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Isolation of Amylolytic bacteria from Gastro-intestinal tract of *Ameiurus melas* (Bullhead Catfish) as probiotic supplement

Research article

Supriya Jadhav, Sameer Sharma and Sibi G*

Department of Biotechnology, Indian Academy Degree College-Autonomous, India

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***Corresponding author:** Sibi G, Head of the Department, Department of Biotechnology, Indian Academy Degree College-Autonomous, India

Abstract

This study primarily focused on isolation and identification of amylase producing bacteria from gastro intestinal tract of *Ameiurus melas* (bull headed catfish). Microbiota was isolated using selective media towards the assessment of amylolytic potential. Based on the morphological and biochemical characterization, the isolate was identified as *Bacillus cereus.* The isolate had exhibited extracellular amylase activity of 18.46 U mL^{-1} and the specific activity was 2.16 U mg^{-1}. Use of isolated bacteria as probiotic supplement in aquaculture could improve growth and health of fish. *Bacillus cereus* with amylase activity isolated from the fish can be beneficially used as a probiotic while formulating the diet for fish, especially in the larval stage. Further, supplementation of isolated bacteria in fish feed could improve gut health and enzyme activity.

Keywords

Amylase, amylolytic, *Bacillus cereus*, Catfish, probiotics

Introduction

Digestive tract of fish contains microorganisms and their presence is due to the aquatic environment and the diet of fish. As the digestive tract is rich with carbohydrates, proteins, enzymes and other nutrients, it is the ideal growth environment for those microorganisms [1]. Study of microflora in fish gut varied life stage [2], trophic level [3], diet, season, habitat, captive-state, sex, and phylogeny. Among the microorganisms present in the fish gut, bacteria are the dominant flora and consist of aerobic, anaerobic and facultative anaerobic bacteria [4-10].

Digestion of food by the fish depends on the enzymes from the intestinal microflora which plays a significant role. Amylase is widely distributed in the intestinal tract of freshwater fish and plays an important role in the digestion of starch. In general, amylase activity in the digestive tract of omnivorous fish is higher than that of carnivorous fish [11,12], but the activity is also affected by dietary manipulation [13,14]. Moreover, it is likely that the activity differs with the structure of the digestive tract, developmental stages and ambient temperatures of fish [15-18]. All fish species investigated per se possess the enzymatic apparatus for hydrolysis and absorption of simple and complex carbohydrates [19]. Digestive α-amylase has been localized throughout the entire GI tract of numerous fish species [20-23]. In catfish, proteolytic and celluloytic bacteria are present in higher numbers within the gut. Amylases are a group of hydrolyses that can specifically cleave the α-1,4 glycosidic bonds in starch and are among the important industrial enzymes.

In this study, an attempt was made to identify the indigenous amylolytic microbiota in the gastrointestinal

tract of catfish. With this objective, the search for extracellular enzyme-producing beneficial gut bacteria could be used as probiotics to improve feeding and other conditions for the intensive rearing of fish.

Materials and Methods

Species used

Black bullhead catfish (*Ameiurus melas*) was used in the study to isolate the amylase producing organisms. Catfish was collected from the local fish market of Satara, Maharashtra (17.6805° N, 74.0183° E).

Sample preparation

A homogeneate solution was made by grinding GI tracts with sterilized 0.85% NaCl solution. Ten fold dilutions were made by mixing this homogenate solution with sterilized distilled water and used as inoculum.

Isolation and culture of gut bacterial flora

To isolate the heterotrophic bacterial population, diluted samples (0.1 ml) were spread on tryptic soy agar to characterize the total viable counts and the plates were incubated at 37°C for 24 hours. The colonies were purified by streaking followed by transferred to starch agar medium followed by incubation at 37°C for 24 hours. Colonies grown on starch agar were considered amylolytic [24,25].

Characterization of the bacterial isolate

Colony characteristics of the isolate from starch agar was observed after growing on a nutrient agar plate at 37°C for 48 h, and cellular morphology was determined using light microscopy and Gram staining. Physiological and biochemical analyses were performed by referring to Bergey's Manual.

Extracellular amylase activity

For the determination of amylase production by the bacterial isolates, pure colonies were inoculated on starch agar plates and incubated at 37°C for 24-48 hours. The culture plates were then flooded with 1% lugol iodine solution to identify amylase activity by formation of transparent zone surrounding the colony.

