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Streptophage-mediated control of off-flavour taint producing streptomycetes isolated from barramundi ponds

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ABSTRACT

Off-flavour taint of aquaculture products is a global issue reducing consumer confidence in the farmed produce as they are taken up via the gills of fish, and deposited in the lipids of the animal. If the fish are not purged, resulting undesirable muddy earthy flavour taint can be tasted by consumers. These undesirable flavour and odour is caused by the terpenoid compounds namely geosmin and 2-methylisoborneol, produced as secondary metabolites by certain bacteria including the cyanobacteria and actinomycetes. Current strategies to remediate the problem rely on treating the symptoms not the cause and involve the use of time consuming purging methods and costly chemicals.

Biological control using bacteriophages, specific to the problem causing bacteria, offers a natural alternative to chemical control, which might reduce further complications of copper based algaecides and its subsequent implications on water quality. In an adaptation of such biological control approach streptomycetes isolated from barramundi ponds were tested for their susceptibility to streptophages to understand whether host destruction via phage lysis would subsequently eliminate off-flavour taint productions by these isolates.

Following the determination of the streptophage susceptibility of the isolates one of the most odourous streptomycete species (USC-14510) was selected to be tested further using different pond simulations resembling real-life applications. Geosmin was tested as the indicator of off-flavour taint production and as it has been previously reported that the cyanobacteria-actinomycete interactions occurring in ponds result in even greater levels of geosmin and 2-methylisoborneol, the geosmin levels for the isolate in the presence of cyanobacteria and streptophages were also tested. Findings indicated that the highly odourous *Streptomyces* species (USC-14510) once infected with streptophages, can lose its capacity to produce off-flavour taints. Pond simulation studies also revealed geosmin production was significantly reduced when streptophages were introduced into the pond water where streptomycete species were grown. The bacteriophage control method developed in the presented study might again confirm significant potential for the bacteriophage-mediated remediation strategy to be adapted by the aquaculture industry.

1. Introduction

Barramundi (*Lates calcarifer*) is an indigenous bony fish species of northern Australia and Asia, currently valued at $25 million with 28% of the total aquaculture produce in Australia [1]. Due to the demand through both the domestic and international markets, wild-captured barramundi supplies are not able to satisfy current market requirements. This demand has pushed for an increase in the production of the fish via terrestrial aquaculture ponds and barramundi aquaculture at present is valued at around $20 million for Queensland alone, with envisaged further increase [2].

However, one of the main issues faced by aquaculturists is the off-flavour taints that affects many freshwater aquaculture

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Table 1
Testing for geosmin and 2-methylisoborneol production by the isolate USC-14510 when treated with and without the phage suspension on agar slants.

Sample type	Phage Application and Fibre exposure
Fibre Blank	Run of blank fibre
Control	Fibre exposed to agar only for 10 min
Streptomycete isolate USC-14510 and daily streptophage inoculation	Daily addition of 100 μL of phage suspension to 100 μL of spread and dried streptomycete suspension, Fibre exposed for 10 min
Streptomycete isolate USC-14510 and single streptophage inoculation	Once only application at Day 0 of 100 μL of phage suspension to 100 μL of spread and dried streptomycete suspension, Fibre exposed for 10 min
Streptomycete isolate USC-14510 only	100 μL streptomycete suspension spread and dried only. Fibre exposed for 10 min

products worldwide. These off flavours are the bio-accumulation of primarily two compounds that are geosmin and 2-methylisoborneol. Geosmin (GSM) and 2-methylisoborneol (2-MIB) are semi-volatile, terpenoid compounds which are secondary metabolites produced by wide range of microorganisms [3] but mainly by cyanobacteria [4] and actinomycetes [5]. The off-flavour taint is a problem for not only barramundi farmers, but for many other freshwater aquaculture species internationally such as catfish and trout, as well as for drinking water supplies [6]. The fish can be unmarketable if the off-flavour taints the flesh, and purging is the only method of removing the off-flavour [7]. This has severe economic implications for the industry due to costs [8].

