

Isolation of Metal Tolerant Microorganisms from the Municipal Solid Waste of Kolhapur

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Abstract: Waste generation and its control have taken an important role in our environment. With the doubling of population and changing lifestyle pattern of the inhabitants the quantity of municipal waste generating is increasing at an alarming rate. Most of this waste is subjected to dumping in specified disposal yards. The greatest challenge to the environmentalists is the eco-friendly management of this waste and application of microorganisms in this context has got an edge over other available technologies. Organic waste is consumed by the bacteria and is no longer present to produce odors, sludge, pollution or unsightly mess.

When bacteria consume waste, they convert the waste into safe by-products and in due course of this conversion they actually produce several metabolites to break down the complex waste into simple compounds. Soil microorganisms are increasingly becoming an important molecule. Extent of microbial diversity in nature is still largely unknown, thus there might be many more useful products yet to be identified from soil microorganisms. In soil 80-90% of microorganisms remain undisturbed whereas these biological communities are known to play a dominant role in maintaining a sustainable biosphere. Today both academic and industrial interest in soil bacteria (due to their several advantages over other microorganisms) is on the rise in search of deriving these unique biologically active metabolites and novel commercially important products from them. Bacteria are present in diverse ecological habitats.

They are considered highly valuable as they are used in fermentation processes such as brewing, baking cheese and butter manufacturing, chemical manufacturing such as ethanol, acetone, organic acid, enzymes, perfumes etc. Microbial mining and they produce various antibiotics, vaccines, steroids as well as other therapeutically useful compound useful components with diverse biological activities. Hence there is an immense possibility to screen effective bacterial strains from waste dump sites with valuable applications. To cope up with the demand for new organisms with properties of production of unique enzymes or molecules for industrial application and waste degradation there have been a constant effort in isolating novel bacteria from diverse environment. Accordingly, the present study was aimed to investigate bacterial strains from a waste dump site with the ultimate objective of waste degradation and discovering novel bioactive compounds for industrial application. In this a heavy metal tolerance of isolated bacteria is seen and also the effect of this isolated strain on the waste water was studied.

Keywords: Metal Tolerant Microorganisms, Municipal Solid Waste

1. Introduction

A. Applications

- The isolated species can be used to treat waste water.
- Waste can be used as a perfect advancement media for development of various microorganisms.
- By fermenting waste various enzymes can be isolated.
- Bioremediation for chemical and petroleum industrial solid wastes.

2. Aim and objective of the work

A. Aim

“Isolation of metal tolerant microorganisms from the municipal solid waste of Kolhapur.”

B. Objective

- To collect the municipal waste sample and isolate the bacteria from this sample.
- To study the morphological characters, Gram nature, and motility of isolated bacteria.
- To study heavy metal tolerance of isolated bacteria.
- To study the degradation of waste from polluted water by organisms.

3. Review of literature

- Golamari Siva Reddy et.al/ J. pharm. sci & vol.9 (9), 2017, 1490-1497. Isolation and characterization of bacteria from compost for municipal solid waste from Gunter and Vijayawada. In this paper the bacterial strains from waste dump destinations with a definitive goal of waste corruption and finding new valuable bioactive mixes for modern application was studied.
- Journal of microbiology and experimentation in this the study of isolation and characterization of bacteria isolated from municipal solid waste for production of industrial enzymes and waste degradation was carried out.
- In research gate the study of isolated and characterization of effective bacteria for solid waste degradation for organic manure was studied.
- In international journal of science inventions today, isolation of metal resistant bacteria from municipal solid

waste dumpsite, Madurai was studied.

- In open access international journal of science and engineering a critical review of biotechnology for solid waste management was studied.

4. Material

A. Study Area

Soil test was gathered from a site of Kolhapur Green energy PVT.LTD (municipal solid wastes management project, Client-Kolhapur municipal corporation.) in Kolhapur district of Maharashtra in India.