Quantitative Amylase Assay

The isolated amylolytic bacteria were inoculated into the starch-agar medium without the agar to quantify amylase activity. Culture media in culture flasks with the bacterial isolate was incubated at 32°C for 24 h. This was followed by centrifugation at 10000 g for 10 mins at 4°C to harvest the cells and the cell free supernatant was analyzed by 3,5-dinitrosalicylic acid (DNS) method to determine the amylase activity using maltose as the standard. Quantitative enzyme activity was expressed as units (U). One amylase unit was defined as the amount of enzyme per millilitre of culture filtrate releasing 1 microgram of reducing sugar per minute. Total protein determination was performed using bovine serum albumin as a standard. Specific activity was defined in terms of the enzyme activity units per mg of total protein.

Results and Discussion

Total bacterial count of 14 CFU/ml was recorded in the GI tract of *Ameiurus melas*. Morphological and physiological characterization of the colonies isolated from starch agar is provided in Table 1. Based on major morphological, physiological and biochemical characters, the isolate was identified as *Bacillus cereus*.

Amylase enzyme activity measured in *B. cereus* was 18.46 U mL^{-1} and the specific activity was 2.16 U mg^{-1}. The results of the present study indicate that the bacteria isolated from the fish digestive tract are capable of producing amylolytic enzymes. There is a considerable body of evidence regarding the endogenous digestive enzymes in fish however, information regarding the enzyme producing intestinal bacteria and their significance in fish is rare. The intestinal microbiota in fish has been classified as indigenous when it is able to colonize the gut ecosystem, or as transient when it only passes through the digestive tract without colonizing. Thus, it seems that bacterial population detected in the present study forms a natural and persistent population (indigenous) in their digestive tracts.

The outcome of the current investigation illustrated that amylolytic bacteria endured in the gastrointestinal tracts of fish species studied, which reinforce the assumptions that the gut bacteria could contribute to digestion in fish [26]. In the current study, the strain isolated from the gut of bullheaded catfish with extracellular enzyme producing capability was classified as aerobic Gram-positive bacilli. Characterization of the isolated bacteria exposed that they might grow within a wide range of temperatures (20-50 °C) and pH (5.0-11.0) depending on the aquatic environment. Equally ranges of temperature and pH tolerance have previously been noted in other strains

Table 1: Morphological, physiological characterization of isolate.

Morphological		Physiological	
Characteristics	**Observations**	**Biochemical Tests**	**Findings**
Colony Size	1-2 mm	Catalase production	+ve
Colony Shape	Round	Citrate utilization	+ve
Colony Margin	Regular	Gelatin liquefaction	-ve
Colony Elevation	Convex	Glucose fermentation	+ve
Colony Texture	Smooth	Indole production	-ve
Appearance	Opaque	Nitrate reduction	+ve
Gram's reaction	Gram positive	Oxidase	+ve
Cell Shape	Rod shaped	Starch hydrolysis	+ve
Motility	Motile	Urease	+ve
Endospore	Present	Voges-Proskauer	-ve
		Oxidase	+ve
		H_2S production	-ve

of the genus *Bacillus* isolated from fish gut [27,28]. The identifications of gut bacteria isolated in the current study based on their biochemical actions with diverse substrates, noted that bacteria within the similar genus shared mutual biochemical properties and it was difficult to differentiate them. The increased interest during the last decade on bacteria in the GI tract of fish is also related to the finding that some of the enzyme producing gut bacteria produce bacteriocins and other compounds that inhibit growth of pathogenic bacteria in vitro. Askarian et al. [29] noted that *Bacillus thuringiensis*, isolated from the gastrointestinal tract of the Atlantic salmon (*Salmo salar L.*) showed extracellular digestive enzyme activities. Physiological and biochemical characterization of the intestinal isolates are important in elucidating their functions in the GI tract.

Earlier investigations have suggested that microorganisms have a beneficial effect in the digestive process of fish. Characterization of the microbial populations in the intestinal microenvironment of fish and understanding the physiological interactions between the indigenous microbiota and the host may have important implications. This study indicated that the enzyme producing gut bacteria can utilize carbohydrates. The isolate bacteria could be introduced as probiotic supplement in fish feed to enrich the fish gastrointestinal tract with amylolytic bacteria thus facilitation the food digestion. Further, the enzyme producing gut bacteria characterized in the present study may be used beneficially for fish especially in the larval stages.

Conclusion

The study primarily focused on isolation and identification of amylase producing bacteria from gastrointestinal tract of bull-headed catfish. Supplementation of isolated bacteria, Bacillus cereus in fish feed could improve gut health and enzyme activity. The present study demonstrates that the *Bacillus cereus* strains isolated from the fish gut shows amylase production which is non-conventional source. This study also demonstrates that the *Bacillus cereus* strains isolated from the fish gut shows amylase production which is non-conventional source.

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