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Biodegradation of Wastes Using Gut Bacteria of *Labeo rohita*

Das Madhuri Kumari* and Saxena Nayni

University Department of Zoology, Ranchi University, Ranchi 834008, India

**Corresponding Author*

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Abstract: Waste management is one of most crucial task to perform in which biodegradable wastes are the most prevalent and generated in every home. These wastes, which mostly consist of fruit and vegetable peels, papers, grains, leaf litters, and so on, are referred to as lignocellulosic biomass. Every year, roughly 220 kilograms of waste per person is generated in metropolitan areas, with 40 to 50% of the waste being biodegradable. To certain extent, effective disposal and land filling solve the problem. However, it takes a long time to degrade and, in the meantime, it produces methane, a greenhouse gas and one of the primary causes of global warming. In such case, microbes can play a crucial role in waste management. Although bacteria are already present in soil but the incorporation of potent bacteria with starch, cellulose, and protein breakdown properties can accelerate the process of degradation. Bacteria in the gut of herbivores and omnivores has the ability to produce extracellular enzymes that breaks down the food into simpler forms.

Bacteria that have been retrieved from the gastrointestinal tract of fish *Labeo rohita* were rod-shaped, Gram-positive and of *Bacillus species*. The bacteria showed significant cellulose, starch and protein breakdown capability *in vitro*. A filter paper degradation test was also done to confirm its efficacy in which a substantial result was observed. The isolated bacteria from the gut of *Labeo rohita* has the ability to breakdown key macromolecules and thus can be used as a bio-augmenting agent to speed up the waste degradation which can ultimately aid in waste management.

Keywords: *Bacillus species*, *Labeo rohita*, Lignocellulosic biomass, Extracellular enzymes

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Introduction

A clean and healthy environment is not the highest goal but most fundamental necessity of living beings. Directly or indirectly we all shares the same environment and are dependent on it. With the world's growing population, wastes has become one of the biggest problems to deal. Though, waste produced by humans has a wide dimension but among them, biodegradable wastes are most common. These waste are produced in every household that mainly contains fruits and

vegetable peels, grains, papers, leaf litters etc. and are termed as lignocellulosic biomass (Knauf and Moniruzzaman, 2004; Maki *et al*., 2009).

Kumar *et al.* (2009) has reported that in metro cities of India, around 220 kg of wastes per person are being produced every year. Among these wastes 40 to 50% are biodegradable wastes. Proper disposal and landfilling solves the problem to some extent however, degradation still takes a long time. In such case microbes can play a significant role in waste degradation. The present study aims to isolate bacteria from the gut of fish *Labeo rohita* that can degrade major biomolecules.

Materials and Methods

Healthy *Labeo rohita* was selected and bought from Fish Farmers Training Centre, Shalimar, Dhurwa, Ranchi. The fish was starved for 24 h in the laboratory so that its gut would get cleaned before dissection (Das and Tripathi, 1991; Madina *et al*., 2023)

Collection of bacteria:

The outer body surface of *Labeo rohita* was thoroughly cleaned with ethanol. With the help of sterilized scissor, ventral portion of fish was opened and gut was removed. After washing the gut thrice with distilled water, outer surface of gut was cleaned with ethanol soaked cotton. 1 g of intestinal portion was homogenized in 9 ml of distilled water. After that homogenate was centrifuged and 1 ml of cell free supernatant (CFS) was collected. This CFS was then serially diluted using serial dilution method followed by Ben and Davidson (2014). The CFS was diluted up to 6^{th} dilution and from the 6^{th} dilution 1 ml of solution was plated on NA media plate and left for incubation for 24 h at 37°C. After 24 h, using streak plate method, the selected bacterial colonies were purified (Ruangpan and Tendencia, 2004).

Identification of bacteria:

Using the Bergey's manual of determinative bacteriology (Bergey, 1994), the isolated bacteria were identified. The identification was done by careful observation of colony morphology of bacteria and Gram staining (Beveridge, 2001).

Screening of cellulose, starch and protein degradation:

Preparation of CMC (carboxymethyl cellulose) media was done using CMC, $MgSO_4$, $NaNO_3$, KCl, yeast extract, glucose and agar. Plates were then prepared and isolated bacteria were inoculated with the help of sterilized inoculation loop. After that, inoculated plates were sealed and incubated at 37°C for 48 h.

After 48 h, bacterial growth was observed. Plates were then flooded with Congo red solution (1%) and left for 2 to 3 min. Then the plates were thoroughly washed 5 to 6 times with one mole (1M) of Sodium chloride solution (NaCl) and at last result was observed.

Screening of starch degradation:

Starch degradation was tested using starch agar media (Lal and Cheeptham, 2012). Isolated bacteria were inoculated on the surface of media and sealed. After 48 hours of incubation at 37°C, iodine solution was poured on the plates and after a minute, solution was drained and result was observed.

Screening of protein degradation:

Gelatin stab was prepared in the test tubes. Gelatin powder, peptone and beef extract was dissolved in distilled water and transferred in test tubes and autoclaved. After cooling the stab, when media gets solidified, one loop full of bacteria was inoculated in the gel up to the depth of half inch and incubated for 10 days at room temperature. Result was observed comparing the control.

