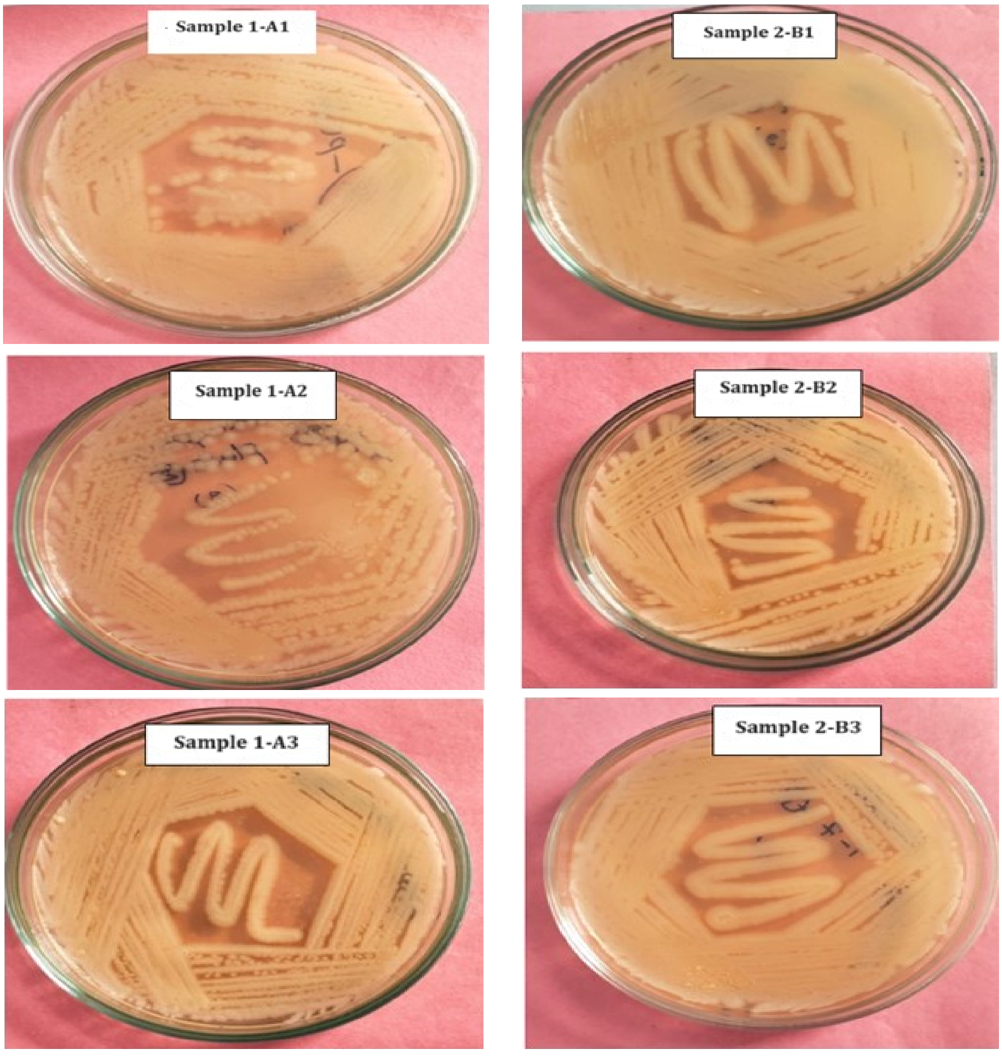

Figure 1. Six bacterial colonies isolated from site 1 and 2.

The isolated bacteria were analysed by biochemical tests which are shown in table 4.

Table 4. Biochemical test for isolated bacteria.

S/N	Indole test	Methyl red test	Voges–Proskauer test	Urease test	Amylase production test	Catalase test	Hydrolysis of gelatin	Hydrogen sulfide production test	Citrate test
A1	+	+	-	-	-	-	+	+	-
A2	-	+	-	+	-	+	+	-	+
A3	-	+	+	-	-	+	+	+	+
B1	-	-	-	+	-	-	-	-	-
B2	+	-	+	-	-	+	-	+	-
B3	-	+	-	-	-	+	-	-	+

3.4. Identification of Heavy Metal Resistant Bacteria by 16s rRNA

From six isolates, B2 isolate showed more resistant to heavy metals. Sample 2 (B2) *Bacillus pseudomycoides* shows higher population by tolerating Pb at 6mM, Zn at 5mM, Mn at 6mM, Cu at 5mM, and Cd at 6mM concentration among the 6 isolates of bacteria (Figure 1).

