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SHORT COMMUNICATION

Characterization of enzyme-producing bacteria isolated from the gut of Asian seabass, *Lates calcarifer* and milkfish, *Chanos chanos* and their application for nutrient enrichment of feed ingredients

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Aquatic bacteria influence the composition of gut microbiota in fish (Cahill 1990). Like all vertebrates and invertebrates, fish also harbour microbial populations in their digestive tracts (Trust & Sparrow 1974). In all higher vertebrates food gets digested by the action of enzyme secreted by host and also enzyme produced by the intestinal microbiota (Kar, Roy, Sen & Ghosh 2008). Endogenous digestive enzyme has been studied by several workers in freshwater fish (Dhage 1968; Das & Tripathi 1991). Few reports are also available with regard to enzyme-producing gut bacteria of freshwater fish (Saha & Ray 1998; Ghosh, Sen & Ray 2002; Kar & Ghosh 2008; Kar *et al.* 2008; Askarian, Zhou, Olsen, Sperstad & Ring 2012; Khan & Ghosh 2012). Different gut microbes having considerable cellulase, amylase and protease activity had been isolated from freshwater fish (Bairagi, Sarkar, Sen & Ray 2004; Saha, Roy, Sen & Roy 2006; Mondal, Roy, Sen & Ray 2008). Gatesoupe, Zambonino Infante, Chu and Quazuguel (1997) reported amylase-producing *Vibrio* spp. isolated from seabass (*Dicentrarchus labrax*) larvae. They, however, did not specify the exact site of the gut (proximal, mid or distal intestine) where the population of these cellulase- and amylase-producing microbes and the enzyme activity is maximum. Reports on the existence of cellulase activity in the digestive system of fish are with contradictory results (Ray, Ghosh & Ring 2012). The ruminants can digest cellulose through the action of cellulose secreted by the anaerobic gut fungi and bacteria present in rumen. There is ongoing interest in screening culturable cellulase-producing microorganisms from different sources. The major aim of this study was to investigate the autochthonous cellulase-producing bacteria in the gastrointestinal tracts of two brackishwater fish namely Asian seabass and milkfish and they were used for nutrient enrichment of the feed ingredients through solid-state fermentation. The adult brackishwater fish 6 nos. of each species) were collected from brackishwater tide fed farm of Kakdwip (Lat. 21°51′15.01″–21°51′30.77″N, Long. 88°10′58.44″–88°11′12.09″E), South 24 Parganas, West Bengal, India. Fish were starved for 48 h to clear their alimentary tracts before dissection (Bairagi, Sarkar Ghosh, Sen & Ray 2002a; Kar *et al.* 2008). During the sampling periods the water temperature varied between 30°C and 35°C. The average weight, length of fish and average weight of digestive tract were recorded (Table 1).

Fish was thoroughly scrubbed with 1% iodine solution after sacrifice (Trust & Sparrow 1974). After dissection on ice the whole alimentary tract was removed and cleaned with chilled sterile physiological saline (0.9% NaCl in PBS buffer, pH 7.2). Subsequently, the pieces of digestive tract were

homogenized with sterilized normal saline solution (NSS). The homogenate was used as inoculum for microbial culture (Das & Tripathi 1991). Likewise, to study the microbial population in different section of digestive tract, the alimentary tract was removed, cut into three portion in equidistance (Proximal intestine, PI; Mid intestine, MI and Distal intestine, DI) and homogenized.

The homogenate was serially diluted in NSS up to 10^{-10} dilution (Beveridge, Sikdar, Frerichs & Millar 1991). The 0.1 mL of each dilution was poured aseptically on Tryptone soya agar (TSA), Carboxymethyl cellulose (CMC) agar, Starch agar (SA), Peptone gelatin agar (PGA) and Tributyrin agar (TBA) for total gut microbial, cellulolytic, amylolytic, proteolytic and lipolytic microbial count, respectively, in duplicate. Plates were incubated at 34°C for 48 h. The colony with different shape, size, colour and transparency was selected as separate isolate and was streaked on TSA, CMC agar and SA plates to obtain the pure culture. Cellulase, amylase and protease activity of whole gut and different portion of gut were assayed following method of Denison and Koehn (1977), Bernfield (1955) and Walter (1984) respectively.