1) Collection of samples

An aggregate of ten waste examples was gathered from dumping site of Garbage in the Kolhapur Green energy PVT. LTD site. Test sample was gathered in sterile zip-lock plastic bag kept in aseptic conditions at 40C and labelled with respect to their source and area. The gathered examples were conveyed to the lab for selection of soil microscopic organisms.



5. Methods

A. Isolation of bacteria from waste samples

For isolation of bacteria serial dilution method is used. In this method take 10 test tubes mark it as 10-1, 10-2....,10-10 and add 10ml sterile distilled water in first tube and 9ml distilled water in remaining tubes. After this 1gm of waste soil sample is weighed and added in the first tube. Shake this tube for some time. Allow the soil to settle and pickup 1ml of water sample and transfer into 2nd tube. The all procedure was repeated up to the last tube. After dilution take 0.1ml of dilution of each tube spread on Nutrient agar plate (It contain 0.5% peptone, 0.3% yeast separate, 0.5% NaCl, 0.25% glucose, 1.5% agar, D/W and pH was adjusted to 7 at room temperature.) and incubate at 370 C for 24 hours. After incubation well isolated colonies were seen from these 5 well isolated colonies were selected and further studied morphological characters, Gram nature and motility. Further this selected colony was restriking on the Nutrient agar slant (stock) and preserve it in Freezer for further studies of this organisms.

1) Metal tolerance assay

Investigation of MIC (Minimum Inhibitory concentration) of substantial metals viz, lend (Pb), zinc (Zn) and Mercury (Hg) was helped out for the bacterial strains through cup Assay

technique. The cleaned Nutrient Agar (NA) medium (it contain 0.5% peptone, 0.3% yeast separate, 0.5% NaCl, 0.25% glucose, 1.5% agar, D/W and pH was adjusted to 7 at room temperature.) was sit up for bacterial development. For this a spread plate method was used. On this with the help of sterile glass borer prepare a wells and on each well mark with particular metal and add 0.01ml of solution of respective metals. After that keep it them in deep freezer for diffusion of metal for 10 minutes. After diffusion put this plate in incubator at 370 C temperature for 24 hours. The inhibitory zones around the well was observed on the plate for some metals.

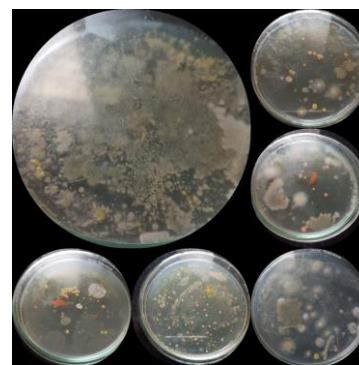
2) Waste water degradation potential of selected bacterial samples

For this a 24 hours of old bacterial colony was used. In this method take 50ml of waste water sample in five conical flasks respectively. Take pH and O.D (optimum density) of the sample. Add the isolated bacterial colony in each Flask of the water sample. Put all this tube at the room temperature for 96 hours and record the O.D. at 530nm and pH after 24 hours, 72 hours and 96 hours.

6. Results and Discussion

- For the isolation of bacteria from municipal solid waste site of Kolhapur serial dilution method is used. In this a nutrient agar medium a well isolated colonies were seen. In this a five specimen namely A, B, C, D and E is selected for the further studies in this the morphological characters and gram nature of the specimen was studied which is as follows:

A. Isolated Species

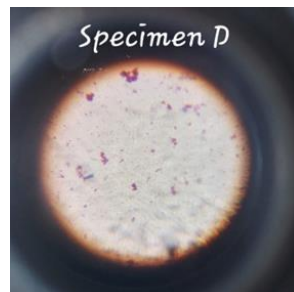
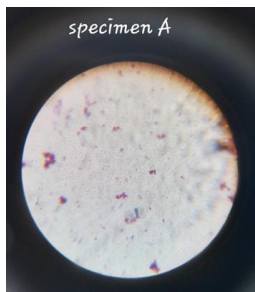


