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International Journal of Marine Science, 2016,Vol.6, No.40, 1-6
http://ijms.biopublisher.ca

ResearchArticle Open Access

Assessment of Phytase Producing Ability of Marine Fish Intestinal Bacteria and Yeasts

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International Journal of Marine Science 2016, Vol.6, No. 40doi: 10.5376/ijms.2016.06.0040
Received: 11 Aug., 2016
Accepted: 14 Oct., 2016
Published: 27 Oct., 2016

Preferred citation for this article:
Ariole C.N., and Uchegbu S.C., 2016, Assessment of Phytase Producing Ability of Marine Fish Intestinal Bacteria and Yeasts, International Journal of Marine Science, 6(40): 1-6 (doi:10.5376/ijms.2016.06.0040)

AbstractThe phytase producing bacteria and yeasts associated with the intestinal tract of two marine fish species (*Liza grandsquamis* and *Ethmalosa fimbriata*) from New Calabar River in the Niger Delta were established. Spread plate technique was employed for isolation of phytase producing bacteria and yeasts using modified phytase-screening medium (MPSM). The pH of the medium was adjusted to 7.0 and 4.5 for isolation of bacteria and yeasts respectively. The isolates were further evaluated for quantitative phytase assay with MPSM broth. The phytase producing bacterial genera in the digestive tract of fish species were identified as *Bacillus*, *Pseudomonas* and *Enterobacter* while the isolated phytase producing yeasts genera were *Saccharomyces* and *Candida*. Among bacterial phytate degraders, *Bacillus* sp. isolated from *Liza grandsquamis* intestine showed highest phytase activity (50.09 ± 0.15 U/ml) while *Enterobacter* sp. isolated from *Ethmalosa fimbriata* intestine showed the lowest phytase activity (2.75 ± 0.32 U/ml). Among yeasts phytate degraders, *Saccharomyces* sp. isolated from *Liza grandsquamis* intestine showed highest phytase activity (12.5 ± 0.27 U/ml) while *Candida* sp. isolated from *Ethmalosa fimbriata* intestine exhibited the lowest phytase activity (3.66 ± 0.71 U/ml). These genera of aerobic microorganisms may take part in phytate degradation in the intestine of marine organisms. Their ability to degrade phytate may provide environmental benefits to defeat the plant phytate anti-nutritional effects.
Keywords Marine fish; Intestinal tract; Bacteria; Yeast; Phytase activity

Introduction

The main phosphorus (P) reservoir in plant feed materials is phytic acid (Myo-inositol 1, 2, 3, 4, 5, 6-hexakis-dihydrogen phosphate) (Oatway et al., 2001). The release of phosphate from phytic acid is initiated by a hydrolytic enzyme known as phytase (E. C. 3. 1. 3. 8. myo-inositol hexaphosphate phosphohydrolase). Phytate, apart from acting as phosphorus reservoirs, bind a significant portion of the micro-elements, proteins, carbohydrates and amino acids transferring them into complex insoluble conglomerates (Onyango and Adeola, 2012). A promising way out to these problems is phytases which have been reported to boost phosphate consumption effectiveness from phytate in feed materials and to reduce phosphorus environmental contamination (Broz et al., 1994; Pen et al., 1993).

A vital nutrient for fish and other livestock is phosphorus which plays a key function in cell formation. It is an important element for fish reproduction, growth and skeletal development (Asgand and Shearer, 1997). The release of elevated concentrations of soluble phosphorus from fish farms into aquatic environment leads to phytoplankton growth which causes concentrations of dissolved oxygen to fluctuate (Li et al., 2004). Inclusion of exogenous phytase in fish (Buruah et al., 2007; Debnath et al., 2005a; b; Sardar et al., 2007; Cao et al., 2008), pig (Han et al., 1997) and poultry (Lei and Stahl, 2000) diets has been reported to improve availability of trace-elements, amino acids, minerals and energy. This also contributes significantly to ecological safety by reducing excretion of phosphorus.

Microbes are the most excellent sources for industrial production of phytases because of their high enzyme production and easy cultivation (Li et al., 2008). Phytase-producing symbiotic bacteria isolated from fish intestine can be used as probiotics in aquaculture to inhibit or decrease the toxicity of phytate in the microenvironment of

the gut (Khan and Ghosh, 2012). Autochthonous phytase producing gut bacteria in freshwater fishes have been reported (Khan and Ghosh, 2012; Khan et al., 2011; Roy et al., 2009). However, information on phytase producing intestinal bacteria and yeasts in marine fish species in the Niger Delta is scarce.

