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LIST OF PUBLICATIONS

- **Panchi Rani Neog** and Konwar, B. K. (2023). The distribution, economic aspects, nutritional, and therapeutic potential of swamp eel *Monopterus cuchia*: A review. ***Fisheries Research***, *261*, 106635. (IF: 2.8)

- **Panchi Rani Neog**, Yadav, M., and Konwar, B. K. (2023). Cloning, expression, and characterization of a surfactant-stable alkaline serine protease ($KNBS^{SP1}$) from *Bacillus safensis* PRN1 with remarkable applications in laundry and leather industries. ***Biocatalysis and Agricultural Biotechnology***, *54*, 102935. (IF: 4)

MANUSCRIPT UNDER COMMUNICATION

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PAPER PRESENTED IN NATIONAL/INTERNATIONAL SEMINARS

- **Neog, P. R.,** and Konwar, B.K. "Nutritional and therapeutic utility of freshwater cuchia Asiatic mud eel: *Monopterus cuchia*" in the national seminar on 'Current Research in Drug Discovery and Development" held during 13th & 14th November, 2019 at Department of Pharmaceutical Sciences, Dibrugarh University.

- **Neog, P. R.,** and Konwar, B.K." Therapeutic utility of Asiatic freshwater mud-eel (*Monopterus cuchia*) and molecular assessment of gut micro-flora" in the national seminar on "Application of Nanotechnology and Biotechnology in daily life" 'held during 26th & 28th September 2019, Sibsagar College.

- **Neog, P. R.,** and Konwar, B.K. Screening and optimization of protease production from gut microbiota of *Monopterus cuchia*" in the national seminar "Advances in Basic and Translational Research in Biology (ABTRiB)" organized by Department of Molecular Biology & Biotechnology, Tezpur University during 11-12th March, 2022.

Appendix

Media Composition

1. **Nutrient Broth**

Ingredients	**Concentration (g/L)**
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Final pH (at 25 °C)	7.4± 0.2

2. **Luria Bertani Broth**

Ingredients	**Concentration (g/L)**
Tryptone	10
Yeast Extract	5
NaCl	10
Final pH (at 25 °C)	7.5 ± 0.2

3. **Phosphate buffer saline (1X)**

Ingredients	**Concentration (g/L)**
NaCl	8
KCl	0.2
Na_2HPO_4	1.44
KH_2PO_4	0.24
pH	7.4

4. **Tryptone Soya Broth**

Ingredients	**Concentration (g/L)**
Tryptone	17.0
Soya peptone	3.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.5
Dextrose	2.5

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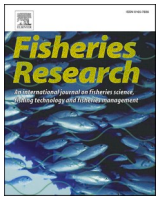

Review

The distribution, economic aspects, nutritional, and therapeutic potential of swamp eel *Monopterus cuchia*: A review

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ABSTRACT

Monopterus cuchia is a freshwater mud eel predominantly found in Bangladesh and the North East Region of India but available in Nepal, Sri Lanka, Pakistan, Indonesia, Philippines, etc. as well. *M. cuchia* is a tasteful, medicinally valuable, nutritionally rich, and economically important fish with high demands in national as well as international markets. The eel is recommended for consumption due to its high protein content and calorific values besides its high erythrocyte indices. The fish is usually available in freshwater ecosystems and shows survivability in extreme environmental conditions like high temperatures, low oxygen levels, high salt conditions, shallow waters, etc. by showing various morpho-histochemical, respiratory, and epidermal adaptations. It plays an important role in the socioeconomic development of a nation by providing employment and means of livelihood to millions of people involved in its production, catching, and marketing. The population of the eel is declining at a faster rate due to aggressive catching and less rearing, habitat destruction, and lack of conservative measures, therefore, requires serious attention in terms of production and conservation. In this regard, the current review gives a deep insight into nutritional, therapeutic and economic importance, habitat, morphology, reproduction, population status, diseases, toxicological studies, and adaptability to extreme environmental cues of the eel. The review also discusses the prevailing socioeconomic status of cuchia catchers and the strategies required to provide them with better employment and boost their social status. The review also discusses several conservative measures of the eel including induced breeding and developing genetic and molecular markers.