The pure colonies were screened and shortlisted based on the morphological, physiological and biochemical tests (Jacob & Gerstein 1960; Teather & Wood 1982; Williams, Sharp & Holt 1986; Saha *et al.* 2006).

Identification based on the phenotypic characters was further confirmed by the analysis of partial 16S rDNA sequences as described by Roy, Mondal and Ray (2009). The PCR products were bidirectionally sequenced using forward, reverse and an internal primer. Sequencing was done with ABI 3130 Genetic Analyser and the sequenced data were aligned and analysed for finding the closest homolog of the microbes with National Centre for Biotechnology Information (NCBI) GenBank database. Phylogenetic trees were made in Seq Scape_v 5.2 software using the Neighbour-Joining method with Bootstrap analysis. The partial sequences of 16S rDNA were deposited in the NCBI GenBank database to obtain accession numbers.

Two locally available fibre rich low cost feed ingredients, rice bran (RB) and sunflower cake (SFC) were selected for solid-state fermentation (SSF) study. The ingredients were fermented with two potential gut bacteria i.e. *Bacillus* sp. DDKRC1. (LC8), isolated from the gut of seabass, *B. subtilis* DDKRC5. (CC8), isolated from milk fish. *Bacillus*

Table 1 Morphometry with microbial population of different fish species

Fish species	Average weight (g)	Average length (cm)	Average weight of digestive tract (g)	Total microbial count in TSA plate(x 10^5) (CFU g^{-1} digestive tract tissue)**	Cellulolytic bacterial count in CMC plate (x 10^3) (CFU g^{-1} digestive tract tissue)**	Amylolytic bacterial count in SA plate (x 10^3) (CFU g^{-1} digestive tract tissue)**	Proteolytic bacterial count in PGA plate (x 10^4) (CFU g^{-1} digestive tract tissue)**	Lipolytic bacterial count in TBA plate (x 10^3) (CFU g^{-1} digestive tract tissue)**
L. calcarifer	903.33 ± 38.44	39.66 ± 0.88	16.17 ± 0.17	60.19 ± 0.27[b]	0.22 ± 0.01[a]	2.68 ± 0.04[a]	6.32 ± 0.10[b]	0.49 ± 0.05[a]
C. chanos	93.00 ± 2.00	23.40 ± 0.32	2.24 ± 0.09	7.72 ± 0.25[a]	15.47 ± 0.22[b]	51.66 ± 0.36[b]	4.39 ± 0.20[a]	3.99 ± 0.11[b]

All values are mean ± S.E. Values bearing different superscripts in a column differ significantly.
TSA, tryptone soya agar; CMC, carboxymethylcellulose; SA, starch agar; PGA, peptone gelatin agar; TBA, tributyrin agar.
**$P < 0.01$.

sp. DDKRC1. and *B. subtilis* DDKRC5. cultures were of 24 h grown culture to obtain an average viable count of 10^7 cell mL^{-1}. Each ingredient was fermented with each bacterium @ 1% (v/w) at four different moisture levels (40, 50, 60 and 70%) for five different hours (0, 24, 48, 72 and 96 h) to optimize these factors with regard to dry matter loss and protein content in the fermented ingredients in triplicate. All the feed ingredients were ground into powder and sterilized in autoclave at 15 lb pressure for 15 min. Sterile distilled water was added to ingredients to bring the desired moisture content. Then, bacterial culture was inoculated and mixed thoroughly and incubated at 34°C in orbital shaker incubator (shaking speed 120 rpm) for different time periods. Dry matter, protein, cellulose and nitrogen free extract (NFE) of feed ingredients were measured (AOAC (Association of Official Analytical Chemists) 1995) before and after incubation.

The experimental data were subjected to analysis of variance (ANOVA) to test the significance among the treatments using SPSS for Windows v. 17.0.

