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Isolation and Antibacterial Activity of a Symbiotic Bacterial Isolate Associated with Loligo Forbesii

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Abstract

The functional source of bioactive metabolites is represented by Symbiotic microorganisms that are associated with marine invertebrates. This research aims to isolate a symbiotic bacterium from Loligo forbesii in order to evaluate the antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. The sample Tissues were concentrated in nutrient broth, streaked for obtaining pure culture and was tested by the agar well diffusion assay. These symbiotic isolates demonstrated different zones of inhibition as 7 mm and 9 mm, respectively. These findings suggest the capability of squid-associated bacteria to release antimicrobial compounds which are of biomedical significance.

1. Introduction

The increase of microbial eco-genomics has reshaped modern biology, emerging the fundamental role of microorganisms in evolution and biological complexity [1,2]. Microbes significantly influence adaptation and diversification without relying solely on traditional vertical inheritance [3].

The interactions between host and microbe are now considered as most significant in development, immunity, and physiology. Microbial cells in humans are outnumbered by host cells which makes the interaction between the host and microbiome critical to health and can cause diseases [4].

Model organisms are required to understand the association between host and microbe. The significant information system includes the Hawaiian bobtail squid *Euprymna scolopes* and the luminous bacterium *Vibrio fischeri*, which is a key model for the study of microbial colonization, specificity of the association, and response of host development [5–7].

Cephalopods, such as *Loligo forbesii* holds a variety of microbial symbionts. The antimicrobial, antiviral and anticancer compounds are derived due to the Marine microbe's synthesis [8–10], yet the full scope of bacteria associated with squid remains unexamined.

Objectives

1. To isolate symbiotic bacteria associated with *Loligo forbesii*.
2. To preserve and analyse pure isolate cultures.
3. To assess antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa.

2. Materials and Methods

- **Sample Collection**

Live specimens of *Loligo forbesii* were collected from a fish market in Thrissur, Kerala, India, around 07:00 am. Samples were transferred to sterile containers and transported under cool temperature for immediate laboratory processing.

- **Isolation of Symbiotic Bacteria**

Specimen tissues were thoroughly rinsed with sterile water and then fragmented. The tissue sample was enriched with nearly 100 mL of nutrient broth and incubated at 37°C for up to 72 hours on a rotary shaker. Once bacterial growth was visible, the aliquots were transferred to a nutrient agar plates with streaking method and was incubated at 37°C for 3 – 7 days. Distinct colonies were categorized by morphology and sub-cultured to get pure isolates.

- **Maintenance of Pure Cultures**

Pure isolates were stored on nutrient agar slants at 37°C and sub-cultured regularly under aseptic conditions.

- **Antibacterial Assay**

Agar well diffusion method was used to evaluate the antibacterial activity of the symbiont. Separate Mueller–Hinton agar plates were inoculated with Pathogenic test strains (*S. aureus* and *P. aeruginosa)*. Wells of 0.85 cm diameter were made using a sterile cork borer. Cell-free supernatant of 10 µL was obtained by centrifugation of broth cultures at 8000 rpm for 5 minutes which was dispensed in to the wells along with positive and negative controls. Incubation of plates were at 37°C for 24 hours and later zones of inhibition were measured.

3. Results

- **Identification of Host Organism**

The identification of freshly collected specimens as *Loligo forbesii* were based on it's morphological characteristics.

Figure 1. Host organism (*Loligo forbesii*)

- **Isolation of Symbiotic Bacteria**

Squid tissues were inoculated and enriched with nutrient broth.

Figure 2. Before incubation: Squid tissue inoculated in nutrient broth

Figure 3. After incubation: Enriched bacterial culture showing turbidity
(Turbidity indicated strong visible bacterial growth after incubation.)

Figure 4. Isolated bacterial symbiont
(Bacterial colonies from streak plates were isolated and purified for subsequent assays.)

