World Academy of Science, Engineering and Technology
International Journal of Marine and Environmental Sciences
Vol:12, No:11, 2018

Isolation and Characterization of a Narrow-Host Range Aeromonas hydrophila Lytic Bacteriophage

Authors : Sumeet Rai, Anuj Tyagi, B. T. Naveen Kumar, Shubhkaramjeet Kaur, Niraj K. Singh

Abstract : Since their discovery, indiscriminate use of antibiotics in human, veterinary and aquaculture systems has resulted in global emergence/spread of multidrug-resistant bacterial pathogens. Thus, the need for alternative approaches to control bacterial infections has become utmost important. High selectivity/specificity of bacteriophages (phages) permits the targeting of specific bacteria without affecting the desirable flora. In this study, a lytic phage (Ahp1) specific to Aeromonas hydrophila subsp. hydrophila was isolated from finfish aquaculture pond. The host range of Ahp1 range was tested against 10 isolates of A. hydrophila, 7 isolates of A. veronii, 25 Vibrio cholerae isolates, 4 V. parahaemolyticus isolates and one isolate each of V. harveyi and Salmonella enterica collected previously. Except the host A. hydrophila subsp. hydrophila strain, no lytic activity against any other bacterial was detected. During the adsorption rate and one-step growth curve analysis, 69.7% of phage particles were able to get adsorbed on host cell followed by the release of 93 ± 6 phage progenies per host cell after a latent period of ~30 min. Phage nucleic acid was extracted by column purification methods. After determining the nature of phage nucleic acid as dsDNA, phage genome was subjected to next-generation sequencing by generating paired-end (PE, 2 x 300bp) reads on Illumina MiSeq system. De novo assembly of sequencing reads generated circular phage genome of 42,439 bp with G+C content of 58.95%. During open read frame (ORF) prediction and annotation, 22 ORFs (out of 49 total predicted ORFs) were functionally annotated and rest encoded for hypothetical proteins. Proteins involved in major functions such as phage structure formation and packaging, DNA replication and repair, DNA transcription and host cell lysis were encoded by the phage genome. The complete genome sequence of Ahp1 along with gene annotation was submitted to NCBI GenBank (accession number MF683623). Stability of Ahp1 preparations at storage temperatures of 4 °C, 30 °C, and 40 °C was studied over a period of 9 months. At 40 °C storage, phage counts declined by 4 log units within one month; with a total loss of viability after 2 months. At 30 °C temperature, phage preparation was stable for < 5 months. On the other hand, phage counts decreased by only 2 log units over a period of 9 during storage at 4 °C. As some of the phages have also been reported as glycerol sensitive, the stability of Ahp1 preparations in (0%, 15%, 30% and 45%) glycerol stocks were also studied during storage at -80 °C over a period of 9 months. The phage counts decreased only by 2 log units during storage, and no significant difference in phage counts was observed at different concentrations of glycerol. The Ahp1 phage discovered in our study had a very narrow host range and it may be useful for phage typing applications. Moreover, the endolysin and holin genes in Ahp1 genome could be ideal candidates for recombinant cloning and expression of antimicrobial proteins.

Keywords : Aeromonas hydrophila, endolysin, phage, narrow host range
Conference Title : ICFAT 2018 : International Conference on Fisheries and Aquaculture Technology
Conference Location : Tokyo, Japan
Conference Dates : November 12-13, 2018