Total microbial count was higher ($P < 0.01$) in digestive tract of *L. calcarifer* as compared with that of *C. chanos* (Table 1). Cellulolytic and amylolytic microbial population was more ($P < 0.01$) in the gut of *C. chanos* (15.47 and 51.66 × 10^3 CFU g^{-1} digestive tract tissue respectively) as compared with that of *L. calcarifer* (0.22 and 2.68 × 10^3 CFU g^{-1} digestive tract tissue respectively). Analysis of microbiota from different portions of the digestive tract of fish revealed that, total microbiota was higher ($P < 0.01$) in MI of *L. calcarifer* and PI of *C. chanos* (Table 2) as compared with that of other sections of gut in respective fish. Amylolytic microbial population was higher ($P < 0.01$) in DI of both the fish species. Cellulolytic microbial population was higher ($P < 0.01$) in the DI of *L. calcarifer* and MI of *C. chanos*. Proteolytic microbial population was higher ($P < 0.01$) in the MI and DI of *L. calcarifer* and in *C. chanos* it was higher ($P < 0.01$) in MI. Lipolytic microbial population was higher ($P < 0.01$) in the MI of both *L. calcarifer* and *C. chanos*. Cellulase and amylase were also estimated in different region of gut and it was observed that cellulase was higher ($P < 0.01$) in PI and DI of *L. calcarifer* but in *C. chanos* it was higher ($P < 0.01$) in MI. In *L. calcarifer*, amylase activity was higher ($P < 0.01$) in DI, but in *C. chanos* it was higher ($P < 0.01$) in MI and DI. Protease activity was higher ($P < 0.01$)

Table 2 Microbial count and digestive enzyme activity in different region of gut of *Lates calcarifer* and *Chanos chanos*

Fish species	Region of Gut	Total microbial count (×10^6 CFU g^{-1} of gut)	Amylolytic microbial count (×10^5 CFU g^{-1} of gut)	Cellulolytic microbial count (×10^5 CFU g^{-1} of gut)	Proteolytic microbial count (×10^4 CFU g^{-1} of gut)	Lipolytic microbial count (×10^5 CFU g^{-1} of gut)	Specific amylase activity*	Specific cellulase activity†	Specific protease activity‡
L. calcarifer	Proximal intestine	0.04 ± 0.00^a	0.92 ± 0.00^a	0.05 ± 0.00^a	0.58 ± 0.03^a	5.56 ± 0.02^b	63.58 ± 0.07^a	23.46 ± 0.80^b	11.10 ± 0.70^b
	Mid intestine	16.00 ± 0.10^c	9.95 ± 0.04^b	0.31 ± 0.00^a	5.27 ± 0.03^c	7.30 ± 0.01^c	65.03 ± 0.20^b	17.24 ± 0.43^a	9.99 ± 0.52^b
	Distal intestine	12.36 ± 0.07^b	51.24 ± 0.20^c	1.90 ± 0.09^b	4.4 ± 0.19^b	1.46 ± 0.07^a	71.77 ± 0.30^c	24.89 ± 1.58^b	1.95 ± 0.00^a
C. chanos	Proximal intestine	4.77 ± 0.03^b	2.05 ± 0.02^a	2.36 ± 0.04^b	0.94 ± 0.01^a	0.03 ± 0.00^a	122.80 ± 1.35^a	50.75 ± 0.34^{bc}	5.72 ± 0.18
	Mid intestine	1.25 ± 0.03^a	6.60 ± 0.05^b	5.82 ± 0.02^c	3.05 ± 0.03^b	0.40 ± 0.01^b	156.44 ± 0.19^b	63.16 ± 0.12^c	6.34 ± 0.11
	Distal intestine	1.68 ± 0.02^a	23.02 ± 0.01^c	1.93 ± 0.02^a	1.60 ± 0.21^a	0.14 ± 0.01^b	159.73 ± 1.30^b	48.05 ± 0.05^a	5.35 ± 0.28

Values bearing different superscripts in a column under each fish species differ significantly ($P < 0.01$).
*Microgram of maltose liberated per mg of protein per min.
†Microgram of D-glucose liberated per mg of protein per min.
‡Microgram of L-tyrosine liberated per mg of protein per min.

in PI and MI of *L. calcarifer*, but in *C. chanos* it was similar in all the region of gut (Table 2).