B. Morphological characters, gram nature and motility of selected species

1) Species A

Size	Shape	Colour	Elevation
4mm	Round	Red	Concave
Margin	Opacity	Surface	Consistency
Entire	Opaque	Rough	Smooth

Gram Nature	Motility
Gram Negative	Actively Motile Cells



2) Species B

Size	Shape	Colour	Elevation
3mm	Irregular	Creamy Pink	Concave
Margin	Opacity	Surface	Consistency
Irregular	Opaque	Rough	Smooth

Gram Nature	Motility
Gram Negative	Actively Motile Cells

5) Species E

Size	Shape	Colour	Elevation
3mm	Round	Orange	Concave
Margin	Opacity	Surface	Consistency
Entire	Opaque	Smooth	Smooth

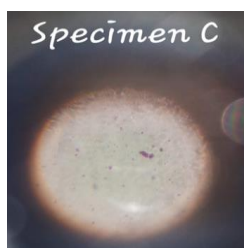
Gram Nature	Motility
Gram Negative	Actively Motile Cells



3) Species C

Size	Shape	Colour	Elevation
3mm	Round	Pink	Concave
Margin	Opacity	Surface	Consistency
Entire	Opaque	Smooth	Smooth

Gram Nature	Motility
Gram Negative	Actively Motile Cells



C. For this three heavy metals such as lead (Pb), Zinc (Zn), and mercury (Hg) was used. In this cup assay technique is used. The results obtain were as follows:

Specimen	Metal Tolerance		
	Lead(Pb)	Zinc(Zn)	Mercury(Hg)
A	Positive	Positive	Negative
B	Positive	Positive	Negative
C	Positive	Negative	Negative
D	Positive	Positive	Negative
E	Positive	Positive	Negative

4) Species D

Size	Shape	Colour	Elevation
2mm	Round	Yellow	Concave
Margin	Opacity	Surface	Consistency
Entire	Opaque	Smooth	Smooth

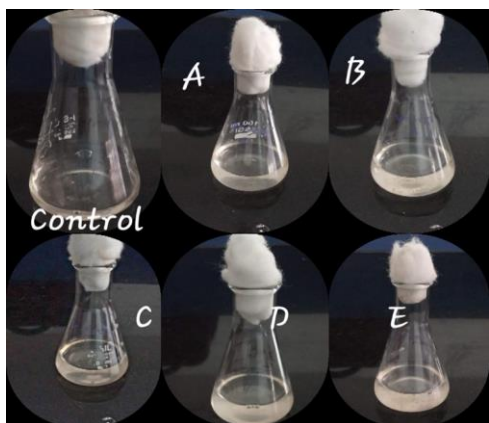
Gram Nature	Motility
Gram Negative	Actively Motile Cells



D. Waste water degradation potential of selected bacterial samples

For this 50ml of waste water sample is placed in conical flask. Check the O.D (optimum density) and PH of the sample. The result obtained were as follows. In this it is prove that the at the incubation period increases isolated species having the ability for the degradation of waste water. The results obtain were as follows:

TIME	SPECIMEN	OPTIMUM DENSITY	pH
After 24 Hours	Control	0.03	7
	A	0.02	7
	B	0.01	7
	C	0.03	7
	D	0.05	7
	E	0.03	7
After 72 Hours	Control	0.03	7
	A	0.02	7
	B	0.01	7
	C	0.02	7
	D	0.02	7
	E	0.01	7
After 96 Hours	Control	0.03	7
	A	0.01	7
	B	0.01	7
	C	0.01	7
	D	0.02	7
	E	0.01	7



7. Conclusion

- Results of the studies shows that the waste contain more number of bacterial species.
- Isolated species has capacity to tolerate some heavy metals like Lead (Pb) & Zinc (Zn).
- Isolated species do not show metal tolerance towards Mercury (Hg).
- Isolated species has potential to degrade waste water.

References

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