Enzymatic Activities of Intestinal Bacteria Isolated from Farmed Clarias gariepinus

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Abstract The culturable bacteria associated with the digestive tract of a freshwater cultured fish, Clarias gariepinus, and their degradative abilities were established. The spread plate method was employed for bacterial isolation. The bacterial isolates were qualitatively screened for extracellular enzyme-producing ability using milk agar, starch agar, egg yolk agar and cellulose agar for protease, amylase, lipase and cellulase activities respectively. A total of 18 bacterial isolates were identified. Bacteria of the genera Bacillus, Staphylococcus, Vibrio, Aeromonas, Pseudomonas, Lactobacillus, Escherichia, Salmonella, Enterobacter, Micrococcus and Flavobacterium were isolated from fish digestive tract at different frequencies with Bacillus predominating. Enzymatic studies indicated that the bacterial isolates possess the ability to degrade proteins, starch, lipids and cellulose. The percentage composition of enzyme-producing bacteria are - protease producing strains (72.2%), lipase producing strains (61.1%), amylase producing strains (55.6%) and cellulase producing strains (38.9%). All the isolates possessed multienzyme activity. An isolate (Bacillus sp. B.) showed activity for protease, amylase, lipase and cellulase enzymes. Therefore, the isolated indigenous multiple enzyme-producing strains can be effectively exploited for use as probiotics while formulating aquafeeds.

Keywords Gut bacterial flora; Freshwater cultured fish; Qualitative enzyme activity

Introduction Aquaculture is an emerging industrial sector which requires continued research with scientific and technical developments and innovation (John and Hatha, 2013). The African catfish, Clarias gariepinus, is of growing economic value in the African aquaculture industry (Aldelhamid, 2009). The fish has become one of the most important freshwater aquaculture species in Nigeria and has a high market value as table fish, being tasty and scaleless. According to Thillaimaharani et al. (2012), the world wide sustainability of the aquaculture industry depends only on the inexpensive high quality feeds. Therefore, microbial enzymes are very much essential for the preparation of high quality functional feeds through bioconversion of cost-effective feed materials.

The intestinal microflora and its metabolic activities can be an important contributing factor in nutrition, physiology and animal welfare (Tanu et al., 2012). This is because the gut microflora in fishes and crustaceans can metabolize several nutrients that the host cannot and can convert them to end products that are beneficial to the host (Suher et al., 2007; Hoyoux et al., 2009). It has been reported that intestinal microorganisms have a beneficial effect on the digestive process of fish such as in the microbial breakdown of cellulose (Saha and Ray, 1998; Bairagi et al., 2002; Saha et al., 2006; Mondal et al., 2008; 2010; Ray et al., 2010), starch (Sugita et al., 1997), protein (Chong et al., 2002; Fu et al., 2005; Silvia et al., 2006) and lipid (Tanu et al., 2012).

The gastrointestinal tract of fish, when compared with the surrounding water, is rich in nutrients and confers a more favourable environment for growth of microorganisms (Saha et al., 2006). The bacterial flora associated with the intestine of tropical estuarine fish species such as Tilapia guineensis has been established (Ariole and Kanu, 2013). The intestinal microflora of fish and shellfish has been reported to aid in the secretion of inhibitory substances that prevent colonization by bacterial pathogens (Berg, 1996; Sugita et al., 1998; Ariole and Anugwa, 2013; Ariole and Nyeche, 2013; Ariole and Oha, 2013). The microbial flora status in Clarias gariepinus hatchery hatchery.
Information on the enzymatic activities of intestinal bacteria from farmed *Clarias gariepinus* is not available. Assessment of the substrate degrading ability of gut microflora is important in understanding the nutrition and physiology of the host organism and may help in formulating appropriate feeds (Tanu et al., 2012). Therefore, the present study was undertaken to establish the culturable bacteria associated with the intestinal tract of farmed *Clarias gariepinus* and their degradative abilities.

1 Materials and Methods

1.1 Sample collection

The African catfish (*Clarias gariepinus*) was obtained from a private fish farm in Port Harcourt, Rivers State of Nigeria.