On the basis of colony morphology, 12 types of isolates i.e. LC1, LC2, LC3, LC4, LC5, LC6, LC7, LC8, LC9, LC10, LC11 and LC12 from the gut of *L. calcarifer* and nine isolates i.e. CC1, CC2, CC3, CC4, CC5, CC6, CC7, CC8 and CC9 from the gut of *C. chanos* were isolated in pure form. Among all isolates from *L. calcarifer,* LC8, LC9 and LC5 exhibited maximum cellulolytic (54.63 U), amylolytic (26.15 U) and proteolytic (6.23 U) activity respectively. Among the isolates from *C. chanos*, CC8 exhibited maximum cellulolytic (15.74 U), amylolytic (26.16 U) and proteolytic (8.03 U) activity (Table 3). Three potential isolates, i.e. LC8, LC9 and CC8 were then characterized for identification.

All the three isolates were Gram positive, rod-shaped, aerobic and endospore producers. They could tolerate a wide range of pH (5–10) and temperature (15–40°C) but salinity tolerance level was up to 2% NaCl concentration, which probably enabled the organisms to adapt to a wide range of temperature and pH (Ghosh *et al.* 2002). They all were capable of utilizing cellulose as sole carbon source and gelatin as amino acid source (Table 4). All the three isolates produced nitrate reductase but could not produce indole from tryptophan. They were capable of producing acid from glucose, sucrose and lactose and showed positive oxidation–fermentation (OF) test of glucose. All the isolates showed negative response to H_2S production. In contrast with these common characteristics, those three isolates differed in many other characters. LC8 produced gas in glucose peptone broth, but LC9 and CC8 did not produce gas in same broth. LC9 and CC8 could utilize citrate whereas LC8 could not.

Based on nucleotide homology and phylogenetic analysis, most potential cellulase-producing bacterial strain LC8 was identified as *Bacillus* sp. DDKRC1. (NCBI Accession no. JN641289) with, match score of 99.1% with *Bacillus* sp. LP1MB. (GU272341). Similarly, LC9 and CC8 were identified as *B. subtilis subsp. subtilis* DDKRC2. (JN641290) and *B. subtilis* DDKRC5. **(**JN641293**)** with 99% similarity with *Bacillus subtilis* JHDC68. (HM585063) and *Bacillus* sp. LS02. (GU972596.1) respectively.

Table 3 Cellulase, amylase and protease activities of different isolates from gut of *Lates calcarifer and Chanos chanos*

	Enzyme activity			
Fish species	**Bacterial isolates**	**Specific Amylase activity**†**	**Specific Cellulase activity**‡**	**Specific Protease activity**§**
L. calcarifer	LC1	–	–	4.73 ± 0.01^{c}
	LC2	–	7.57 ± 0.05^{b}	5.56 ± 0.02^{f}
	LC3	17.69 ± 0.04^{c}	45.53 ± 0.03^{e}	7.05 ± 0.13^{h}
	LC4	19.90 ± 0.05^{d}	44.24 ± 0.11^{e}	4.97 ± 0.01^{d}
	LC5	17.55 ± 0.05^{c}	41.42 ± 0.03^{d}	6.23 ± 0.05^{g}
	LC6	20.08 ± 0.07^{e}	–	3.04 ± 0.03^{a}
	LC7	14.72 ± 0.03^{b}	7.60 ± 0.02^{b}	5.15 ± 0.02^{e}
	LC8	20.80 ± 0.05^{e}	54.63 ± 0.04^{f}	6.09 ± 0.02^{g}
	LC9	26.15 ± 0.02^{g}	15.93 ± 0.05^{c}	4.70 ± 0.01^{c}
	LC10	–	43.56 ± 0.04^{e}	5.02 ± 0.01^{de}
	LC11	13.86 ± 0.08^{a}	1.09 ± 0.05^{a}	4.51 ± 0.00^{b}
	LC12	23.57 ± 0.07^{f}	6.90 ± 0.02^{b}	6.17 ± 0.01^{g}
C. chanos	CC1	23.25 ± 0.06^{d}	7.30 ± 0.07^{c}	5.59 ± 0.07^{e}
	CC2	10.26 ± 0.08^{a}	1.23 ± 0.05^{a}	4.76 ± 0.01^{b}
	CC3	19.96 ± 0.03^{c}	4.61 ± 0.03^{b}	4.75 ± 0.02^{b}
	CC4	18.98 ± 0.02^{bc}	7.56 ± 0.06^{c}	6.40 ± 0.01^{f}
	CC5	17.81 ± 0.02^{b}	–	3.66 ± 0.01^{a}
	CC6	–	2.45 ± 0.01^{ab}	4.90 ± 0.02^{c}
	CC7	–	3.35 ± 0.02^{b}	5.47 ± 0.02^{d}
	CC8	26.16 ± 0.038^{e}	15.74 ± 0.03^{d}	8.03 ± 0.02^{h}
	CC9	20.43 ± 0.017^{c}	–	6.60 ± 0.01^{g}

Values bearing different superscripts in a column under each fish species differ significantly.

