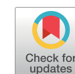
Aquaculture Reports 12 (2018) 30–35

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Marine protease-producing bacterium and its potential use as an abalone probiont



Muhamad Amin

Fisheries Faculty, University of 45 Mataram, West-Nusa Tenggara, Indonesia

ARTICLE INFO

Keywords:
Bacillus amyloliquefaciens MA228
Protease

ABSTRACT

Gastrointestinal tracts (GITs) of aquatic animals were considered as ideal sources for isolating aquaculture probiotic candidates. Thus, this study aimed to isolate protease-producing bacteria from GITs of hybrid abalone (*Haliotis rubra x Haliotis laevigata*), and screened them for their potential uses as abalone probionts. Ten proteolytic bacterial strains were isolated, and all isolates phenotypically referred to genus *Bacillus*. Among the 10 pure strains, MA228 had the highest ability to hydrolize casein and gelatine. Through 16S rRNA sequence (accession number MG976611), the isolate showed 99% similarity to *Bacillus amyloliquefaciens*. Further *in vitro* assays indicated that the bacterial strain had good viability and stability in manufactured pellets stored at 4 °C, seawater with a salinity of 35 mg/L, and simulated gastrointestinal tract conditions of abalone. Additionally, another *in vitro* study indicated that protease produced by the probiotic candidate could significantly hydrolase protein content in manufacture pellet of abalone. These results suggest that *B. amyloliquefaciens* MA228 is a potential probiont for increasing protein digestibility in hybrid abalone.

1. Introduction

Abalone aquaculture is increasingly important because of the progressive decline of wild-catch abalone overtime, yet the world's demand for the highly nutritional seafood is continuously increasing. However, the slow growth rate has delayed the development of abalone aquaculture (Amin et al., 2017; Dunstan et al., 2007). Previous studies suggest that the slow growth related to the ability of abalone to digest and harvest nutrient contents from their feed (Ray et al., 2012). Other studies described that digestion and harvesting rates highly depend on the quality and quantity of digestive enzymes in their gastrointestinal tracts (GITs) (Bedford, 1996; Lemieux et al., 1999; Ray et al., 2012). Since then, many studies have been investigating the work of enzymes in feed digestions including how to increase the enzyme quantity in GITs of cultured species.

There are two common ways to increase enzyme quantity in GITs of aquaculture animals; (1) the supplementation of exogenous enzymes to feed (Ai et al., 2007; Hlophe-Ginindza et al., 2016; Lu et al., 2014), and (2) the application of enzyme-producing bacteria (Amin et al., 2017; Hamza et al., 2016; Reda, Selim, 2015; Sha et al., 2016; Yang et al., 2014). These approaches have been confirmed to not only increase nutrient digestibility, but also decrease the amount of organic waste from undigested feed. Nevertheless, the utilization of exogenous enzymes has been criticized due to expensive and their stability at various processing condition including the heat, pressure and moisture (Pavasovic et al., 2004; Shi et al., 2016). The high temperature and pressure during the feed processing may cause damage to the dietary enzymes (Shi et al., 2016). Meanwhile, the use of protease-producing bacteria has become increasingly accepted for growth enhancement due to: easy in the application, and being considered as an ecofriendly approach. As a consequence, the interest in isolating bacterial strains which has capacity to synthesize protease to be used as probiotic candidates is increasing.

There is a general consensus that probiotic strains should be isolated from GIT of animals where they will be applied (Ambas et al., 2015; Amin et al., 2017). Thus, this study aimed to isolate protease-producing bacteria from GITs of hybrid abalone, and asses their suitability as a probiotic strain for abalone culture. The GIT was selected as a source of bacterial isolation, because GITs are important sites where digestion and absorption of feed nutrients occur (Ray et al., 2012). There were several criteria to select probiotic candidates for abalone in this study: the capacity to produce protease, high viability and stability in feed, rearing water, being tolerance to GITs conditions, non-toxic to the cultured animals and does not carry antibiotic-resistance genes. In addition, the capacity of protease to digest commercial feed of abalone was also investigated by *in vitro* study.

E-mail address: muhamad.amin@utas.edu.au.

https://doi.org/10.1016/j.aqrep.2018.09.004
Received 30 May 2018; Received in revised form 31 July 2018; Accepted 16 September 2018
2352-5134/ © 2018 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license
(http://creativecommons.org/licenses/BY-NC-ND/4.0/).

2. Material and methods

2.1. Isolation of protease-producing bacteria

Isolation of protease-producing bacteria was conducted using an enrichment culture method according to Amin (2016). Briefly, five g of pooled GITs were homogenized in 45 mL sterile seawater (32 mg/L) with a stomacher (Lab-Blender 400, Townson & Mercer Pty. Ltd, N.S.W). Ten mL of the homogenate was then inoculated into 500 mL sterile seawater (32 mg/L) with the following substrate: 2.5% casein (2330, Ajax Chemicals, Australia) and 2.5% gelatin (1080, Ajax Chemicals, USA). After 14-day incubation, 1 mL aliquot was serially diluted and 100 μL of each dilution was spread on agar plates consisting of: 2% (w/v) yeast extract (LP0021, Oxoid UK), 0.3% (w/v) casein, 0.2% (w/v) $MgSO_4$ (A648487, BDH England), 0.1% (w/v) glucose (10117, Merck Australia), 0.5%(w/v), 0.3% (w/v) gelatin, 0.0.1% (w/v) $FeSO_4$ (28400, BDH Australia), 0.02% (w/v) K_2HPO_4, and 1.5% (w/v) bacteriological agar (LP0011, Oxoid UK). The plates were afterward incubated at room temperature aerobically for 2 days. Well-separated colonies with different morphological appearance were purified by repeatedly streaking on tryptone soya agar (TSA; CM0131, Oxoid, UK). Pure colonies were preserved in 15% glycerol stock (24,388.295, VWR Belgium) and stored at −20 °C.

