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# Isolation of protease producing lactic acid bacterial isolates from shrimp

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**Abstract**
*In-vitro* studies were conducted to isolate protease producing lactic acid bacterial isolates from shrimp. Twenty seven lactic acid bacterial isolates were isolated from shrimp and shrimp waste using MRS agar media. Among the 27, 10 isolates were found to be proteolytic. Finally, the best isolate (LABS-06) was selected by calculating the protease enzyme activity. The highest extracellular protease enzyme produced was (23.84 U/ml) by the isolate LABS-06.

**Keywords:** MRS agar, lactic acid bacteria, protease enzyme, enzyme activity, enzyme potency

## Introduction
Proteolytic enzymes catalyze the cleavage of peptide bonds in proteins. Proteases are degradative enzymes which hydrolyse peptide bonds in proteins and generate amino acids and small molecular weight peptides. They are also called peptidases or proteinases. They are ubiquitous in nature. They are found in all living organisms such as plants, animals and microbes. However, the microbial production of proteases is preferred over other sources because microbes can be grown quickly and require a small place (Kauffman *et al*., 2007) [3].
The inability of the plant and animal proteases to meet current world demands has led to an increased interest in microbial proteases. Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity and their susceptibility to genetic manipulation. Microbial proteases account for approximately 40 per cent of the total worldwide enzyme sales (Anbu *et al*., 2017) [1]. Proteases from microbial sources are preferred over the enzymes from plant and animal sources since they possess almost all the characteristics desired for their biotechnological applications because of their rapid growth, the limited space required for their cultivation, and the ease with which they can be genetically manipulated to generate new enzymes with altered properties that are desirable for their various applications (Godfrey and West, 1996) [2].
Shrimp processing is one of the major food industries in India and its waste is the single largest industrial fish waste in the country, causing diverse environmental problems. Shrimp shell waste is a good economic source of protein, lipid, calcium carbonate, chitin and carotenoids. A recent innovation in fisheries and shrimp waste management is the astaxanthin sorption process, where a solid waste like fish scales is used as a natural adsorbent for a carotenoid pigment astaxanthin from the seafood industry wastewater (Stepnowski *et al.*, 2004) [6]. Utilization of shrimp processing waste also provides an ample scope for value addition and enhances its value as an agricultural commodity.

## Material and Methods
The experiment was conducted in the Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Dharwad.

### Isolation and screening of efficient proteolytic bacteria
### Sample collection
Shrimp and shrimp waste was collected from M/s Fouress Foods Pvt. Ltd., Madanageri, Ankola, Uttara Kannada, Karnataka and was stored under refrigerated condition.

### Reference proteolytic strain collection
The reference strain for proteolytic activity *Bacillus* sp. (MTCC-1747) was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh**.**

**Qualitative screening**

Lactic acid bacteria (LAB) from shrimp and shrimp waste were isolated using MRS agar media. After isolation, these LAB isolates were plated out on casein agar media. A clear zone around the bacterial colony growth indicated proteolytic activity of the isolates. The zone of hydrolysis was noted for each colony and the protease enzyme potency was calculated. The promising bacterial isolates (10) were selected based on the protease enzyme potency values. The proteolytic enzyme potency was calculated using the formula given below (Vijayshree *et al*., 2015) [7].

$$\text{Protease enzyme potency (\%)} = \frac{\text{Total diameter} - \text{Diameter of the colony}}{\text{Diameter of the colony}} \times 100$$

**Quantification of the enzyme**

Protease enzyme activity by selected isolates was quantified using the method followed by Khembavi *et al*. (1993) [4]. An aliquot of 0.5 ml of enzyme solution was mixed with 0.5 ml substrate (1% Hammerstein casein in 50 mM Tris-Cl buffer, pH 9) and incubated at 60 ºC for 30 min. The reaction was stopped by the addition of 0.5 ml 20 per cent trichloroactic acid and kept for 10 min at 25 ºC in order to complete the reaction, centrifuged at 10,000 rpm at 4 ºC for 15 min and the absorbance was measured at 280 nm. One unit of protease activity is defined as the amount of enzyme required to liberate 1 µg tyrosine per ml per minute. The best isolate showing the highest activity was selected for further studies.

Protease enzyme activity was calculated using the formula given below.

$$\text{Enzyme activity (Units/ml)} = \frac{\text{Micro mole tyrosine equivalent released} \times \text{total volume of assay}}{\text{Volume of enzyme taken} \times \text{incubation time}}$$

## Results

**Isolation of proteolytic lactic acid bacteria from shrimp**

The colonies which were positive for proteolytic activity had shown zone of hydrolysis on casein agar media were purified on MRS agar media. A total of 27 LAB isolates were isolated among which 10 isolates showed proteolytic activity.

**Qualitative screening**

Of the 27 LAB isolates, 10 isolates showed zone of protein hydrolysis on casein agar media in varied levels. The enzyme potency varied from 23.50 to 81.10 per cent. Results are depicted in Table 1.