Filter paper degradation test:

Bacteria that were isolated from the gut of *Labeo rohita* was tested to see filter paper degrading capacity. Two conical flasks were taken and 25 ml of distilled water was poured in each conical flask. Whatman filter paper was cut into 8×1 cm and placed in the flask. Each flask containing 2 strips of filter paper of weight 186 mg.

Fig. 1: Growth of bacterial colonies.

Table 1: Showing result of different tests

S. No.	Test	Isolate 1	Isolate2
1.	Gram staining	+	+
2.	Cellulose degradation test	-	+
3.	Starch degradation test	+	+
4.	Protein degradation test	+	+

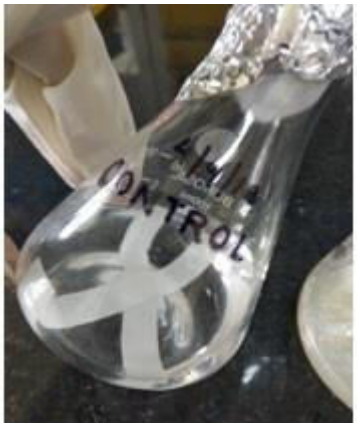

Fig. 2: Filter paper degradation test (control).

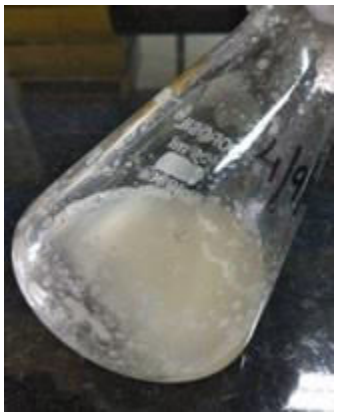

Fig. 3: Filter paper degradation test (Day 12).

In one flask five loop full of each isolated bacteria was inoculated and another flask was kept as control. The initial weight of two filter papers was 186 mg. Filter paper was weighed in four days of interval until degraded completely.

Results

Morphological analysis of isolated bacterial colonies (Fig. 1) revealed that the two varieties of bacteria were isolated from the gut of *Labeo rohita*, that were labelled as Isolate 1 and Isolate 2. Colony morphology of Isolate 1 was quite large, circular, smooth edged and clear white in colour with little elevation, whereas, colonies of Isolate 2 had only difference in coloration which was of cream colour. Gram staining revealed that both the bacteria were gram positive *Bacillus* bacteria.

Cellulose, starch and protein are the major biomolecules that have a specific molecular structure and arrangement. Cellulose degradation test showed a clear zone around the bacterial growth of Isolate 2 after staining with Congo red (1%), which indicated the positive cellulose degradation capacity of bacteria whereas, Isolate 1 did not show any such clear zone, hence showed a negative result. A positive result was found in starch degradation test in which a clear zone was observed around the bacterial growth of both the

Table 2: Showing result of filter paper degradation in (mg)

S. No.	Days	Weight of filter paper(control)	Weight of filter paper(test)
1.	Day 0	186 mg	186 mg
2.	Day 4	186 mg	121 mg
3.	Day 8	186 mg	73 mg
4.	Day 12	186 mg	00 mg

isolates after treating with iodine solution (Table 1).

So far, the protein degradation test is concerned, the gelatin stab were found to be liquefied in case of both the bacteria whereas, no liquefaction was observed in control, which indicated a positive test result.

Whatman filter paper is mainly composed of cellulose (~98%). The isolated bacteria showed significant degradation of filter paper in 12 days (Figs. 2, 3). The result was confirmed by comparing the test with the control as shown in Table 2. The weight of filter paper was also taken at intervals to authenticate the result.

Discussion

In past few years, production of waste has become one of the biggest issue for the environment. Generation of waste is directly proportional to the human population. According to United Nation data, India has now become one of the most populated country in the world. Thus, it would not be an exaggeration if we say that much more waste could be generated in near future. Proper waste disposal helps to certain extent however, it is a slow process. The most suitable and eco-friendly way of waste management would be natural decomposition.

In such case, bacteria having the capacity to degrade the waste can be used in degradation process. This addition of microbes is termed as bio-augmentation. It has been reported that bacteria has the ability to degrade major biomolecules (Ray *et al*., 2012; Liu *et al*., 2016). Haque *et al*. (2022) and Arun *et al*. (2021) have reported that certain bacteria are able to degrade heavy metals as well.

Biodegradable wastes mainly contain fruits and vegetable peels, papers, leaf litters, fabrics etc. The major biomolecules in such wastes are starch, cellulose, protein. Though degradation takes place naturally in environment but it is a time taking process, in this case bio-augmentation of potent bacteria can be helpful. The two isolated bacteria in the present investigation showed significant degradation of biopolymers.

The bacteria were Gram positive and of *Bacillus species*. Considering the fast degradation property of the bacteria, it can be used as a bio-augmenting agent which can play a significant role in waste management.

Conclusion

In this study focus was basically put on the isolation of such bacteria that are able to degrade natural polymers found in wastes. Lignocellulosic biomass is the biodegradable component that usually degrade naturally, but the rate of degradation is quite slow. In present investigation the isolated Gram positive *Bacillus* bacteria from the gut of *Labeo rohita* showed the positive starch, cellulose and protein degradation test result. Filter paper degradation test also showed significant result. Thus, due to the noteworthy ability of degradation, the isolated bacteria can be utilised as a bio augmenting agent that will lead to the breakdown of waste in an proficient manner.

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