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Investigating quorum-quenching marine bacilli as potential biocontrol agents for protection of shrimps against Early Mortality Syndrome (EMS)

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Early Mortality Syndrome (EMS) has been a major problem for shrimp aquaculture in Southeast Asia due to its epizootic prevalence within the region since the first reported case in 2009. This study explores the application of halophilic marine bacilli isolated from coral mucus and their quorum-quenching abilities as potential biocontrol agents in aquaculture systems to combat the causative agent of EMS, *Vibrio parahaemolyticus*. *N*-acylhomoserine lactone (AHL)-degrading (AiiA) activity was first screened by PCR then confirmed by bio-reporter assay, and a combination of 16S rDNA sequence analysis and quantitative phenotype assays including biofilm-formation and temperature-growth responses were used to demonstrate diversity amongst these quorum-quenching isolates. Three phenotypically distinct strains showing notable potential were chosen to undergo co-cultivation as a method for strain improvement via long term exposure to the pathogenic *V. parahaemolyticus*. The novel approach taken led to significant improvements in antagonism and quorum quenching activities as compared to the ancestral wild-type strains and offers a potential solution as well as pathway to improve existing beneficial microbes for one of the most pressing issues in shrimp aquacultures worldwide.

The production of seafood has undergone a dramatic transition since the 1980s to include fish farming or aquaculture. One of the most lucrative and widely traded aquaculture products are shrimps, with the commercial sale of shrimp a substantial source of revenue for many developing countries and Asia producing almost 90% of all aquaculture products worldwide^{1–3}. China is the world's largest aquaculture producer and alone produces 50 percent of penaeid shrimps globally⁴, with tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Penaeus vannamei*) among the common cultured species^{5,6}. Persistent diseases and related issues have, however, reduced the production of farmed shrimp in China and Vietnam¹ as the nature of intensive shrimp farming makes shrimps highly susceptible to diseases such as Early Mortality Syndrome (EMS; or Acute Hepatopancreatic Necrotic Syndrome, AHPNS) which occurs within the first 35 days after stocking cultivation ponds⁷. EMS is caused by *Vibrio parahaemolyticus* which can also produce gastroenteritis in people through poorly prepared seafood and contaminated water^{8,9}. *V. parahaemolyticus* utilises quorum sensing (QS) to regulate the expression of virulence factors including the production of toxins and biofilm-formation.

Bacterial communication systems depend on autoinducers (AIs) as signalling molecules. Three major classes of AIs are known: *N*-acyl Homoserine Lactones (AHLs) or Autoinducer-1, Autoinducing Peptides (AIPs) and Autoinducer-2 (AI-2)¹⁰. QS systems are widespread amongst *Vibrio* spp.; addition of the signalling molecule C6-HSL to bacterial media, for example, led to an increase in biofilm formation by 29%¹¹. They act through complex signal transduction pathways involving the synthesis and detection of *N*-acylhomoserine lactone (AHL) signal molecules known as autoinducers which lead to the expression of virulence factors only when high cell densities are achieved¹².

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