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Isolation Of Decomposing Bacteria from Humus Sample and Their Application

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ABSTRACT

An investigation was carried out to study the activity of decomposing bacteria on plastic and leaves. To isolate bacteria that effectively degrade waste, a sample of humus soil was collected from the ground of Shri Guru Ram Rai medical college. Three bacterial strains, namely IsoA, IsoB, and IsoC, were obtained and cultured on Nutrient agar medium (NAM). The characteristics of these strains were examined through visual observation of colonies, assessment of their cultural properties, microscopic examination, and biochemical tests for identification. A bacterial suspension was prepared to observe the impact of these bacteria on plant leaves and plastic waste. During the degradation process, noticeable changes were observed in the odour, weight, and pH of the bacterial suspension. The findings of this study suggest that beneficial bacteria can be isolated from the surrounding environment, such as humus soil, to facilitate the eco-friendly conversion of waste materials.

1. Introduction

The degradation of organic waste materials, such as plastic and plant leaves, is a significant environmental concern. The accumulation of non-biodegradable waste in landfills and natural habitats has detrimental effects on ecosystems and human health. Therefore, there is an urgent need to develop eco-friendly methods for waste management and disposal.

One potential solution lies in harnessing the power of decomposing bacteria. These microorganisms possess the enzymatic capability to break down complex organic compounds into simpler, more environmentally friendly forms. By isolating and studying these bacteria, we can gain insights into their mechanisms of action and potentially utilise them for the efficient degradation of waste materials.

Humus soil, rich in organic matter and microorganisms, can serve as a potential source for the isolation of decomposing bacteria. The diverse microbial community in humus soil is likely to harbour bacteria with the ability to degrade various types of waste, including plastic and plant leaves. By isolating and characterising these bacteria, we can identify potential candidates for eco-friendly waste management strategies.

The objective of this thesis is to isolate decomposing bacteria from a humus soil sample collected from the ground of Shri Guru Ram Rai medical college and study their application in the degradation of waste materials. By studying the cultural characteristics, microscopic features, and biochemical properties of the isolated bacteria, we aim to identify their taxonomic classification and evaluate their potential for waste degradation.

Furthermore, we will investigate the effect of the isolated bacteria on the degradation of plant leaves and plastic waste through observations of changes in odour, weight, and pH of bacterial suspensions during

the degradation process. The goal of this research is to add to the expanding corpus of information about the possible use of beneficial bacteria in environmentally friendly waste management techniques.
By understanding the capabilities and limitations of these decomposing bacteria, we can explore innovative strategies for waste management and contribute to the development of sustainable solutions for environmental preservation. The findings of this study may have implications for industries, municipalities, and individuals seeking to minimise their environmental impact through effective waste management practices

2. Material & methods

2.1. Sample Collection

A humus sample was collected from Medical college ground of Shri Guru Ram Rai University Dehradun, Uttarakhand. A 100gm sample was collected with the help of sterile spatula in a cap tube and marked accordingly.

2.2 Culture Method

A dilution was prepared of 10-1 to 10-6 and marked sequentially, soil was added in 10-2 dilution blank and then vigorously shaken so that the soil mixed up properly. After 1 minute with a sterile pipette, 1 ml of dilution from 10-2 is transferred to 10-3 tube then from 10-3 tube to 10-4 , then from 10-4 tube to 10-5 tube and then lastly from 10-5 tube to 10-6 tube.
Following this procedure, 1 cc of dilution fluid from each dilution tube was transferred to several culture plates, and these were then incubated for 24-48 hours at 37 °C to ensure optimal growth. After proper growth of microorganisms in culture plates. Viable growth of different colonies were observed and marked IsoA, IsoB and IsoC; their cultural characteristics were observed and afterwards Gram's staining was performed and cellular morphology and Gram's reaction was observed under the microscope.

2.3 Pure culture

The marked colonies were re-streaked as a primary inoculant on different Nutrient agar plates to obtain a pure culture of them and named plates accordingly i.e IsoA, IsoB & IsoC and incubated for 24 hours at 37o C to obtain proper growth. After that again their cultural characteristics were observed and Gram's staining was performed to Re-check the identical cell morphology to do comparison with the original colony and pure cultures were obtained. After achieving the pure cultures, isolated bacteria slants were prepared for further experiments.

2.4 Biochemical test

2.4.1 Catalase test

In this test enzyme catalase helps in breakdown of hydrogen peroxide into oxygen and water. 2H2O2 2H2O + O2 (gas bubbles) When the small bacterial inoculum is introduced into the hydrogen peroxide, the sudden development of oxygen bubbles indicates the presence of catalase enzymes. Lack of oxygen bubbles indicates the lack of catalase enzymes. Not all bacteria possess the catalase enzyme.