†Microgram of maltose liberated per mg of protein per min.

‡Microgram of D-glucose liberated per mg of protein per min.

§Microgram of L-tyrosine liberated per mg of protein per min.

$^{**}P < 0.01$.

Table 4 Biochemical characteristics of LC8, LC9 and CC8

Tests	Characteristics		
	LC8	LC9	CC8
Gas production from glucose	+	–	–
Methyl red test	+	+	+
Voges Proskauer test	–	+	+
Indole test	–	–	–
Nitrate reduction test	+	+	+
Oxidation/fermentation of glucose	+	+	+
Oxidase test	–	+	+
Casein hydrolysis	+	+	+
H_2S production	–	–	–
Citrate utilization test	–	+	+
Catalase test	+	+	+
Urea hydrolysis	–	–	–
Starch hydrolysis	+	+	+
Cellulose hydrolysis	+	+	+
Gelatin hydrolysis	+	+	+
Tributyrin hydrolysis	–	+	+
Acid production from carbohydrates			+
Glucose, sucrose, lactose	+	+	+
Mannitol, xylose	+	+	

–, negative; +, positive.

Among these three promising isolates, two isolates i.e. LC8 with peak cellulase activity (54.63 U) and CC8 with peak amylase (26.16 U) and protease (8.03 U) activity were used for solid-state fermentation study.

From solid-state fermentation study, it was observed that 60% moisture and 48 h incubation was optimum for fermentation of rice bran with *Bacillus* sp. DDKRC1. and *B. subtilis* DDKRC5. In case of sunflower cake, 50% moisture and 72 h incubation was optimum for fermentation with *Bacillus* sp. DDKRC1. and 60% moisture and 48 h incubation was optimum for *B. subtilis* DDKRC5. with regard to dry matter loss and increase ($P < 0.05$) in protein content. Fermentation of rice bran and sunflower cake with *Bacillus* sp. DDKRC1. for optimum period at optimum moisture level resulted in increase in protein content by 19% and 8.32%, reduction in cellulose content by 6.6% and 14.65% and reduction in NFE content by 6.67% and 7.14% in fermented rice bran and sunflower cake, respectively, when compared with the control i.e. fermentation at zero hour (Table 5). Whereas fermentation with *B. subtilis* DDKRC5. increased the protein content of 10.46% and 5.70%, reduced the cellulose content of 2.42% and 6.25% and reduced NFE content of 9.86% and 10.31% in fermented rice bran, and sunflower cake, respectively, when compared with the control.

In this study, autochthonous bacteria strongly adhered to the gut having cellulolytic and amylolytic activity were isolated after 48 h of starvation.

Higher cellulolytic bacterial population in the gut of *C. chanos,* herbivore fish, as compared with *L. calcarifer*, carnivore fish, in this study clearly indicated that gut bacterial population changes with feeding behaviour of host animal (Kar & Ghosh 2008).

In general, bacteria are predominant in the environment in which fish live and it is impossible to avoid them being a component of their diet and they have a beneficial effect in the digestive processes of fish (Ring, Strm & Tabachek 1995).

