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Isolation of Chitinase Producing Microbes from Gastrointestinal Tract of Gift Tilapia (*Oreochromis Niloticus*)

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ABSTRACT
Present study was designed to isolate chitinase producing microbes from gastrointestinal tract of GIFT tilapia and to optimize the incubation period for the growth of chitinase producing microbes. Fingerlings of GIFT tilapia were ascetically dissected and total 6 chitinase producing colonies were isolated on Nutrient agar with 1% colloidal chitin supplement. The colonies were selected on the basis of zone of hydrolysis around the colony. On the basis of biochemical test results colony C:1, C:5, and C:6 showed similarity with Pseudomonas species, colonies C:2 and C:4 showed similarity with bacillus group andC: 3 showed similarity with Aeromonas species. For optimization of incubation period 24 hours, 48 hours and 72 hours the results of optical density for broth at 550 nm showed the maximum growth at 48 hours. The findings of microbial diversity of gastrointestinal tract of GIFT tilapia indicates that gut of tilapia can be a good source for isolation of chitinase producing microbes which helps the fish to digest chitin containing food items. The bacterial chitinases can also be a potential fungicide for agriculture and aquaculture sectors.
Keywords*: Chitinase, gastrointestinal tract, GIFT tilpia, Colloidal chitin, aquaculture*

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INTRODUCTION

India is the second largest fish producer of the world in inland sector and third in marine sector [1]. The fisheries sector contributes 5.15% of agricultural GDP and 1.1% of the national GDP of India. Aquaculture has been the fastest growing food producing sector globally for over half a century, with production growing at an average rate of 8.1% per year since 1961 representing almost 50% of the human consumed fish [2, 3]. Tilapia accounted for 7.4 % of global aquaculture production and tilapia is the second important finfish species group cultured world wide n terms of production [4]. To maximize the tilapia production genetically improved farmed tilapia (GIFT) strain, a synthetic strain of *Oreochromis niloticus* was developed through selection of several generations from a base involving eight different strains of nile tilapia (*O. niloticus*). Eknath [5] and Eknath and Acosta [6] reported that the GIFT strain showed 12-17% average genetic gain per generation over five generations, an average 60% faster growth and 50% better survival at harvest than most commonly farmed strain of tilapia. In aquaculture, intestines, gills, the skin mucus of aquatic animals, and habitats or even culture collections and commercial products, can be ideal sources for isolating appropriate probiotics [7]. Many of the researchers isolated extracellular enzyme producing microbes from gastrointestinal tracts of fishes as potential probiotics Saha *et al.* [8]; Sarkar and Ghosh, 2014 [9]; Ghosh *et al.* [10]; Ghosh *et al.* [11]; Chellaram, and Krithika [12]and Mondal *et al.,* 2008 [13]. Chitin is component of exoskeleton of various arthropods, yeast and algae. Shrimp waste contains 20% - 60% chitin and possible to be source of chitinolytic bacteria [14]. The shrimp shell waste is one of the major marine wastes which may cause environmental issues due to its rapid deterioration. Though this shell waste can be degraded through chemical process like demineralization and deproteinization but it causes corrosion, low yield and high cost. So, an ecofriendly approach is needed which can utilize this crustacean waste safely and maintain the carbon-nitrogen balance in the environment [15]. Chitin obtained from shrimp shell waste has wide application in nanotechnology for

nanofertilizer development for agriculture. Chitinases can be also used as potent anti fungal agent (as the cell wall of fungi is made up of chitin), biocontrol agent for insects and pests in crops, single cell protein development and mosquito control [16, 17]. The major crustacean species that contain chitin exoskeleton are penaeid shrimps, squilla and crab. The shell of these crustaceans contain good amount of the natural long polymer of N-acetylglucosamine or chitin [18]. Chitinase are the chitin degrading enzymes contributes to generate carbon and nitrogen in environment. Chitinases are the second most abundant natural polymer after cellulose in nature, which are considered as potential biopestcide as it can digest the cell wall of the fungi (22-44%) and insects. Chitinases are very helpful in moulting in shrimps and can be a potential growth promoter in shrimp's aquaculture and in the field of medical science. Chitinases are constituents of several bacterial species; the study of some of the best known genera includes *Aeromonas*, *Serratia*, *Vibrio*, *Streptomyces* and *Bacillus*. The current research work focuses on isolation and characterization of chitinase producing bacteria from gastrointestinal tract of GIFT tilapia (*Oreochromis nlioticus*).

MATERIAL AND METHODS

Conditioning of Gift tilapia and isolation of chitinase producing bacteria

Fingerlings of *Oreochromis niloticus* (GIFT tilapia) were procured from Manikonda tilapia hatchery, near Vijaywada, Andhra pradesh. The fishes were treated with 2 ppm potassium permanganate to avoid infection due to injury and transportation stress. Then these fishes were slowly released into plastic tank, pre-treated with potassium permanganate and acclimatized in plastic tanks with adequate aeration and feeding. Fingerlings of GIFT tilapia (*O. niloticus*) were randomly selected for isolation of symbiotic bacterial flora from gastrointestinal tracts. Selected fishes were starved for 24 hrs before dissection [19]. The fishes were dissected aseptically within laminar airflow on ice slabs and gastrointestinal tract was emptied, thoroughly rinsed (five times) in sterile 0.9% saline in order to remove non-adherent bacteria. Then it was separately homogenized with 10 parts of chilled 0.89% sodium chloride solution [20] for sample preparation.The culture media used for isolation was nutrient agar with 1% colloidal chitin.

