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## Investigating antagonistic activity of fish gut bacterial isolates against aquaculture pathogens: Short Communication

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**Abstract** Aquaculture worldwide suffers significant economic losses and high fish mortality due to bacterial diseases. Antibiotics have been used extensively to combat these infections owing to the overuse of antibiotics, Drug-resistant bacteria can emerge. Studies on probiotics, which provide improved growth and disease resistance, are becoming more and more popular as an antibiotic substitute, Bacteria derived from a marine setting such as fish gut might be putative candidate for aquaculture probiont. In vitro antagonistic activity against fish pathogens has been used to assess the antimicrobial properties of bacteria isolated from the gut of *Sardinella longiceps* and *Rastrelliger Kanagurta*.

**Keywords:** Aquaculture, Antibiotic, Fish health, Probiotics

### Introduction

Bacterial infections stand as a primary contributor to fish mortality rates and the substantial economic losses witnessed in global aquaculture (Swain *et al.*, 2007). Among the prominent pathogenic microorganisms responsible for these losses are *Aeromonas* spp. and *Vibrio* spp. In efforts to mitigate the impact of such bacterial pathogens, antibiotics have been extensively employed. However, the indiscriminate use of antibiotics poses risks, including the emergence of drug-resistant bacteria in fish and their surrounding environments (Thornber *et al.*, 2022). Consequently, there is a growing focus on seeking alternative strategies for pathogen control related to these issues. Probiotic research emerges as a promising avenue in this regard, offering viable alternatives to antibiotics with significant potential

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benefits. Probiotics; particularly bacterial probiotics, are gaining traction as a means of controlling pathogens, offering enhanced disease resistance and growth advantage (Hai *et al.*, 2015). The emphasis in probiotic research lies in leveraging microbial antagonism against pathogenic bacteria, given its potential efficacy in aquatic environments. This approach capitalizes on the production of antimicrobial compounds distinct from traditional antibiotics to inhibit pathogen proliferation.

## **Materials and methods**

### ***Collection of samples***

Fresh fishes namely *Rastrelliger Kanagurta* (Indian Mackerel) and *Sardinella longiceps* (Oil Sardines) were collected at Vizhinjam a major fishing region in Thiruvananthapuram, Kerala, India. Fishes were surface sterilised using 70% Ethanol. The intestines were aseptically excised (Vignesh *et al.*, 2019) and surface sterilised with 70% ethanol and stored in a transport media at 4°C, the intestines were homogenized using mortar and pestle. 1 ml from the homogenized intestines was serially diluted (1:10) in distilled water. 0.1ml of the aliquot from  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  were spread plated onto nutrient agar (optimised with 50% Sea water) 6.8pH in triplicates and incubated at 37°C for 24-48 hours. The distinctively morphological colonies were selected and streaked separately on nutrient agar plates to get pure cultures and retained in a refrigerator (4°C) for further study.

### ***Antagonistic activity***

The antagonistic activity of the isolates was screened using the agar well diffusion method (Bhatnagar and Lamba, 2015). Muller Hinton Agar (MHA) plates were prepared and inoculated with pathogen cultures such as *Vibrio alginolyticus*, *Vibrio harveyii*, *Vibrio vulnificus* and, *A. Salmonicida* (0.5 MacFarland). The supernatant obtained from 24 hour cultures of the bacteria was grown at 37°C in 100 ml nutrient broth and obtained via centrifugation at 10,000 rpm for 10 minutes (Giri *et al.*, 2011). 100µl of the culture supernatant was added to wells created in the inoculated MHA plates. Control wells containing Nutrient broth were negative controls, while wells containing Nalidixic acid antibiotics ( $2\mu\text{g } \mu\text{l}^{-1}$ ) served as positive controls. The diameter of the clear zone of inhibition around each well was measured following a regulated incubation period of 24 hours at 37°C.

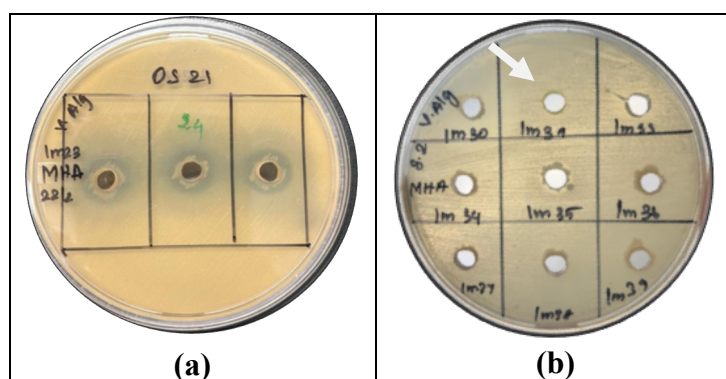
## **Results**

### ***Isolation of the bacteria from fish gut***

After incubation, the examination of microbial populations a total of 144 strains were isolated, 75 from *Sardinella longiceps* (OS) and 68 from *Rastrelliger Kanagurta* (IM) based on the morphological differences. Among these isolates, 6 strains displayed inhibitory activity against selected fish pathogen. The antagonistic activities of the isolated bacteria from the fishes were performed by agar well diffusion method (Bhatnagar and Lamba, 2015).

**Table 1.** Antagonistic activity of the fish gut isolates

Bacterial Isolate	Zone of Inhibition (mm) against fish pathogens			
	<i>V. harveyi</i>	<i>V. vulnificus</i>	<i>V. alginolyticus</i>	<i>A. salmonicida</i>
IM30	15	13	28	12
IM23	0	0	16	12
OS18	13	0	0	0
OS21	13	0	14	0
OS16	9	0	0	0
OS40	12	0	10	8



**Figure 1.** Inhibition zones of the isolates against fish pathogens (a) Activity of OS21 and (b) Activity of IM30

## Discussion

Marine ecosystem has always been a niche for bio-active products. In this study the intestinal microbiota of economically important fishes was examined for potential probiotic strains for aquaculture. The extra-cellular components of the isolated bacteria had shown to be varying degrees of antagonistic activity on various fish pathogenic strains, the maximum zone of inhibition was found against *V. alginolyticus* (28mm) by IM30 and the minimum zone of inhibition was found against *A. salmonicida* (9mm) by OS40. The bacterial isolate IM30 exhibited clear zones of inhibition against all fish pathogens. Probiotic bacteria have been demonstrated to be an antibacterial effect, earlier studies have supported the probiotic bacterium's capacity to stop the growth of both gram negative and gram-positive

pathogenic bacteria (Midhun *et al.*, 2017) from *Anabas testudineus* (Bloch, 1792) against Vibrios (Austin *et al.*, 1995), *Streptomyces* is isolated from fish gut that also possess antimicrobial activity and anti-quorum sensing activity (Vignesh *et al.*, 2019). This might be attributed to the diet, several environment factors as well as synthesis of bacterions, siderophores and lysosomal enzymes, either individually or in combination. The isolated bacteria are a potential candidate for probiotic development which will be further studied under several parameters and assays followed by partial sequencing.

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