Therefore, in the present study, we evaluated the phytase producing ability of intestinal bacteria and yeasts isolated from two marine fish species (*Liza grandsquamis* and *Ethmalosa fimbriata*) from New Calabar River in the Niger Delta.

1 Materials and Methods

1.1 Collection of Sample

Healthy live marine fish species *(Liza grandsquamis* and *Ethmalosa fimbriata*) were collected from New Calabar River in Nigeria with the assistance of a local fisherman and transported to the laboratory in sterile plastic bags.

1.2 Isolation of phytase producing intestinal bacteria and yeasts

Phytase-producing bacteria and yeasts were isolated using a modified phytase-screening medium (MPSM) described by Khan and Ghosh, 2012. The medium contain: 0.01 g $FeSO_4$ $7H_2O$, 10 g glucose, 10 g urea, 2 g sodium citrate, 3 g citric acid, 1 g $MgSO_4$ $7H_2O$, 1 g $(NH_4)_2SO_4$, 3 g sodium phytate and 20 g agar per litre of deionized water.

Two marine fish species (*Liza grandsquamis* and *Ethmalosa fimbriata*) were examined. For each replicate, three samples of fish were used. The average weight/length of *Liza grandsquamis* and *Ethmalosa fimbriata* were 22 g/10 cm and 11 g/8 cm respectively. The six fish samples were killed by physical damage of the brain. Prior to dissection, 70% ethanol was used to clean the fish superficially. From each collective gut contents, 1.0 g was taken with sterile precaution and suspended in 9.0 ml sterile normal saline. Serial dilution up to 10^{-6} was then carried out with the homogenate.

For isolation of phytase producing bacteria, 0.1 ml of each dilution was inoculated in triplicate on MPSM (pH 7.0) plates. The plates were incubated at 37°C for 72 h. Colonies with different morphological appearances from a particular plate were streaked singly on MPSM plates to obtain uncontaminated cultures. Based on their morphological and biochemical characteristics, the purified isolates were identified to generic level (Holt et al., 1994; Garrity et al., 2005; de La Maza et al., 2013).

For isolation of phytase producing yeasts, 0.1 ml of each dilution was inoculated in triplicate on MPSM (pH 4.5) plates. The plates were incubated at 28°C for 5 days. A portion of each yeast colony which developed was aseptically subcultured into fresh MPSM (pH 4.5) plates for purification. Characterization of yeasts isolates were based on colonial and microscopic examination as well as biochemical features. Identification to generic level was performed using the keys provided by Samson and De Boer, 1995.

1.3 Quantitative assay for extracellular phytase production

Phytase-producing bacterial and yeast strains isolated from the fish species examined were further evaluated for quantitative phytase assay with MPSM broth. The method described by Yanke et al., 1998 was employed. Broth culture (1.5 ml) was centrifuged at 18,000 g. The cells were washed with 0.1 M sodium acetate (pH 5.0) and then suspended in 750 µl buffer. Thereafter, 600 µl 0.2% (w/v) sodium phytate which was prepared with 0.1 M sodium acetate buffer (pH 5.0) was added to 150 µl of the cell suspension. It was incubated for 30 min at 39°C. Then 750 µl 5% (w/v) trichloroacetic acid and 750 µl phosphomolybdate colour reagent were added to stop the reaction. The colour reagent was newly prepared by adding 4 vols 1.5% (w/v) ammonium molybdate solution in 5.5% (v/v) sulphuric acid to 1 vol. 2.7% (w/v) ferrous sulphate solution. The colour was allowed to develop for 5 min. Then, the liberated inorganic phosphorous was measured at A_{840} with a Spectronic UV Spectrophotometer. One unit (U) of phytase activity was defined as 1µg of inorganic phosphorous released per 1 ml of culture filtrate per 1 min (Yanke et al., 1999).

1.4 Statistical analysis

Standard deviations for each of the experimental results were calculated using Excel Spreadsheets, with Microsoft excel software. Differences between treatments were examined for significance by one-way ANOVA and P = 0.05 was considered to be statistically significant.

2 Results

2.1 Phytase producing intestinal bacteria

Phytase producing intestinal bacteria from *Liza grandsquamis* were identified as *Bacillus* (2 strains), *Pseudomonas* (1 strain) and *Enterobacter* (1 strain) (Table 1) while phytase producing intestinal bacteria from *Ethmalosa fimbriata* were identified as *Bacillus* (2 strains) and *Enterobacter* (1 strain) (Table 2).

