Natural v/s Commercial Trypsin Isolated from Liver Waste of *Labeo Rohita*

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Research Article

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Abstract

The mounting human population and the massive amount of waste generated from the same is receiving particular attention towards valorization of waste. According to the annual report of FAO, (2018) the human consumption of fish protein has reached 87% in 2016 from 67% during 1960s. Aquaculture only has contributed to 5.8% annual growth rate among food sectors in the past decade. In this milieu, disposal of fish visceral waste is becoming a major menace to fishery industries exerting a great economic and environmental impact. Being perishable in nature, the organic portion of the waste decomposes rapidly and acts as a breeding ground for microbes. Moreover, the hefty and indiscriminate use of antibiotics and disinfectants in farmed animals is developing resistant strains, thus raising environment and ecological concerns. In order to solve such problem, the present investigation focussed upon employing the visceral trypsin as a cell dissociating agent. The efficacy of trypsin obtained from viscera of *Labeo rohita* upon KB cell line (Doubling time 50 hrs) was assessed in terms of cell viability. The cytotoxic effect of the visceral trypsin at 0.01%, 0.1% and 1% concentration were investigated at three time points (10 sec, 15 sec and 20 sec). Commercial (bovine) trypsin was considered as control.

A time dependent decrease in cell viability upon gradually increasing the concentration was observed in all groups of treatment. The lowest reduction in cell viability (2%) was observed with 1% concentration at 15 sec and 20sec. Although, commercial trypsin was found more efficient than trypsin isolated from waste during this study but the potency of visceral trypsin observed cannot be ruled out. Thus, the application of this enzyme as a cell-dissociating agent suggested it as a comparable candidate with commercial trypsin.

1. Introduction

Fishery sector is contributing towards the global economy since time immemorial so as to sustain the livelihood and maintain the nutritional status. Moreover, Idowu et al., (2019) have highlighted over the nutritional contents in fishes (protein, omega-3, vitamins, fatty acids, minerals etc.) fundamental for human health. According to the annual report of FAO, (2018) the human consumption of fish protein has reached 87% in 2016 from 67% during 1960s, and the annual waste discard is around 9.1 million tons (FAO, 2019). The consumption will continue to rise as fish and fishery by-products are easily accepted by major religions such as Jews, Buddhists, Muslims, Christians and Hindus (Nawaz *et al.*, 2020). Moreover, prevailing pandemic conditions have aroused people's seriousness towards protein-rich diet. However, this sector has several challenges which includes improved recovery and upgradation of waste. "Fish waste" is in itself a broad term that includes dead/damaged fish, irrelevant species, fish trimmings, tissues, blood, bones *etc.* While the former denotes by-catch, the remaining represents discards generated during processing. This includes viscera, head, bones, skin, fins, blood, liver frame, gonads, gut *etc.* (Ahuja et al., 2020). Both these side-stream biomass accounts for around 25-70% of the global fish processing (Ucak et al., 2021). Being perishable in nature the organic portion of fish waste is more prone to bacterial decomposition and undergoes rapid autolysis and lipid oxidation. Moreover, Lulijwa et al., (2020) have highlighted over the indiscriminate practise of antibiotics, disinfectants and chemotherapeutic drugs

against pathogens in farmed animals leading to development of resistant microorganisms. Such species further raising ecological and economic impressions. Using technical advancements, this waste can be valorised to obtain industrially important products, thereby lessening the waste burden over the environment. Among the various value-added products, enzymes are the most attractive and promising one. Thus, the premise of present study was to focus on cost effective ways to obtain trypsin from visceral waste of widely consumed freshwater carp- *Labeo rohita*, which was purified, characterized and applied as a tissue dissociating agent.

2. Materials And Methods

2.1 Collection of samples

The fish waste was procured in an ice-box from local fish market of Shahpura, Bhopal and immediately transferred to Molecular Biology Laboratory, Department of Biotechnology, Barkatullah University, Bhopal. The fish was identified as *Labeo rohita* (Fig.1) using an online database, Fish Base. 100g liver (Axis LC/GC weighing balance) was excised, washed with 0.8% saline to remove blood and debris, labelled and stored at – 20°C for further use.