Cellulolytic and amylolytic activity has been observed in different microbes isolated from the gastrointestinal tract of different fish e.g. tilapia (*Oreochromis mossambica*), Chinese grass carp (*Ctenopharyngodon idella*) and common carp *(Cyprinus carpio)* (Bairagi *et al.* 2002a and Saha *et al.* 2006). Ghosh *et al.* (2002) studied the enzymatic potentialities of *B. circulans, B. pumilus* and *B. cereus* isolated from the gut of *Labeo rohita*. Bairagi *et al.* (2002a) could not detect cellulolytic bacteria in the gastrointestinal tract of carnivorous catfish and murrels. However, the result of the present investigation showed that the presence of cellulolytic bacteria in carnivores *L. calcarifer* which might be due to the fact that carnivores may get it from other invertebrates that harbour the cellulolytic bacteria (Stickney 1975). In the present investigation, cellulase activity was higher in PI and DI of *L. calcarifer* and MI of *C. chanos* which might be due to the presence of higher microbial cellulase secreted by the cellulolytic microbiota present in the respective region of *L. calcarifer* and *C. chanos*. Shcherbina and Kazlauskiene (1971) also reported cellulose digestion in posterior portion of the digestive tract of carp indicating the presence of microbial cellulase in this region. Amylase activity was higher in DI of *L. calcarifer* and MI and DI region of *C. chanos* but population of amylolytic microbiota was higher in DI in both the species. Higher protease activity in PI and MI of *L. calcarifer* might be due to more endogenous secretion in those regions. The results of this study indicated that there was a distinct microbial source of digestive enzymes apart from the endogenous sources in fish digestive tracts. Similar findings were also reported by Mondal *et al.* (2008).

Table 5 Change of nutrient content of the feed ingredients fermented with potential gut microbes

		Name of bacteria	
Name of Ingredients	**Parameters**	***Bacillus* sp. DDKRC1.**	***B. subtilis* DDKRC5.**
Rice bran	Optimum fermentation condition	60% moisture & 48 h incubation	60% moisture & 48 h incubation
	Increase in CP%	19.00 ± 0.12^{b}	10.46 ± 0.14^{a}
	Decrease in Cellulose%	6.60 ± 0.22^{b}	2.42 ± 0.15^{a}
	Decrease in NFE%	6.67 ± 0.11^{a}	9.86 ± 0.06^{b}
Sunflower cake	Optimum fermentation condition	50% moisture & 72 h incubation	60% moisture & 48 h incubation
	Increase in CP%	8.32 ± 0.05^{b}	5.70 ± 0.12^{a}
	Decrease in Cellulose%	14.65 ± 0.21^{b}	6.25 ± 0.08^{a}
	Decrease in NFE%	7.14 ± 0.05^{a}	10.31 ± 0.14^{b}

Values bearing different superscripts in a column and row differ significantly $P < 0.05$.

The result of this study indicated that cellulolytic, amylolytic and proteolytic bacteria exist in the digestive tract of Asian seabass and milkfish and supported the hypothesis that bacteria contribute to the production of cellulase, amylase and protease in fish gut. These extracellular enzymes from the intestinal microbiota potentially could have a significant role in digestion, especially for substrates such as cellulose, which few animals can digest (Smith 1989).

Reduction in cellulose and NFE content of the fermented ingredients in solid-state fermentation (SSF) study might be due to secretion of cellulase and amylase enzyme by the bacteria which could help to reduce cellulose and available carbohydrate, NFE. The increase ($P < 0.05$) in protein content of the fermented feed ingredients may be attributed to the proportional dry matter loss during fermentation and also due to efficient bioconversion of highly polymerized carbohydrates into microbial protein and the production of different types of enzymes (Vijayakumar 2003; Bhatnagar 2004; Joseph, Paul & Bhatnagar 2008). Fermentation thus resulted in nutrient enrichment of rice bran and sunflower cake. The results of several feeding trials indicated that the crude fibre level of feed ingredients can be considerably reduced after being fermented with extracellular enzymes specially cellulase-, amylase- and protease-producing bacterial strains in solid-state fermentation process (Bairagi, Sarkar, Sen & Ray 2002b; Bairagi *et al.* 2004; Ramachandran, Bairagi & Ray 2005; Saha & Ray 2011). Joseph *et al.* (2008) reported that there was initial decrease in crude protein content in fermented ingredient mixture due to utilization of available nitrogen of ingredients by *Aspergillus niger* for its vegetative growth, followed by the synthesis of protein through the process of bioconversion resulting in an increase in protein content of substrate with extended duration of fermentation. Similar observations in protein enrichment were also reported by Singh, Linden, Johnson and Tengerdy (1990) and Arora, Sehgal and Thapar (2000) for fermented potato process waste using *Rhizopus oryzae*.