1.2 Enumeration and isolation of culturable intestinal bacteria

Fifty live fish with an average weight of approximately 25g were killed by physical destruction of the brain. Before dissection, the fish were externally cleaned with 70% ethanol. Pooled samples of 10 fish were used for each replicate. From each pooled gut contents, 1.0g was taken aseptically and homogenized with 9.0ml sterile physiological saline. The homogenate was serially diluted up to $10^{-6}$ dilution. Then 0.1ml of each dilution was plated in triplicate onto different media using the spread plate method. The media chosen were Nutrient agar (Oxoid), MacConkey agar (BIOTECH), Thiosulphate citrate bile salt sucrose (TCBS) agar (Oxoid), Salmonella-Shigella agar (Fluka), Aeromonas medium with Ampicillin supplement (Ryan) (Oxoid), Mannitol salt agar(Lab M), Pseudomonas cetrimide agar (Oxoid) and de Man Rogosa and Sharpe(MRS) agar (Oxoid).

1.3 Screening for amylase-producing strains

Bacterial isolates were screened for amylolytic properties by starch hydrolysis test on starch agar plates. The plates were incubated at 37°C for 24h. The plates were flooded with 1% prepared iodine solution at the end of incubation. A clear zone of hydrolysis surrounding the growth indicates positive result while the presence of blue colour around the growth indicates a negative result.

1.4 Screening of potent alkaline protease producing strains

Bacterial isolates were screened for extracellular protease production by streaking onto skim milk agar plates. The plates were incubated at 37°C for 24h. Protease production was demonstrated by the clearing of opaque milk proteins in the area surrounding the colony.

1.5 Screening of lipolytic bacteria

The isolates were screened for lipolytic activity by streaking on Egg Yolk Agar. The plates were incubated at 37°C for 24-48h. The formation of a thin iridescent layer overlying the colonies was considered as positive result.

1.6 Screening for cellulolytic bacteria

Isolates were screened for cellulose activity by streaking on Cellulose Agar. Plates were incubated for 24-48h at 37°C. At the end of the incubation period, the plates were flooded with 1% congo red. Appearance of clear zone around the colony showed the presence of cellulase.

2 Results

2.1 Bacterial count and isolation

The total heterotrophic bacterial count was $3.8\pm0.02 \times 10^{8}$ cfu/g in the intestine of farmed *Clarias gariepinus*. A total of 18 bacterial isolates were identified according to Holt et al. (1994). Bacteria of the genera *Bacillus*, *Staphylococcus*, *Vibrio*, *Aeromonas*, *Pseudomonas*, *Lactobacillus*, *Escherichia*, *Salmonella*, *Enterobacter*, *Micrococcus* and *Flavobacterium* were isolated from the fish gut at different frequencies with *Bacillus* (22.2%) predominating (Table 1).

| Genus             | Number of isolates |
|-------------------|--------------------|
| Bacillus          | 4 (22.2)           |
| Staphylococcus    | 2 (11.1)           |
| Vibrio            | 2 (11.1)           |
| Aeromonas         | 2 (11.1)           |
| Pseudomonas       | 2 (11.1)           |
| Lactobacillus     | 1 (5.6)            |
| Escherichia       | 1 (5.6)            |
| Salmonella        | 1 (5.6)            |
| Enterobacter      | 1 (5.6)            |
| Micrococcus       | 1 (5.6)            |
| Flavobacterium    | 1 (5.6)            |
| Total             | 18 (100)           |

Note: Numbers in parentheses represent percentage frequencies
2.2 Screening of enzyme-producing strains

The composition and substrate degrading ability of bacteria isolated from the intestinal tract of farmed *Clarias gariepinus* is shown in Table 2. The percentage composition of enzyme-producing bacteria are: Protease producing strains (72.2%), lipase producing strains (61.1%), amylase producing strains (55.6%) and cellulase producing strains (38.9%). All the isolates possessed multienzyme activity. An isolate (*Bacillus* sp. B1) showed activity for protease, lipase, amylase and cellulase enzymes.