The ten proteolytic isolates were selected for screening the quantity of protease produced, *viz*., LABS-01 (30.4%), LABS-05 (44.9%), LABS-06 (81.1%), LABS-08 (23.5%), LABS-09 (77.5%), LABS-11 (75.1%), LABS-13 (74.1%), LABS-15 (60%), LABS-18 (72.2%) and LABS-24 (35.2%).

**Quantification of protease enzyme activity**

Enzyme quantification was done for the ten proteolytic isolates which were selected based on the qualitative protease enzyme screening. Isolate LABS-06 was found to produce the highest protease enzyme activity (23.84 U/ml) followed by the isolate LABS-09 (22.59 U/ml) and the least (9.35 U/ml) by isolate LABS-08. Results are depicted in Table 2.

## Discussion

In the present study, protease producing lactic acid bacteria were isolated using shrimp and shrimp waste. As many as 27 LAB were isolated among which 10 were found to be proteolytic and their enzyme potency recorded.

The 10 protease producing LAB isolates were quantified for their protease enzyme activity. Similarly (Pallavi *et al.*, 2014) [5], a total of 23 protease positive bacteria were isolated and based on the zone of hydrolysis, top five protease positive isolates were selected for enzyme quantification.

In the present study, the isolate LABS-06 showed the highest enzyme activity (23.84 U/ml) after 48 h of incubation (Table-4). In the media, casien was used as the protein source.

**Table 1:** Proteolytic activity of the isolated LAB isolates

| Sl. No. | Isolate code no. | Proteolytic activity | Total diameter (mm) | Colony diameter (mm) | Zone of hydrolysis (mm) | Enzyme potency (%) |
|---|---|---|---|---|---|---|
| 1 | LABS-01 | + | 15.0 | 11.5 | 3.5 | 30.40 |
| 2 | LABS-02 | - | | | | |
| 3 | LABS-03 | - | | | | |
| 4 | LABS-04 | - | | | | |
| 5 | LABS-05 | + | 10.0 | 6.9 | 3.1 | 44.90 |
| 6 | LABS-06 | + | 23.0 | 12.7 | 10.3 | 81.10 |
| 7 | LABS-07 | - | | | | |
| 8 | LABS-08 | + | 13.1 | 10.6 | 2.5 | 23.50 |
| 9 | LABS-09 | + | 22.9 | 12.9 | 10.0 | 77.52 |
| 10 | LABS-10 | - | | | | |
| 11 | LABS-11 | + | 22.6 | 12.9 | 9.7 | 75.19 |
| 12 | LABS-12 | - | | | | |
| 13 | LABS-13 | + | 21.6 | 12.4 | 9.2 | 74.19 |
| 14 | LABS-14 | - | | | | |
| 15 | LABS-15 | + | 20.0 | 12.5 | 7.5 | 60.00 |
| 16 | LABS-16 | - | | | | |
| 17 | LABS-17 | - | | | | |
| 18 | LABS-18 | + | 20.5 | 11.9 | 8.6 | 72.27 |
| 19 | LABS-19 | - | | | | |
| 20 | LABS-20 | - | | | | |
| 21 | LABS-21 | - | | | | |
| 22 | LABS-22 | - | | | | |
| 23 | LABS-23 | - | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 24 | LABS-24 | + | 23.0 | 17.0 | 6.0 | 35.20 |
| 25 | LABS-25 | - | | | | |
| 26 | LABS-26 | - | | | | |
| 27 | LABS-27 | - | | | | |
| 28 | *Bacillus* sp. (MTCC-1747) | + | 22.0 | 13.0 | 9.0 | 69.20 |

**Note:**
LABS – Lactic acid bacteria from shrimp
+ Positive for the test
- Negative for the test

**Table 2:** Quantity of protease produced by the selected LAB isolates

| Sl. No. | Isolate code no. | Quantity of tyrosine released (µM) | Protease enzyme activity (U/ml) |
|---|---|---|---|
| 1 | LABS-01 | 192.12 | 9.63 |
| 2 | LABS-05 | 204.23 | 10.23 |
| 3 | LABS-06 | 476.26 | 23.84 |
| 4 | LABS-08 | 186.68 | 9.35 |
| 5 | LABS-09 | 451.38 | 22.59 |
| 6 | LABS-11 | 440.57 | 22.07 |
| 7 | LABS-13 | 433.60 | 21.72 |
| 8 | LABS-15 | 214.23 | 10.73 |
| 9 | LABS-18 | 412.47 | 20.65 |
| 10 | LABS-24 | 195.00 | 9.77 |
| 11 | Reference strain (*Bacillus* sp.) (MTCC-1747) | 385.00 | 19.28 |
| | S. Em. ± | | 0.0017 |
| | CD (P = 0.01) | | 0.0071 |

**Note:**
LABS – Lactic acid bacteria from shrimp

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