The outcome of this study indicated that these two bacteria isolated from the gut of Asian seabass and milk fish were capable of producing cellulolytic, amylolytic and proteolytic enzymes in varying quantities and can be beneficially applied for nutrient enrichment of different feed ingredients. There is scope for refinement of fermentation process using both the bacteria together for nutrient enrichment of different feed ingredients. The potentiality of these enriched ingredients to replace fish meal in diet of different aquaculture species and effect of live supplementation of these two bacteria in fish performance is a subject for further study to make the aquaculture economically viable.

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References

AOAC (Association of Official Analytical Chemist (1995) *Official Methods of Analysis*. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Arora M., Sehgal V.K. & Thapar V.K. (2000) Production of fungal protein and amylases by solid substrate fermentation of potato-waste. *Indian Journal of Microbiology* **40**, 259–262.

Askarian F., Zhou Z., Olsen R.E., Sperstad S. & Ring E. (2012) Culturable autochthonous gut bacteria in Atlantic salmon (*Salmo salar* L.) fed diets with or without chitin. Characterization by 16S rRNA gene sequencing, ability to produce enzymes and in vitro growth inhibition of four fish pathogens. *Aquaculture* **326–329**, 1–8.

Bairagi A., Sarkar Ghosh K., Sen S.K. & Ray A.K. (2002a) Enzyme producing bacterial flora isolated from fish digestive tracts. *Aquaculture International* **10**, 109–121.

Bairagi A., Sarkar Ghosh K., Sen S.K. & Ray A.K. (2002b) Duckweed (*Lemna polyrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu (*Labeo rohita* Ham.) fingerlings after fermentation with a fish intestinal bacterium. *Bioresource Technology* **85**, 17–24.

Bairagi A., Sarkar Ghosh K., Sen S.K. & Ray A.K. (2004) Evaluation of nutritive value of *Leucaena leucocephala* leaf meal inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings. *Aquaculture Research* **35**, 436–446.

Bernfield P. (1955) Amylases, α and β. In: *Methods of Enzymology*, Vol. I (ed. by S. P Colowick & N. O Kaplan), p. 149. Academic Press, New York.

Beveridge M.C.M., Sikdar P.K., Frerichs G.N. & Millar S. (1991) The ingestion of bacteria in suspension by the common carp *Cyprinus carpio* L. *Journal of Fish Biology* **39**, 825–831.

Bhatnagar D. (2004) Amylase and protease production by solid state fermentation using *Aspergillus niger* from mangrove swamp, M. F. Sc. (Mariculture) Dissertation, Central Institute of Fisheries Education, Mumbai, India, 63pp.

Cahill M.M. (1990) Bacterial flora of fishes: a review. *Microbial Ecology* **19**, 21–41.

Das K.M. & Tripathi S.D. (1991) Studies on the digestive enzymes of grass carp *Ctenopharyngodon idella* (Val.). *Aquaculture* **92**, 21–32.

Denison D.A. & Koehn R.D. (1977) Cellulase activity of *Poronia Oedipus*. *Mycologia* **69**, 592–601.

Dhage K.P. (1968) Studies of the digestive enzymes in the three species of the major carps of India. *Journal of Biological Science* **11**, 63–74.

Gatesoupe F.J., Zambonino Infante J.L., Chu C. & Quazuguel P. (1997) Early weaning of sea bass larvae, *Dicentrarchus labrax*: the effect of microbiota, with particular attention to iron supply and exoenzymes. *Aquaculture* **158**, 117–127.

Ghosh K., Sen S.K. & Ray A.K. (2002) Characterization of Bacilli isolated from the gut of rohu, *Labeo rohita,* fingerlings and its significance in digestion. *Journal of Applied Aquaculture* **12**, 33–42.

Jacob M.B. & Gerstein M.J. (1960) *Handbook of Microbiology*. D Van Nostrand Co. Inc., Princeton, New Jersey.

Joseph I., Paul Raj R. & Bhatnagar D. (2008) Effect of solid state fermentation on nutrient composition of selected feed ingredients. *Indian Journal of Fisheries* **55**, 327–332.

Kar N. & Ghosh K. (2008) Enzyme producing bacteria in the gastrointestinal tracts of *Labeo rohita* (Hamilton) and *Channa punctatus* (Bloch). *Turkish Journal of Fisheries and Aquatic Sciences* **8**, 115–120.