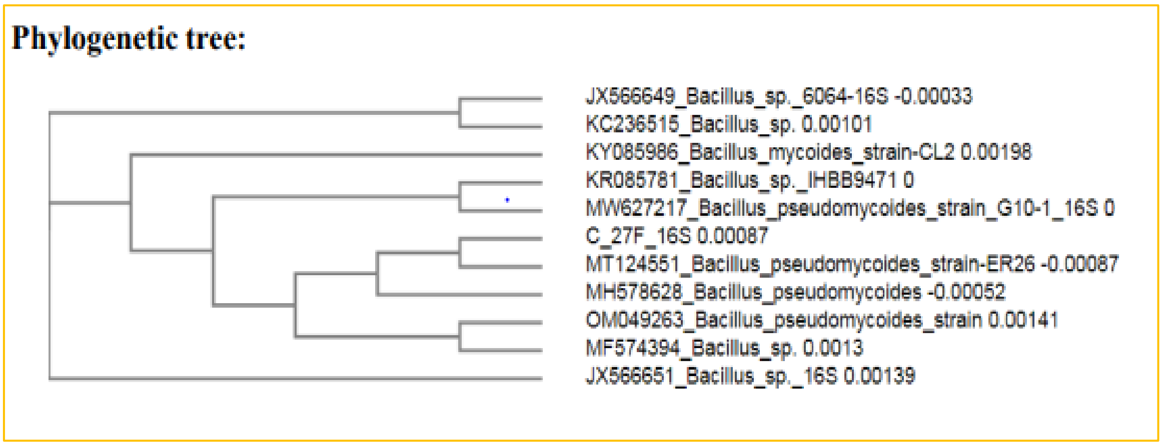

Figure 2. Phylogenetic tree of heavy metal resistant bacteria (*Bacillus pseudomycoides*) from site 1.

4. Conclusion

Heavy metals are stable and persistence environmental contaminant of sewage, which results in total loads of pollutants being delivered to the environment. Heavy metal pollution in aquatic environment and subsequent uptake in food chain by aquatic organisms and humans put public health at risks. However, even at lower concentrations heavy metals like Cd, Ag, Mn, Zn, Cu, Fe and Pb may exhibit extreme toxicity under certain condition. Thus, this makes regular monitoring of aquatic environment to be more imperative and necessary. The present study was designed with an aim to discover and identify bacterial strains from sewage samples of Yogi Vemana University (girls and boys hostels). We used serial dilution technique for isolation of sewage bacteria, streak plate technique used for screening and biochemical assays for characterization and the 16S rRNA sequencing approach for phylogenetic characterization. Heavy metals (iron, manganese, zinc, copper, cadmium, lead, and silver) are identified to know the effect of heavy metals toxicity on bacterial population growth. Among the six isolated bacterial cultures, the organism from the sample 2 (organism 3) shown higher tolerance to heavy metals and showed higher growth population than the other 5 organisms. The organism-B3 from sample 2 showing higher growth population was identified by molecular characterization as *Bacillus pseudomycoides.*

Declarations

Acknowledgements: We would like to thank the head, Department of Biotechnology and Bioinformatics and Department of Botany for providing necessary facilities during the course of this study.

Author Contributions: SS: Concept, design, literature survey, prepared first draft of manuscript, data collection, data analysis, manuscript preparation and submission of article; MK: Concept, literature survey,

manuscript revision; PNS: Concept, design, literature survey, manuscript preparation, manuscript review; MM: Concept, design, manuscript revision.
Conflict of Interest: The authors declare no conflict of interest.
Consent to Publish: The authors agree to publish the paper in International Journal of Recent Innovations in Academic Research.
Data Availability Statement: The data presented in this study are available upon request from the corresponding author.
Funding: This research received no external funding.
Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.
Research Content: The research content of manuscript is original and has not been published elsewhere.

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Citation: Shaik Shahina, Meenugula Kana, Philomina, N.S. and Madakka, M. 2024. Isolation, Identification and Characterization of Heavy Metal Bacterial Communities from Sewage. International Journal of Recent Innovations in Academic Research, 8(6): 1-7.