Queensland has one sea cage operation [2] and six to eight pond based barramundi aquaculture systems, which are freshwater, estuarine and salt systems [9]. Most ponds are unlined, earth ponds, and are located north of the Mackay area (21.1411°S, 149.1861°E), above the tropic of Capricorn, an area with average temperatures of 13–23 °C in July and 23–30 °C in December and January [10]. With such high temperatures and excessive nutrients from both fish waste and fish meal feedstock, and direct soil contact, barramundi ponds are conducive to actinomycete growth and cyanobacterial blooms.

When aquaculture ponds are constructed in Australia as bare earth ponds, with no lining, metabolically active component of the pond microflora produce the GSM and 2-MIB that are released into the pond water. As both GSM and 2-MIB are lipophilic, they are taken up via the gills of fish, and deposited in the lipids of the animal [11,12]. If the fish are not purged, consumers can taste these compounds, recognised as an undesirable muddy earthy flavour taint [13]. Purging adds a delay of 3–5 days before the fish are marketable, plus the need to have the area for a depuration pond and availability of clean water, free of the impurities and their producers [7].

Current strategies to remediate the problem rely on treating the symptoms not the cause as well as the use of time consuming purging methods and costly chemicals. Other methods of prevention of tainting the fish include applications of copper sulphate as an algaecide, the use of recirculation tanks where the pond water in continually filtered and the mechanical methods used for the removal of cyanobacteria after a bloom occurs [14]. Yet such human intervention still is not effective in preventing the release of the metabolites to the pond impacting product quality. Preventing the formation of the taint is therefore the ideal solution, however, the dynamic environment of the aquaculture ponds is conducive to high algal and bacterial growth, and the use of products harmful or able to be accumulated by the fish, to control the algae or bacteria is limited. One environmentally-friendly approach might be the use of bacteriophages specifically targeting the odour causing microorganisms since bacteriophages have been proven effective in different environmental setting to control targeted bacteria [15–17]. Currently, the use of bacteriophages in aquaculture industry is gaining importance such as their application in the fishery industry for infection control in fish and shellfish [18–20]. Actinophages are viruses that infect actinomycetes and they were reported to comprise six morphological types and belong to three different viral families: *Myoviridae, Siphoviridae and Podoviridaes*. Streptophages are the ones specific for the members of the family *Streptomycetaceae* and mostly belong to the *Siphoviridae* group with a long and non-contractile tail [21] and they usually show a high degree of polyvalency within family [22,23].

In the light of above presented information the following research study was designed to investigate the potential use of streptophages as biological control agents to eliminate odourous streptomycetes in laboratory and simulated settings with a long-term objective to reduce or eliminate off-flavour taints produced by these bacteria in barramundi ponds.

2. Methods and materials

2.1. Isolation and characterization of the streptomycete isolates

Streptomycetes were isolated from barramundi ponds using mud samples collected from barramundi ponds located in northern Queensland, Australia using the methods described by Küster and Williams [24] and stored at the University of the Sunshine Coast, Microbial Library at −25 °C in frozen glycerol suspension [25].

Isolates were identified using molecular sequencing method [26]. The QIAGEN HotStarTaq® Multiplex PCR Kit and the primers B27F and 1492R were used to obtain PCR products, which were subsequently were sequenced at Macrogen Inc. (South Korea, http://www.macrogen.com/). 16S rRNA sequences were subsequently deposited in the GenBank (http://www.ncbi.nlm.nih.gov/genbank/). 16S rRNA gene sequences were aligned in the same orientation with the online implementation of the SINA aligner (http://www.arb-silva.de/aligner) [27]. The sequences were then de-gapped and subsequently de-replicated by clustering at 99.9%

Table 2
Experimental design details of the pond simulation tests.

1	2	3	4	5	6
Sand	Sand	Sand	Sand		
Pond water	Pond water	Pond water	Pond water	Pond water	Pond water
Streptomycete isolate	Streptomycete isolate	Streptomycete isolate in sand	Streptomycete isolate in sand	Streptomycete isolate	Streptomycete isolate
Phage		Phage in sand		Phage	
		Rubber lining	Rubber lining	Rubber lining	Rubber lining