Kar N., Roy R.N., Sen S.K. & Ghosh K. (2008) Isolation and characterization of extracellular enzyme producing bacilli in the digestive tract of Rohu, *Labeo rohita* (Hamilton) and Murrel, *Channa punctatus*(Bloch). *Asian Fisheries Science* **21**, 421–434.

Khan A. & Ghosh K. (2012) Characterization and identification of gut-associated phytase-producing bacteria in some freshwater fish cultured in ponds. *Acta Ichthyologica et Piscatoria* **42**, 37–45.

Mondal S., Roy T., Sen S.K. & Ray A.K. (2008) Distribution of enzyme-producing bacteria in the digestive tracts of some freshwater fish. *Acta ichthyologica et piscatoria* **38**, 1–8.

Ramachandran S., Bairagi A. & Ray A.K. (2005) Improvement of nutritive value of grass pea (*Lathyrus sativus*) seed meal in the formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings after fermentation with a fish gut bacterium. *Bioresource Technology* **96**, 1465–1472.

Ray A.K., Ghosh K. & Ring E. (2012) Enzyme producing bacteria isolated from fish gut: a review. *Aquaculture Nutrition*, **18**, 465–492.

Ring E., Strm E. & Tabachek J.A. (1995) Intestinal microflora of salmonids: a review. *Aquaculture Research* **26**, 773–789.

Roy T., Mondal S. & Ray A.K. (2009) Phytase-producing bacteria in the digestive tracts of some freshwater fish. *Aquaculture Research* **40**, 344–353.

Saha A.K. & Ray A.K. (1998) Cellulase activity in rohu fingerlings. *Aquaculture International* **6**, 281–291.

Saha S. & Ray A.K. (2011) Evaluation of nutritive value of water hyacinth (*Eichhornia crassipes*) leaf meal in compound diets for rohu, *Labeo rohita* (Hamilton, 1822) fingerlings after fermentation with two bacterial strains isolated from fish gut. *Turkish Journal of Fisheries and Aquatic Sciences* **11**, 199–207.

Saha S., Roy R.N., Sen S.K. & Roy A.K. (2006) Characterization of cellulase-producing bacteria from the digestive tract of tilapia, *Oreochromis mossambica*

(Peters) and grass carp, *Ctenopharyngodon idella* (Valencinnes). *Aquaculture Research* **37**, 380–388.

Shcherbina M.A. & Kazlauskiene O.P. (1971) The reaction of the medium and the rate of absorption of nutrients in the intestine of carp. *Journal of Ichthyology* **11**, 81–85.

Singh K., Linden C.J., Johnson E.J. & Tengerdy P.R. (1990) Bioconversion of wheat straw to animal feed by solid substrate fermentation or ensiling. *Indian Journal of Microbiology* **30**, 201–208.

Smith L.S. (1989) Digestive functions in teleost fishes. In: *Fish Nutrition* (2nd edn) (ed. by J.E. Halver), pp. 331–421. Academic Press, San Diego.

Stickney R.R. (1975) Cellulase activity in the stomachs of freshwater fishes from Taxas. *Proceedings of southeast Association of Game fish Commission* **29**, 282–287.

Teather R.M. & Wood P.J. (1982) Use of Congo-red polysaccharide interaction in enumeration and charecterization of cellulolytic bacteria from the bovine rumen. *Applied Environmental Microbiology* **43**, 777–780.

Trust T.J. & Sparrow R.A.H. (1974) The bacterial flora in the alimentary tract of freshwater salmonid fishes. *Canadian Journal of Microbiology* **20**, 1219–1228.

Vijayakumar M. (2003) Solid state fermentation of oil cakes and wheat flour and evaluation of the products in shrimp feed. M.F.Sc. (Mariculture) Dissertation, Central Institute of Fisheries Education, Mumbai, India, 85pp.

Walter H.E. (1984) *Methods of Enzymatic Analysis*. Verlag Chemie, Weinheim.

Williams S.T., Sharp M.E. & Holt G., (eds.) (1986) *Bergey's Manual of Systematic Bacteriology*, Vol. I, Williams and Wilkins, Baltimore, USA.

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