2.4.2 Indole test

The capacity of a microbe to convert tryptophan into indole is ascertained by a biochemical test conducted

on bacterial species. Tryptophan is basically a type of amino acid which go through deamination by bacteria that have tryptophan enzyme.

Positive result – The appearance of red or red-violet colour ring.
Negative result – The appearance of a yellow colour ring.

2.4.3 MR-VP Test

The Methyl Red test is carried out using an infected tube of MR-VP broth and the pH indicator methyl red. The medium's buffers will be overwhelmed by the acids if the organism adopts the mixed acid fermentation strategy to produce stable acidic end products, which will result in an acidic environment. The VP test detects microorganisms that produce acetone via the butylene glycol route. Following inoculation with the organism that employs the butylene glycol route, the VP reagents are added to MR-VP broth. Potassium hydroxide (KOH) oxidises the acetoin end product, resulting in diacetyl.

2.4.4 Citrate utilisation test

The main goal of the citrate test is to find out whether bacteria can utilise inorganic ammonium hydrogen phosphate (NH4H2PO4) as a source of nitrogen and sodium citrate as their sole supply of carbon this is only feasible if the bacteria are able to ferment citrate.

Positive result – colour change is observed that is green to blue due to the reagent used Bromophenol blue.
Negative result – colour of the media remains same no colour change occurs that means very less or no growth in the medium.

2.4.5 Urease test

The enzyme urease hydrolyzes urea to produce ammonia and carbon dioxide.
Positive result : colour change is observed that is pink colour.
Negative result : no colour change means the culture medium remains yellowish**.**

2.4.6 Amylase test

Many bacteria possess the enzyme which helps to catalyse a chemical reaction outside the cell. Conversely, sources of nutrients such as carbohydrates are too large to be absorbed. Smaller molecules may separate from the cell membrane.
Positive result – After adding iodine solution, a clear zone forms around the bacterial colony line, indicating that the bacteria have hydrolyzed starch.
Negative result – Purple, Blue or black colouration of the medium depends upon the bacteria.

2.4.7 Triple sugar iron Test

This test is used in presumptive identification based on the fermentation of sucrose, glucose, and lactose, which releases gases and also shows the generation of H2S. The production of H2S production may be inhibited on TSI for organisms that utilise sucrose and inhibit the enzyme mechanism that eventually result in production of H2S. Sodium thiosulfate is converted by some bacteria in the medium to H2S, which combines with ferric ions to generate iron sulphide, an uncontrollable black precipitate.

- Yellow and the red slope : If the bacteria only uses the glucose

- Bottom and the slope turn yellow : if the bacteria use the glucose/sucrose/lactose
- Detachment of agar : production of gases like CO2 and O2
- Black precipitate : it indicates the production of H2S.

2.4.8 Casein test

By generating the proteolytic exoenzyme caseinase, some microbes are able to break down the casein protein. Casein, a protein substrate, is added to milk to augment NAM in the medium.
Positive result – There is clearing under or around the settlement.
Negative result– There's no clearance to be seen**.**

3. Result and discussion

3.1 Cultural and microscopic characteristics of the isolates

Isolated stains	**Cultural characteristics**	**Microscopic characteristics**
Iso A	Milky, Elevated	Gram positive - Long rod chain
Iso B	Yellowish elevated	Gram positive- Short and long chains
Iso C	Filamentous, Irregular, Sticky	Gram negative - Rods

3.2 Biochemical test

Experimental stains	**Biochemical tests**								
	Macconkey agar	**Catalase test**	**Indole test**	**MR-VP test**	**Citrate utilisation test**	**Urease test**	**Amylase test**	**TSI**	**Casein test**
Iso A	Lactose fermenting	(+)	(-)	(-) (-)	(+)	(-)	(+)	Acid/acid– Indicates the fermentation of dextrose, lactose/ sucrose	(+)
Iso B	Non – Lactose fermenting	(-)	(-)	(+) (-)	(+)	(-)	(+)	Yellow slant /yellow butt – Indicates the fermentation of	(+)

								dextrose, lactose/ sucrose	
Iso C	Non – Lactose fermenting	(++)	(-)	(-) (-)	(+)	(-)	(+)	Red slant/ slightly yellow butt – Indicates the fermentation of dextrose.	(+)

4. Effect of decomposing bacteria on leaves & plastics

4.1. Initial observation (at T)

	Isolated stains	**Colour**	**pH**	**Weight (gm) (Broth + Sample)**
Leaves	Iso A	Green	7	6
	Iso B	Green	7	6
	Iso C	Green	6	6
Plastic	Iso A	White	7	6
	Iso B	White	6	6
	Iso C	White	6	6
Plastic + Leaves	Iso A	Green & White	6	6
	Iso B	Green & White	7	6
	Iso C	Green & White	6	6