Preparation of specific media for culture

Colloidal chitin was used as substrate for growth of chitinase producing microbes, so colloidal chitin was prepared according modified method described by (Faramarzi *et al.* 2009) [21]. Ten grams of chitin from shrimp shell flake was added into 100 ml concentrated HCl (36%) and kept in vigorous stirring for 2 h in thermal shaker at 37˚C or until chitin completely dissolved. The suspension was precipitated by adding 500 ml of ice-cold absolute ethanol slowly. Then pH of suspension was neutralized with the help of 10 N NaOH. Suspension was centrifuged at 4500 rpm for 25 min and the precipitate was stored at 4˚C for further use. For preparation of nutrient agar with 1 % of colloidal chitin; 1 % of colloidal chitin was added in nutrient agar (Himedia) during media preparation.

Screening of chitinase producing microbes

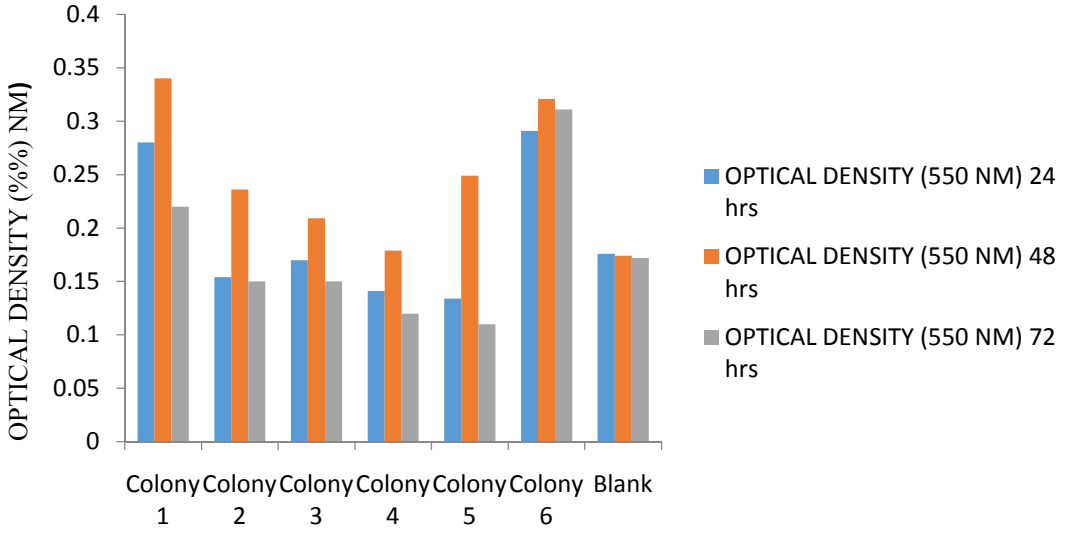
For screening of chitinolytic microbes, 200µl of respected dilution (10^{-3}, 10^{-4} and 10^{-5}) was used for spreading in media (duplicate) aseptically. The inoculated plates were then incubated in inverted position in BOD incubator at 37˚ C for 24 hours. Total 6 bacterial colonies were observed to degrade chitin. So the 4 bacterial colonies that's shows zone of hydrolysis in its surrounding were supposed to be chitinase producing and selected for further analysis.

Biochemical tests for different isolates

S.NO.	BIOCHEMICAL TEST	C:1	C:2	C:3	C:4	C:5	C:6
1	Gram's staining	-	+	-	+	-	-
2	Catalase activity	+	+	+	+	+	+
3	Oxidase activity	+	+	+	_	+	+
4	Starch hydrolysis	+	+	+	+	+	+
5	Nitrate reduction	+	-	+	-	+	+
6	Citrate utilization	+	_	+	+	+	+
7	Lipase activity	+	+	+	+	+	+
8	Protease activity	+	+	+	+	+	+
9	Endospore formation	_	+	_	+	-	-

Optimization of microbial growth with different incubation period

To get optimum incubation period for maximum growth of chitinase producing microbes the all the colonies were inoculated in nutrient broth with 1% colloidal chitin as substrate and incubated at 37˚C for 24hrs, 48 hrs and 72 hours and absorbance was measured at 550 NM).



RESULTS AND DISCUSSION

The findings of the above study showed 6 unique chitinase producing colonies were selected on the basis of maximum zone of hydrolysis on nutrient agar with 1% colloidal chitin media. First colony C:1, C:5, and C:6 showed similarity with *Pseudomonas species*[22], Colonies C:2 and C:4 showed similarity with *bacillus group*[23] for bacillus group) as several species of *Bacillus* have been studied to produce chitinase, which includes, *Bacillus thuringiensis, Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus magaterium*, *Bacillus laterosporus*, *Bacillus pabuli*, *Bacillus stearothermophilus*, *Bacillus subtilis* and *Bacillus circulans*[24]. While C:3 showed similarity with *Aeromonas species*[25] on the basis of biochemical analysis the study shows similarity with the findings of Tao Yong *et al.,* 2005[26], they studied that Chitinase can be found in a wide range of organisms which includes Bacillus, *Aeromonas, Vibrio, Pseudomonas, Serratia, Enterobacter, Actinomycete* species in bacterial group and *Trichoderma* and *Aspergillus* species in the group of fungi. The results of the current study also indicate that the optimum incubation period for maximum growth of microbes is 48 hours.

CONCLUSION

The findings of the current study indicate that gastrointestinal tract of GIFT tilapia contains considerable numbers of chitinase producing microbes, which helps this omnivorous fish to digest chitin containing feed ingredients. The isolation of these microbes can help in quantification of bacterial chitinase from gut microbes of GIFT tilapia and can be further used as potential fungicide for agriculture and aquaculture sector.

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