Table 1: Phytase activity of bacterial isolates from *Liza grandsquamis* intestine

Bacterial isolates	**Phytase activity (U/ml)**
Bacillus sp.	50.09 ±0. 15
Bacillus sp.	36.06 ± 0. 21
Pseudomonas sp.	8.44 ±0. 34
Enterobacter sp.	6.30 ±0. 41

Table 2: Phytase activity of bacterial isolates from *Ethmalosa fimbriata* intestine

Bacterial isolates	**Phytase activity (U/ml)**
Bacillus sp.	30.81 ±0. 11
Bacillus sp.	25.31 ±0. 51
Enterobacter sp.	2.75 ±0. 32

2.2 Phytase producing intestinal yeasts

The phytase producing intestinal yeasts from *Liza grandsquamis* were identified as *Saccharomyces* (2 strains) and *Candida* (1 strain) (Table 3). The phytase producing intestinal yeasts from *Ethmalosa fimbriata* were identified as *Saccharomyces* (2 strains) and *Candida* (1 strain) (Table 4).

Table 3: Phytase activity of yeast isolates from *Liza grandsquamis* intestine

Yeast isolates	**Phytase activity (U/ml)**
Saccharomyces sp.	12.5 ±0. 27
Saccharomyces sp.	8.82 ±0. 39
Candida sp.	5.1 ±0. 47

Table 4: Phytase activity of yeast isolates from *Ethmalosa fimbriata* intestine

Yeast isolates	**Phytase activity (U/ml)**
Saccharomyces sp.	7.12 ±0. 62
Candida sp.	3.66 ±0. 71

2.3 Quantitative phytase activity

Among bacterial phytate degraders, *Bacillus* sp. isolated from *Liza grandsquamis* intestine showed highest phytase activity (50.09 ± 0.15 U/ml) while *Enterobacter* sp.isolated from *Ethmalosa fimbriata* intestine showed the lowest phytase activity (2.75 ± 0.32 U/ml) (Tables 1 and 2). Among yeasts phytate degraders, *Saccharomyces* sp. isolated from *Liza grandsquamis* intestine showed highest phytase activity (12.5 ± 0.27 U/ml) while *Candida* sp. isolated from *Ethmalosa fimbriata* intestine showed the lowest phytase activity (3.66 ± 0.71 U/ml) (Tables 3 and Table 4).

3 Discussion

Three bacterial genera *Bacillus*, *Pseudomonas* and *Enterobacter* isolated from *Liza grandsquamis* intestine (Table 1) and two bacterial genera *Bacillus* and *Enterobacter* isolated from *Ethmalosa fimbriata* intestine (Table 2) were found to utilize phytate for growth. A phytase producing bacteria such as *Bacillus licheniformis* isolated from *Labeo rohita* (Roy et al., 2009) and *Rhodococcus* sp. MTCC 9508 isolated from *Catla catl*a (Khan et al., 2011) have been reported. It has also been reported that *Acinetobacter* sp., *B. subtilis*, *B. cereus*, *B. thuringiensis* and *Bacillus* sp. isolated from the gastrointestinal tract of Atlantic salmon (S*almo solar*) that was fed with or without diet supplemented with chitin demonstrated phytase activity (Askarian et al., 2012). Phytase producers such as *Brochothrix thermosphacta* and *Brochothrix* sp. from gastrointestinal tract of Atlantic cod were also reported (Askarian et al., 2013).*Bacillus atropheus* and *Bacillus subtilis* isolated from *Gudusia chapra* and *Labeo bata* respectively also exhibited phytase activity (Khan and Ghosh, 2012). Several authors suggested that enzymes produced by such intestinal microorganisms may have a major function in digestion (Ariole et al., 2014; Ghosh et al., 2002a; b; Ray et al., 2010; 2012).

Two yeast genera *Saccharomyces* and *Candida* isolated from *Liza grandsquamis* (Table 3) and *Ethmalosa fimbriata* (Table 4) were found to utilize phytate for growth. A number of marine yeast strains from the intestine of marine fish (*Acanthogobius hasta* and *Hexagrammos otakii*) and sea cucumber (*Holothuria scabra*) have been recognized as phytase producers (Li et al., 2008). Such phytase producing microorganisms might aid in breakdown of phytate in the intestines of the host animal (Li et al., 2008).

The measured levels of phytase activity (Tables 1, 2, 3 and 4) suggest that fish gut bacteria and yeasts are a prospective source of phytase that can be commercially developed. Exploitation of such gut microbiota detected in the present study for breakdown of phytate in feed materials of plant source is possible for the improvement of the nutritive value of feeds rich in phytate. Results of the current study suggest that symbiotic phytase-producing bacteria and yeasts from fish intestine can be exploited as probiotics in aquaculture that may reduce the toxicity of phytate in the microenvironment of the gut and reduce faecal phosphorus pollution into the aquatic environment.