4.2 Final observation (at T+30)

	Isolated stains	**Colour**	**pH**	**Weight (gm) (Broth + Sample)**
Leaves	Iso A	Blackish green	8	1.7747
	Iso B	Blackish green	8	0.264
	Iso C	Blackish green	7.5	0.5301
Plastic	Iso A	Pale yellow	8	0.3021

	Iso B	Pale yellow	8.5	1.4839
	Iso C	Pale yellow	9	1.0521
Plastic +Leaves	Iso A	Blackish green + Pale yellow	8	0.16
	Iso B	Blackish green + Pale yellow	8	1.6265
	Iso C	Blackish green + Pale yellow	8	1.6658

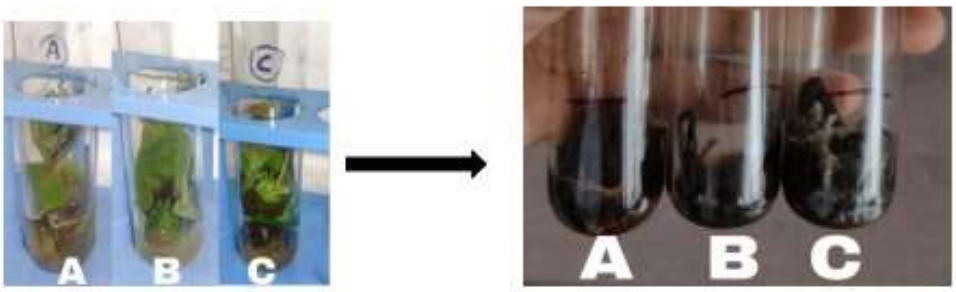

Fig. Before Fig. After

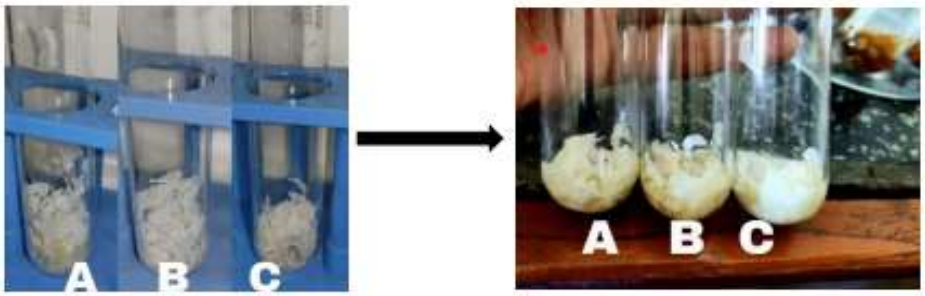

Fig. Before Fig. After

Fig. Before Fig. After

5. Conclusion

In conclusion, the microbial degradation of plastic and plant waste presents a promising environmental strategy for improving waste management without causing harm to nature and ecosystems. The advancements in biotechnology and microbiology offer new perspectives and innovative ideas for the

management and degradation of plastic and plant waste. The isolated bacteria, identified as Iso A - Bacillus species, Iso B - Clostridium species, and Iso C - Pseudomonas species, have demonstrated their potential as effective decomposers of plant and plastic waste. This study has significant implications for economically managing waste while protecting the environment. By isolating decomposing bacteria from the surrounding environment, it is possible to utilise their bio-conversion abilities for the safe disposal of solid waste, benefiting both human health and the environment. The study also observed the effects of the isolated strains, by creating bacterial suspensions, on the degradation of plant leaves and plastic waste.

6. References

1. Rahman, M. H. 2004 Composting of solid waste in Bangladesh. Proceedings of the 19th International Conference on Solid Waste Technology and Management. Philadelphia, USA)
2. (Olsen,1963; Rodin and bazilevich, 1967
3. https://www.researchgate.net/publication/serialdilution
4. KMITL Sci. Tech. J. Vol. 8 No. 2 Jul. - Dec. 2004
5. Book of biochemical tests for identification of medical bacteria by Jean F. McFadden
6. https://microbiologyinfo.com/indole-test-principle-reagents-procedure-resultinterpretation-and-limitations
7. Tille P.M. 2014. Bailey and Scott's diagnostic microbiology. Thirteen editions. Mosby, Inc., an affiliate of Elsevier Inc. 3251 Riverport Lane. St. Louis. Missouri 63043
8. https://www.austincc.edu/microbugz/starch_hydrolysis.php
9. https://www.himedialabs.com/TD/M107.pdf