Identification and Characterization of Marine Bacteria Associated with *Lytechinus variegatus*

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Abstract

This study aims to identify and characterize bacterial flora that coexists on the surface and test of the sea urchin, Lytechinus variegatus. Several bacterial strains were isolated from wild type of sea urchins by gently swabbing their external surfaces and plating them onto marine agar plates. 14 were chosen for identification and characterization. Gram staining of these isolated bacteria revealed all were gram negative rods. They are oxidase positive and do not ferment lactose. One of the isolated bacteria was agarolytic in nature. Chromosomal DNA of all the 14 bacteria were isolated, and the conserved 500 bp of the 16S rRNA gene was amplified by PCR. Samples will be sequenced and subjected to phylogenetic analysis using QUIIME software. Bacteria will also be tested for antimicrobial properties, antibiotic resistance and the presence of bioactive compounds.

Introduction

Marine invertebrates, particularly sessile ones, are rich sources of unusual metabolites. Many of these metabolites have great potential for development as drugs and research tools. These metabolites are not made by the invertebrates. They are made by the symbiotic bacteria that coexist on the surface of marine invertebrates. Identifying these bacteria will contribute to marine diversity, symbiotic relation between host and bacteria and development of metabolites useful in biotechnology. The Sea Urchin, Lytechinus variegatus, is a complex eukaryotic organism with highly limited mobility and photosensitivity. It is typically green in color, round and covered in spines. As Deuterostomes, sea urchins are close relatives to many higher organisms, such as humans and other mammals. Due to its lack of mobility, it is more likely that the Sea Urchins are in a symbiotic relationship with bacteria that aid in its survival. Focus of the current project is to characterize and identify bacteria living on the surface of Lytechinus variegatus by genetic and biochemical tests.

Methods

1. Sea urchins were purchased from Carolina Biological and were kept under controlled and carefully monitored conditions.
2. Using sterile swabs, the test, mouth and gonopore of urchins and the water in which they arrived were swabbed and streaked onto marine agar plates.
3. Bacteria that grew were re-streaked to isolate for single colonies. Differentiation was based on color, size, and presence of pigments.
4. The isolated colonies were gram-stained and tested for the presence of plasmid DNA.

4A. The colonies will be tested for the presence of antimicrobial and /or bioactive metabolites

4B. Isolated bacteria were analyzed by biochemical tests

4C. The bacterial tests will be analyzed by 16S rDNA sequencing

Figure 1:
Sea Urchin Lytechinus variegatus in the lab

Results

1. BACTERIA SELECTED FOR FURTHER IDENTIFICATION

Out of 76 isolates, 14 bacteria were chosen for further analysis. The nomenclature was based on the urchin number and the surface from where they were isolated. Bacterial isolates by the assigned nomenclature: U2Ta, U9Ga, U7M, U1Ta, U8T, U6G, U5Mc, U6T, U1Mc, U9Ma, U5Mb, Lu80, U10Wagl, U13G.

U2Ta	U9Ga	U7M	U1Ta	U8T	U6G	U5Mc
U6T	U1Mc	U9Ma	U5Mb	LU80	U10Wagl	U13G

2. GRAM STAINING

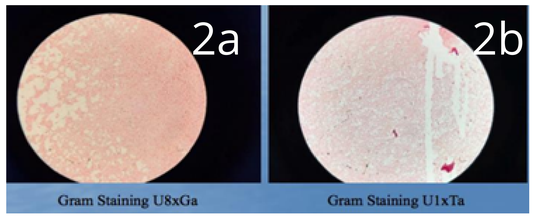


Figure 2a. U8xGa (obtained from the gonopore/anus urchin#8)
Figure 2b. U1xTa (obtained from the test of urchin #1)

3. BIOCHEMICAL TEST

Bacteria isolates were analyzed by biochemical tests as outlined in Bergey's manual.

a. OXIDASE TEST:

Bacteria that produce the enzyme cytochrome C oxidase will react with the redox reagent to give a purple color within 10-15 seconds.

b. LACTOSE FERMENTATION

Bacteria that digest lactose and produce acids will lower the pH, thereby changing the color of the indicator phenol from red to yellow. All bacteria were non-lactose fermenting