1. Introduction

The freshwater mud eel *Monopterus cuchia* is an amphibious fish commonly known as gangetic mud eel, rice eel, or swamp eel belonging to Synbranchidae (Table 1) (Jahan et al., 2019). It is a freshwater air-breathing fish commonly found in freshwater and brackish water with an altitudinal range of 76–1350 m above sea level (Nahid et al., 2020). *M. cuchia* is naturally distributed across North-East and Northern India, Pakistan, Bangladesh, Nepal, Sri Lanka, Indonesia, Philippines, and New Guinea (Roy et al., 2016; Chanda, 2018). The fish is nocturnal and carnivorous in feeding habit, therefore, prefers animal-based food like small fish, amphibians, crustaceans, insect larvae, echinoderms, aquatic invertebrates, earthworms, snails, tubifex, fish fingerlings, insect pupae, slaughterhouse waste, etc. (Abedin et al., 2020; Nahid et al., 2020). The proteinaceous food is digested by the presence of endogenous enzymes produced in the digestive tract of the fish. Tsen and Wang (1982) identified pepsin, trypsin, and chymotrypsin as the major proteases in the gastrointestinal tract of Japanese eel. A noticeable amount of sand and mud are found in the gut of the fish which suggests that *M. cuchia* is a bottom feeder (Kurbah and Bhuyan, 2019). Furthermore, during day time they usually hide under mud and stones depicting their adaptability to various environmental conditions. The fish is pollution resistant and can also adapt to various adverse situations like low oxygen concentration, shallow water, and high temperature (Abedin et al., 2020). *M. cuchia* is a delicious, tasty, nutritionally rich, economically and medicinally important freshwater fish that could play an important role in the growth and development of the human body (Begum et al., 2017). It has been reported that the average protein content and calorific value per 100 g of cuchia flesh are as high as 14 g and 303 kcal, respectively (Alam et al., 2010). Additionally, *M. cuchia* plays an important role in the socioeconomic development of developing countries by generating employment and boosting the economy. It has been reported that a large section of people is involved in its production, marketing, and other associated business (Menezes et al., 2013). Tribal communities viz. Hajong, Garo, Monipuri, Shaotal, and Rajbongshi of North East India traditionally use *M. cuchia* for the treatment of various

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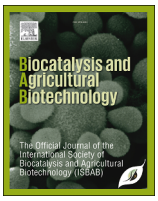

Cloning, expression, and characterization of a surfactant-stable alkaline serine protease (KNBS^{SP1}) from *Bacillus safensis* PRN1 with remarkable applications in laundry and leather industries

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ABSTRACT

Proteases represent an important category of proteolytic enzymes with vast biotechnological applications in various industries like detergent, leather, agriculture, poultry, pharmaceutical, food, etc. The present study proposes an eco-friendly enzyme-mediated approach as opposed to conventional chemical approaches in detergent and laundry industries that are hazardous to human health and the environment. Accordingly, the *knbs*^{SP1}gene, which encodes the trypsin-like serine protease KNBS^{SP1}, from *Bacillus safensis* PRN1 was isolated, cloned into an expression vector (PET-28a), and expressed in the *Escherichia coli* cells BL21 (DE3). A single Ni-NTA affinity chromatography step was utilized to purify the recombinant protease (rKNBS^{SP1}). The molecular weight of the rKNBS^{SP1} was approximately ~33 kDa determined in Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The rKNBS^{SP1} showed optimal activity at 60 °C and pH 8. It retained above 70% of its enzyme activity at pH 10 and 70 °C. The protease was strongly inhibited by phenylmethylsulfonyl fluoride (PMSF) and diisopropyl fluorophosphate suggesting its inclusion in the serine family of protease. The rKNBS^{SP1} demonstrated outstanding compatibility and stability with metal ions, commercial detergents, and surfactants, exhibiting 97.8 ± 2.5% stability with Surf Excel, a commercial laundry detergent, and 96.9 ± 2.0% stability with SDS. Interestingly, the protease demonstrated blood-stained removal and goat skin dehairing (8 h) properties. All these remarkable characteristics make this protease a potential eco-friendly candidate in the detergent formulation and leather processing industries.

1. Introduction

Enzymes are biological catalysts that attract researchers for their huge catalytic power and green nature. Certain characteristics of enzymes *viz.,* nominal by-product formation, moderate reaction conditions, and substrate specificity make them the most important entities for the bioprocessing industry (Ravindran and Jaiswal, 2016). The widespread use of noxious chemicals in various industries has affected human health and the ecosystem (Boyce and Walsh, 2012). Traditional leather processing generates huge quantities of liquid wastes, solid wastes (lime and chrome dirt) and emits toxic gases such as volatile organic compounds, ammonia, hydrogen sulfide, etc. causing immense environmental problems (Kanagaraj et al., 2020; Sivasubramanian et al., 2008). Furthermore, conventional detergents are mainly composed of xenobiotic compounds and hazardous chemicals such as bleaching agents, whitening agents, bluing agents, perfumers, anti-re-deposition agents, and linear alkyl benzene (LAS) contaminating the soil and aquatic fauna (Paul et al., 2016). These noxious chemicals enter the human body through various routes and cause various skin and lung diseases

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