Table 2 Composition and substrate degrading ability of bacteria isolated from the intestinal tract of farmed *Clarias gariepinus*

| Bacterial isolates            | Enzymes                        |
|------------------------------|--------------------------------|
|                              | Protease (72.2%) | Lipase (61.1%) | Amylase (55.6%) | Cellulase (38.9%) |
| *Bacillus* sp. B1            | +                | +              | +               | +                |
| *Bacillus* sp. B2            | +                | +              | -               | -                |
| *Bacillus* sp. B3            | +                | +              | -               | -                |
| *Bacillus* sp. B4            | -                | +              | -               | -                |
| *Staphylococcus aureus*      | +                | -              | -               | +                |
| *Staphylococcus sp.*         | +                | -              | -               | +                |
| *Vibrio* sp. V1              | -                | +              | +               | -                |
| *Vibrio* sp. V2              | -                | -              | +               | -                |
| *Aeromonas hydrophila*       | +                | -              | +               | -                |
| *Aeromonas* sp.              | +                | -              | +               | -                |
| *Pseudomonas aeruginosa*     | +                | +              | -               | -                |
| *Pseudomonas* sp.            | +                | +              | -               | -                |
| *Lactobacillus* sp.          | +                | +              | -               | -                |
| *Escherichia coli*           | +                | +              | -               | -                |
| *Salmonella* sp.             | +                | +              | -               | +                |
| *Enterobacter* sp.           | -                | -              | +               | +                |
| *Micrococcus* sp.            | -                | +              | -               | +                |
| *Flavobacterium* sp.         | +                | -              | -               | -                |

Note: (+) indicates positive activity; (-) indicates negative activity; Numbers in parentheses represent the percentage composition of enzyme-producing strains

3 Discussion

The total heterotrophic bacterial count of 3.8±0.02 x 10^8 cfu/g in the intestinal tract of farmed *Clarias gariepinus* reveal that dense bacterial population occur in the digestive tract of fish. This result is in agreement with that of Shangong et al. (2010) who reported a total viable count of 3.4 x 10^8 cfu/g in the intestinal content of yellow catfish (*Pelteobagrus fulvidraco*). Al-Harbi and Udin (2005) reported that the presence of a high bacterial load in gill and intestine of fish might be due to high metabolic activity of fish associated with increased feeding rates at higher temperature. They also reported that pond water and sediment bacteria influenced the bacterial composition of gills and intestine of tilapia.

The genera of bacteria (Table 1) isolated from the intestine of farmed *Clarias gariepinus* are not uncommon to the aquatic environment and have been isolated by other workers (Al-Harbi and Udin, 2004; Pond et al., 2006; Hovda et al., 2007; Kim et al., 2007 and Ariole and Kalu, 2013). The bacteria ingested by the fish along with their diet may adapt themselves to the environment of the gastrointestinal tract and form a symbiotic association (Ringø and Birkbeck, 1999).

Enzymatic studies indicated that the bacterial isolates possess the ability to degrade proteins, lipids, starch and cellulose (Table 2). Protease activity was exhibited by a majority of the isolates (72.2%) reflecting that the bacterial flora associated with the intestinal tract of *Clarias gariepinus* are capable of digesting foods rich in proteins. Some authors have also established that bacteria in the digestive tract of fish demonstrated proteolytic, lipolytic, amylolytic and cellulolytic activities (Ghosh et al., 2002; Saha et al., 2006; Ray et al., 2010; Sumathi et al., 2011 and Ariole and Kalu, 2013). The presence of these enzymes is important for the digestion of proteins, lipids, starch and cellulose, which are the major components of fish feed.
microorganisms in the digestive tract indicates a significant role played by them during digestion of food.

The activity for four enzymes (protease, lipase, amylase and cellulase) showed by an isolate (Bacillus sp. B1) is not surprising because diverse strains of exo-enzyme producing Bacillus spp. have been identified from the gastrointestinal tract of freshwater teleosts (Ray et al., 2012). The bacterial flora of the gastrointestinal tract with diversified enzymatic potential plays a vital role in major part of the metabolism of the host animal (Clements, 1997). The digestive enzymes present in fish digestive tract can elucidate some aspects of their nutritive physiology and thus be supportive to develop nutritional strategies for fish feeding and diet formulation (Alexander et al., 2002; Ghosh et al., 2002; Nibeta and Ghosh, 2008 and Ray et al., 2010). Therefore, the indigenous multiple enzyme-producing bacteria can be effectively exploited for use as probiotics while formulating cost-effective aquafeeds.

Authors’ contributions

CNA contributed during conception and design, sample collection, analysis and interpretation of results and write-up of the manuscript. HAN and PWC contributed during sample analysis and acquisition of data. All the authors read and approved the final manuscript.

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