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LACTOCOCCUS LACTIS STRAINS FROM INTESTINAL ORGAN OF BLACK TIPS SHARK CARCHARHINUS LIMBATUS PRODUCING NISIN- LIKE BACTERIOCIN ACTIVE AGAINST SHRIMP AND FISH PATHOGENS (VIBRIO PARAHAEMOLYTICUS AND VIBRIO ALGINOLYTICUS)

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Shark can be a potential source for host-associated probiotics destined to be used in aquaculture. In this study, intestinal tissues of Black tip shark (*Carcharhinus limbatus*) was used as the source sample for inhibition screening based on spot-on-lawn and agar well diffusion methods. Out of the 80 isolates, only four showed antagonistic activities against selected indicator strains. All of the 4 isolates FA1, FA2, FA3 and FA4 were found to be Gram positive coccus, non-spore former, oxidase and catalase negatives, as well as lactose fermenters. The isolate FA1, FA2, FA3 and FA4 were found to demonstrate broad range of inhibitory spectrum on gram-positive indicator bacterium (*Staphylococcus aureus* and *Bacillus cereus*) and the gram-negative bacterium (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus*); with the highest inhibition zone at 20.0 ± 0.1 mm, recorded on *V. parahaemolyticus*. The genotype of the isolates was characterized using 16S rRNA sequencing. Each sequence with a given GenBank (NCBI) accession number (MN975529 for FA1; MN982712 for FA2; MN982711 for FA3; and MN982710 for FA4) showed at least 99% similarity with *Lactococcus lactis* subspecies. The crude cell free supernatant (CFS) of the isolates potentially contained a putative bacteriocin displaying nisin-like properties. The pH and catalase treatments showed that neither organic acid nor hydrogen peroxide (H_2O_2) was the inhibitory component. An almost 95% inactivation of the CFS's antimicrobial activity was observed following treatment with trypsin suggested a presence of proteinaceous agent. Growth and inhibition studies on all isolated strains demonstrated that the inhibition properties were growth associated, with maximum inhibition achieved at $\sim 20.0 \pm 0.1$ mm (or $\sim 168 \pm 17$ AU/ml) at 17 hours by FA2 strain against *V. parahaemolyticus*. A study on the mode of inhibition demonstrated a bactericidal killing against *V. parahaemolyticus*. The isolation of *Lactococcus lactis* strain from shark is rather unique since it was commonly reported to be isolated from human and animals. These strains show bacteriocinogenic properties, broad spectrum inhibition, and more importantly, they are able to antagonise some pathogens implicated in fish or shrimp diseases. Therefore, these strains have potential for use as probiotics in aquaculture. © 2020. All Rights Reserved.

Author keywords

lactic acid bacteria; *Lactococcus lactis*; nisin; probiotic

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