2.2 Isolation of trypsin

According to the method of Simpson and Haard, (1985) crude enzyme extract was prepared, clarified, purified and characterised as trypsin as described by Batav and Gothaiwal, (2020).

2.3 Efficacy of trypsin

The efficacy of purified trypsin in animal cell culture was estimated by the method given by Freshney (2006). As cancerous cell line does not exhibit strict growth conditions and contact inhibition, the cell line KB (HeLa derivative, National Centre for Cell Sciences, Pune) was chosen for the study. Varying concentrations (0.01%, 0.1% and 1%) of the enzyme samples was inoculated to cover the cell monolayer. Incubation at room temperature was given for 10sec, 15sec and 20sec intervals for each set of experiment. After incubation, the solution was removed and cells were suspended into 10ml of growth media. To check cell viability in each case, 10 µl of inoculum (20,000 cells after 48hrs) was incubated with 10 µl trypan blue solution at room temperature for 1 min. Number of cells per ml was calculated using Hemocytometer as,

$$\text{cells per ml} = \text{viable cells} * D * 5000$$

Where, D = dilution factor Viability (%) was calculated as,

$$\text{viability} = \left(\frac{\text{number of live cells}}{\text{number of dead cells}} \right) * 100$$

The cell viability was compared with that of commercial trypsin.

3. Results And Discussions

The efficacy of *Labeo* trypsin over KB cell line was determined by measuring cell viability at different concentrations (0.01%, 0.1% and 1%) and time intervals (10sec, 15sec and 20ssec). Commercial (bovine) trypsin was considered as control. A time-dependent decrease in cell viability was observed with gradual increase in concentration of the enzyme which was in accordance with the observations of Sutradhar *et al.*, (2010). Plate 1 exhibits cell viability at 0.01% concentration of the two trypsinising agents (in lag phase) at different time intervals. From Fig. 2 it is apparent that fish derived trypsin resulted into 82%, 80% and 75% cell viability, viability at 0.1% concentration was found to be 95%, 90% and 97% (Fig.3) and viability at 1% concentration was 93%, 98% and 98% (Fig.4) respectively at three time points. However, the sample showed a variation in reduction of cell viability (13%, 10% and 5% respectively). Further, the sustainability of the source of the enzyme needs to be studied.

Although, commercial trypsin was found more efficient than trypsin isolated from waste during this study but the source of enzyme obtained during this study cannot be ruled out. The visceral trypsin studied showed similar kinetic properties and structural resemblance with other fish trypsin and commercial (bovine) trypsin. The enzyme was also found capable as a cell-dissociating agent.

4. Conclusion

The enzyme trypsin isolated from fish visceral waste was found equally effective cell dissociating agent as commercial trypsin. It is anticipated to be more economic as compared to commercial trypsin. On the other hand, the processing of viscera into trypsin could be a practical alternative of waste management. Moreover, fish processing industries could endorse this enzyme by setting up a unit in the vicinity of fish catch and landings to immediately collect the waste generated.

Declarations

5. CONFLICTS OF INTEREST

The authors declare no conflict of interest.

6.1 Ethics Approval and consent to participate - N.A.

6.2 Consent for publication – The authors agree to publish the manuscript and give their consent.

6.3 Availability of data and materials – The authors declare that the data is available.

6.4 Competing interests – The authors declare no competing interest.

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6.6 Authors' contributions – The author Dr. Charu Batav has conducted the study and prepared the manuscript under the guidance of Prof. Ragini Gothwal, and is the corresponding author as well.

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6.8 Authors' information – Dr. Charu Batav is a guest faculty in the Department of Biotechnology, Barkatullah University, Bhopal and has conducted the study as a part of her Ph.D. degree.

Prof. Ragini Gothwal is the Head of the Department of Biotechnology, Barkatullah University, Bhopal and has supervised the research work. Her area of research is Environmental Biotechnology and has worked extensively on Cyanobacteria during her Ph.D. and Post-Doc.

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Figures

Figure 1

Labeo rohita with liver waste

Figure 2

Time dependent trypsinisation on cell viability using 0.01% Concentration

Figure 3

Time dependent trypsinisation on cell viability using 0.1% concentration

Figure 4

Time dependent trypsinisation on cell viability using 1% concentration

Supplementary Files

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