2.2. Quantification of protease activity

Protease activity was measured in terms of hydrolytic capacity (HC) according to Taechapoempol et al. (2011). In brief, the pure bacterial isolates were cultured in tryptone soya broth (TSB; CM0129, Oxoid, UK). After two-day incubation, a solution with OD at 600 nm of 0.2 was set, of which two μl was pipetted out and dropped on the centre of casein-gelatine agar plate in duplicates. After 2d incubation, diameters of bacterial colonies and related-clearance zones were measured. Hydrolytic capacity (HC) was calculated using a formula below. Colony with the highest HC was selected for further characterization.

$$HC = \frac{Diammeter\ of\ Clearence\ zone\ (mm)}{Diammeter\ of\ bacterial\ colony\ (mm)}$$

2.3. Identification of protease-producing bacteria

Bacterial strain with the highest protease activity was identified using both phenotypic and genotypic assays as described by Amin, Bolch, Adams, Burke (2017). Phenotypically, the selected isolate was subjected to Gram-staining, catalase and oxidase assays. Genotypically, the bacterial strain was identified using a colony polymerase chain reaction (PCR) by targeting the bacterial 16S rDNA sequence. A pair of primers used in this study was 27 F (5′-AGA GTT TGA TCC TGC CTC AG-3′) and 907R (5′−CCG TCA ATT CCT TTG AGT TT-3′). Purified PCR products were sent for sequencing and the sequenced isolate was compared to published sequences using the BLAST search algorithm to confirm its identity.

2.4. Phylogenetic analysis

Sequence data was examined for sequence homology with the archived 16S rDNA sequences from GenBank at https://blast.ncbi.nlm.nih.gov/Blast.cgi. Then, multiple alignments of sequences were performed with the ClustalW2 program. A phylogenetic tree was constructed using the neighbor-joining DNA distance using Geneious software version 5.3.6. The resultant tree topology was evaluated by bootstrap analysis of neighbor-joining data sets based on 100 resamplings.

2.5. GenBank submission

The partial sequence of the 16S rRNA gene of bacterial strain was submitted to NCBI GenBank and the assigned accession number was MG976611.

2.6. Screening of potential putative strain

In order to simulate the passage through several environmental conditions and reach GIT sites in which food digestion occurs, MA288 was exposed to four environmental conditions: commercial pellets stored at 4 °C, rearing seawater, simulated stomach juice (SSJ), and simulated intestinal juice (SIJ). In addition, toxicity assay, susceptibility to antibiotics and *in vitro* digestion of abalone commercial pellet using crude protease produced by the selected strain were also performed.

2.6.1. Viability and stability in commercial pellets and rearing water

These assays were performed according to Amin, Bolch, Adams, Burke (2017). In brief, an overnight culture of the selected isolate with OD of 0.15 at 600 nm was firstly sprayed onto duplicate of 50 g sterile commercial abalone pellet, and air dried for 30 min before being stored at 4 °C. In addition, 50 μl of the bacterial aliquot was incubated in duplicates of 10 mL of seawater with salinity of 35 mg/ L for 8 h which at 17 ± 0.6 °C. The viable cells were monitored after 24 h in the feed, and 8 h in rearing water.

2.6.2. Tolerance to gastrointestinal tract conditions

This study was performed according to a modified protocol of Amin, Adams, Bolch, Burke (2016) with some modifications. Briefly, the probiotic candidate was exposed to a simulated stomach juice (SSJ) and simulated intestinal juice (SIJ) for 4 and 8 h respectively. pH of the SSJ and SIJ was adjusted to 5 dan 6.65 which were actual pH of stomach and intestine of hybrid abalone (Harris et al., 1998). Subsequently, 200 μL of fresh bacterial solution (OD_{600}: 0.2) was inoculated into duplicates of either 20 mL SSJ or SIJ. The viable cells were counted by plating onto duplicate TSA plates at 0 h and 4 h (SSJ) or 8 h (SIJ).

2.6.3. In vitro digestion of protein by protease

Digestibility of crude protein content in the abalone feed was measured in terms of optical density (OD) value at 280 nm wavelength according to a protocol of Lan, Pan (1993) with a slight modification. The OD value represented the amount of free amino acids (FAA) degraded from crude protein in the abalone feed. In brief, 5 mL supernatant of the protease-producing bacterium was mixed with 3.5 gr of abalone feed in four replicates. Afterward, 5 mL of 40% trichloroacetic acids (TCA) was added to the mixture to stop protease activity (Tonheim et al., 2007). Then, the mixture was centrifuged at 500 x *g* for 30 min to collect the supernatant. Absorbance was measured with a spectrophotometry at A280 nm wavelength for detecting the FAA and small peptides.

2.7. Toxicity assay

Twenty juvenile hybrid abalone weighing 0.47 ± 0.12 g were divided into two experimental groups in a small-scale, *in vivo* experiment. Each group had two rearing tanks and each tank had five juvenile abalone. The abalone were fed with 1.5% $BW.day^{-1}$ with commercial pellets which were previously impregnated with the protease-producing bacterium ($\sim 1.0 \times 10^9$ CFU g^{-1} feed). The animals were reared in 10 L filtered seawater (35 mg/L) for 14 days, and the rearing water was replenished 100% every three days. Mortality was recorded daily, and dead abalone was removed immediately from the tanks.

2.8. Susceptibility to antibiotics

Susceptibility of the selected bacterial strain to six antibiotics:

Download English Version:

https://daneshyari.com/en/article/11030415

Download Persian Version:

https://daneshyari.com/article/11030415

Daneshyari.com