- **Antibacterial Activity**

The cell-free supernatant from the symbiotic isolate inhibited both the test pathogens. Agar well diffusion plates exhibited various zones of inhibition.

Test pathogen	**Inhibition Zone**
Staphylococcus aureus	7 mm
Pseudomonas aeruginosa	9 mm

Figure 5. Antibacterial activity of symbiotic bacterial isolate against *Staphylococcus aureus*

Figure 6. Antibacterial activity of symbiotic bacterial isolate against *Pseudomonas aeruginosa*

4. Discussion

Marine symbiotic bacteria are significant sources of bioactive compounds, such as antimicrobials with high ecological roles and biomedical importance [5–7,11].

The isolate effectively inhibited both Gram-positive and Gram-negative pathogens by potentially reflecting in natural defence mechanism and competitive functions of the squid microbiome. Symbiont induced responses of the host including the altered development are documented in cephalopod bacteria systems [10–12].

Future investigations should include:

- Species identification using 16S rRNA gene sequencing
- Analysis of chemical composition of metabolites
- Analysis of cytotoxicity
- genomic exploration and utilization for biosynthetic pathways.

5. Conclusion

This study was able to successfully isolate a symbiotic bacterial strain from *Loligo forbesii* and to demonstrate its antibacterial potential against *S. aureus* and *P. aeruginosa*. The growing evidence suggests that bacteria associated with marine host are key sources of natural antimicrobial compounds. Further biochemical and molecular research may find new metabolites with pharmaceutical compounds.

References

1. Pace NR, Sapp J, Goldenfeld N. Phylogeny and beyond: scientific, historical, and conceptual significance of the first tree of life. Proc Natl Acad Sci U S A. 2012;109:1011–8.
2. Koonin EV. The Logic of Chance: The Nature and Origin of Biological Evolution. Upper Saddle River (NJ): FT Press; 2011.
3. Gevers D, Knight R, Petrosino JF, Huang K, McGuire AL, et al. The human microbiome project. PLoS Biol. 2012;10:e1001377.
4. McFall-Ngai MJ, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, et al. Animals in a bacterial world. Proc Natl Acad Sci U S A. 2013;110:3229–36.
5. Stabb EV, Visick KL. Vibrio fischeri: a bioluminescent symbiont of Euprymna scolopes. In: Rosenberg E, et al., editors. The Prokaryotes. 4th ed. Springer; 2014.
6. Ruby EG. Symbiotic conversations under genetic interrogation. Nat Rev Microbiol. 2008;6:752–62.
7. Nyholm SV, Stabb EV, Ruby EG, McFall-Ngai MJ. Establishment of an animal–bacterial association. Proc Natl Acad Sci U S A. 2000;97:10231–5.

8. Douglas AE. The Symbiotic Habitat. Princeton: Princeton University Press; 2010.
9. Foster JS, McFall-Ngai MJ. Bacteria-induced apoptosis in host epithelium. Dev Genes Evol. 1998;208:295–303.
10. Montgomery MK, McFall-Ngai MJ. Embryonic development of the squid light organ. Biol Bull. 1993;184:296–308.
11. Montgomery MK, McFall-Ngai MJ. Symbiont-induced organ morphogenesis. Development. 1994;120:1719–29.
12. Lee PN, McFall-Ngai MJ, Callaerts P, de Couet HG. Euprymna scolopes as a model system. Cold Spring Harb Protoc. 2009;4:1394–408.
13. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, et al. Gut microbiome richness and metabolic markers. Nature. 2013;500:541–6.
14. Simpson JH. Mapping neural circuits in Drosophila. Adv Genet. 2009;65:79–143.
15. Heath-Heckman EAC, et al. Symbionts entrain host circadian rhythms. mBio. 2013;4:e00167–13.
16. Ruby EG, Urbanowski M, Campbell J, et al. Complete genome sequence of Vibrio fischeri. Proc Natl Acad Sci U S A. 2005;102:3004–9.