Authors' contributions

SCU collected the samples and performed the experiment. CNA conceived, designed and supervised the study and also wrote the manuscript. All the authors read and approved the final manuscript.

References

Ariole C.N., Nwogu H.A., and Chuku P.W., 2014, Enzymatic activities of intestinal bacteria isolated from farmed *Clarias gariepinus*, International Journal of Aquaculture, 4(18): 108-112
http://dx.doi.org/10.5376/ija.2014.04.0018

Asgard T., and Shearer K.D., 1997, Dietary phosphorus requirement of juvenile Atlantic salmon, *Salmo salar* L, Aquaculture Nutrition, 3: 17-23
http://dx.doi.org/10.1046/j.1365-2095.1997.00069.x

Askarian F., Zhou Z., Olsen R.E., Sperstad S., and Ringø E., 2012, Culturable autochthonous gut bacteria in Atlantic salmon (*Salmosalar* 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
http://dx.doi.org/10.1016/j.aquaculture.2011.10.016

Askarian F., Sperstad S., Merrifield D.L., Ray A.K., and Ringø E., 2013, The effect of different feeding regimes on enzyme activity of gut microbiota in Atlantic cod (*Gadusmorhua* L.),Aquaculture Research, 44(5): 841-846
http://dx.doi.org/10.1111/j.1365-2109.2011.03079.x

Broz J., Oldale P., Perrin-Voltz A. H., Rychen G., Schulze J., and SimoesNunes, C., 1994, Effects of supplemental phytase on performance and phosphorus utilisation in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates, Brazilian Journal of Poultry Science, 35: 273-280
http://dx.doi.org/10.1080/00071669408417691

Baruah K., Sahu N.P., Pal A.K., Jain K.K., Debnath D., and Mukherjee S.C., 2007, Dietary microbial phytase and citric acid synergistically enhances nutrient digestibility and growth performance of *Labeo rohita* (Hamilton) juveniles at sub-optimal protein level, Aquaculture Research, 38: 109-120
http://dx.doi.org/10.1111/j.1365-2109.2006.01624.x

Cao L., Yang Y., Wang W.M., Yakupitiyage A., Yuan D.R., and Diana J.S., 2008, Effects of pre-treatment with microbial phytase on phosphorous utilization and growth performance of Nile tilapia (*Oreochromis niloticus*), Aquaculture Nutrition, 14: 99-109

http://dx.doi.org/10.1111/j.1365-2095.2007.00508.x

Debnath D., Pal A.K., Sahu N.P., Jain K.K., Yengkokpam S., and Mukherjee S.C., 2005a, Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings, Aquaculture Research, 36: 180-187
http://dx.doi.org/10.1111/j.1365-2109.2004.01203.x

Debnath D., Sahu N.P., Pal A.K., Jain K.K., YengkokpamS., and Mukherjee S.C., 2005b, Mineral status of *Pangasius pangasius* (Hamilton) fingerlings in relation to supplemental phytase: Absorption, whole body and bone mineral content, Aquaculture Research, 36: 326-335
http://dx.doi.org/10.1111/j.1365-2109.2004.01204.x

de La Maza L.M., Pezzlo M.T., Shigei J.T., Tan G.L., and Peterson E.M., 2013, Colour Atlas of Medical Bacteriology, 2nd edition, American Society for Microbiology (ASM) Press, Washington, DC, 328.
http://dx.doi.org/10.1128/9781555814755

Garrity G.M., Bell J.A., and Lilburn T., 2005, The revised road map to the manual. In: Brenner, Krieg, Stanley and Garrity (eds.),Bergey's Manual of Systematic Bacteriology. The Proteobacteria, Part A, Introductory Essays. Springer, 2(2): 159-220
http://dx.doi.org/10.1007/0-387-28021-9_21

Ghosh K., Sen S.K., and Ray, A.K., 2002a, Characterization of bacilli isolated from the gut of rohu, *Labeo rohita*, fingerlings and its significance in digestion, Journal of Applied Aquaculture, 12(3): 33–42
http://dx.doi.org/10.1300/J028v12n03_04

Ghosh K., Sen S.K., and Ray A.K., 2002b, Growth and survival of rohu, *Labeo rohita* (Hamilton) spawn fed diets supplemented with fish intestinal microflora, Acta Ichthyologica et Piscatoria, 32(1): 83–92
http://dx.doi.org/10.3750/AIP2002.32.1.07

Han Y.M., Yang F., Zhou A.G., Miller E.R., Ku P.K., Hogberg M.G., and Lei X.G., 1997, Supplemental phytases of microbial and cereal sources improve dietary phytate phosphorus utilization by pigs from weaning through finishing, Journal of Animal Science, 75: 1017–1025
http://dx.doi.org/10.2527/1997.7541017x

Holt J. G., Krieg N. R., Sneath P. H. A., Stanley J. T., and Williams S. T., (eds.), 1994, Bergey'smanual of determinative Bacteriology 9th ed. Williams and Wilkins Baltimore, Maryland, U. S. A.

Khan A., and Ghosh K., 2012, Characterization and identification of gut-associated phytase-producing bacteria in some freshwater fish cultured in ponds, Acta Ichthyologica et Piscatoria, 42(1): 37-45
http://dx.doi.org/10.3750/AIP2011.42.1.05

Khan A., Mandal S., Samanta D., Chatterjee S., and Ghosh K., 2011, Phytase-producing *Rhodococcus* sp. (MTCC 9508) from fish gut: A preliminary study, Proceedings of the Zoological Society, 64(1): 29-34
http://dx.doi.org/10.1007/s12595-011-0004-1

Lei X.G., and Stahl C.H., 2000, Nutritional benefits of phytase and dietary determinants of its efficacy, Journal of Applied Animal Research, 17: 97-112
http://dx.doi.org/10.1080/09712119.2000.9706294

Li M.H., Manning B.B., and Robinson E.H., 2004, Summary of phytase studies for channel catfish, Mississippi Agricultural & Forestry Experimental Station Research Report 23: 1–5

Li X., Chi Z., Liu Z., Yan K., and Li H., 2008, Phytase production by a marine yeast *Kodamea ohmeri* BG3, Applied Biochemistry and Biotechnology, 149 (2): 183–193
http://dx.doi.org/10.1007/s12010-007-8099-6

Oatway L., Vasanthan T., and Helm J.H., 2001, Phytic acid, Food Reviews International, 17(4):419–431
http://dx.doi.org/10.1081/FRI-100108531

Onyango E.M., and Adeola O., 2012, Inositol Hexaphosphate increases mucin loss from the digestive tract of ducks, Journal of Animal Physiology and Animal Nutrition, 96(3): 416-420
http://dx.doi.org/10.1111/j.1439-0396.2011.01157.x

Pen J., Verwoerd T.C., van Paridon P.A., Beudeker R.F., van den Elzen P.J.M., Geerse K., van der Klis J.D., Versteegh H.A.J., van Ooyen A.J.J., and Hoekema A., 1993, Phytase- containing transgenic seeds as a novel feed additive for improved phosphorus utilization, Nature Biotechnology, 11: 811-814
http://dx.doi.org/10.1038/nbt0793-811

Ray A.K., Roy T., Mondal S., and Ringø E., 2010, Identification of gut-associated amylase, cellulase and protease producing bacteria in three species of Indian major carps, Aquaculture Research, 41 (10): 1462–1469.

Ray A.K., Ghosh K., and Ringø E., 2012, Enzyme-producing bacteria isolated from fish gut: a review, Aquaculture Nutrition, 18(5): 465-492
http://dx.doi.org/10.1111/j.1365-2095.2012.00943.x

Roy T., Mondal S., and Ray A.K., 2009, Phytase-producing bacteria in the digestive tracts of some freshwater fish, Aquaculture Research, 40 (3): 344–353
http://dx.doi.org/10.1111/j.1365-2109.2008.02100.x

Samson R.A., and De Boer E., 1995, Introduction to food-borne fungi, 4th rev. ed. Koninklijke Nederlandse Akademie van Wetenschappen, Centralbureau voor Schimmelcultures, Baarn, 322.

Sardar P., Randhawa H.S., Abid M., and Prabhakar S.K., 2007, Effect of dietary microbial phytase supplementation on growth performance, nutrient utilization, body compositions and haemato-biochemical profiles of *Cyprinus carpio* (L.) fingerlings fed soyprotein-based diet, Aquaculture Nutrition, 13 (6): 444–

456
http://dx.doi.org/10.1111/j.1365-2095.2007.00497.x

Yanke L.J., Bae H.D., Selinger L.B., and Cheng K.J., 1998, Phytase activity of anaerobic ruminal bacteria, Microbiology, 144 (6): 1565–1573
http://dx.doi.org/10.1099/00221287-144-6-1565

Yanke L.J., Selinger L.B., and Cheng K.J., 1999, Phytase activity of*Selenomonas ruminantium*: a preliminary characterization, Letters in Applied Microbiology, 29 (1): 20–25
http://dx.doi.org/10.1046/j.1365-2672.1999.00568.x