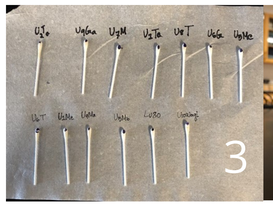


Figure 3: Oxidase test (marine bacterial isolate)

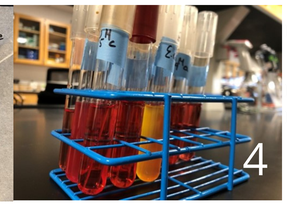
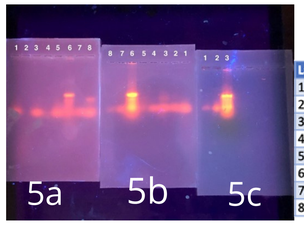


Figure 4:
Lactose Fermentation Test

4. 16S rDNA AMPLIFICATION AND SEQUENCING

Chromosomal DNA was isolated from all the 14 bacteria, and the CONSERVED 500 BPS of 16s rDNA region was amplified using Thermo Fisher 16s rDNA microSEQ kit. The proximate length of the DNA fragment was determined by running a gel alongside a DNA ladder. PCR fragments were sequenced using reverse and forward primers and sent to the DNA sequencing facility at the University of Illinois at Urbana-Champaign

Figure 5: Gel Electrophoresis (Marine Bacterial Isolate)



5a

Lane	Sample
1	U6G
2	U5Mc
3	U6T
4	U1Mc
5	U9Ma
6	DNA Ladder
7	U5Mb
8	Lu80

5b

Lane	Sample
1	(+) Control
2	(-) Control
3	U2Ta
4	U9Ga
5	U7M
6	DNA Ladder
7	U1Ta
8	U8T

5c

Lane	Sample
1	U10Wagl
2	U13G
3	DNA Ladder

5. ENZYMATIC ASSAY of α AMYLASE

Bacterial samples may secrete amylase into the environment. The amalyse degrade the starch into smaller molecules. This enzyme assay will test for the presence of enzyme activity.

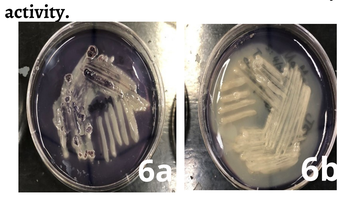


Figure 6: α-Amylase assay of bacterial isolates
·Figure 6a U2Ta (only isolate to have shown lack of α-amylase breakdown)
·Figure 6b U9Ma (isolate to have shown a presence of α-amylase breakdown)

6. ENZYMATIC ASSAY OF CELLULASE

Cellulase activity is determined by its effect on microcrystalline cellulose with respect to glucose formation. Carboxymethylcellulose (CMC) was used to detect cellulolytic enzymes by staining the bacterial streaks of agar-based CMC plates with Gram's iodine. The staining intensity negatively correlates with enzyme activity.

Figure 7: Enzymatic Cellulase Test

7. ANTIBIOTIC RESISTANCE

The bacterial samples collected display diversity among their resistances and sensitivities to common antibiotics.

Bacteria / Antibiotic	Lu80	U9Ma	U1Tc	U5Mc	U6G	U2Ta	U10Wagl	U7M	U13G	U9Ga	U6T	U8T	U1Mc	U5Mb
Bacitracin	+	-	+	+	-	-	-	-	+	+	+	-	+	-
Ciprofloxacin	-	+	-	-	+	-	+	+	+	+	-	-	+	+
Oxytetracycylin e	+	-	+	+	-	-	-	-	-	+	+	-	+	-
Erythromycin	-	+	-	-	+	-	-	+	-	-	-	+	-	+
Trimethoprim	+	-	-	+	-	+	+	+	-	-	+	-	-	-
Vancoprin	+	+	-	-	-	+	+	+	-	-	-	-	-	+
Kanamycin	-	+	-	-	-	-	+	+	-	-	+	+	+	+
Streptomycin	-	+	-	-	-	-	+	+	-	-	+	+	+	+

Conclusion and Discussion

1. All bacterial isolates tested were gram negative rods
2. All of them tested positive for oxidase test
3. They are lactose non fermenters
4. They show resistance to one or more common antibiotics.
5. They also harbor cryptic plasmids.

Based on these results it appears that these bacteria could belong to the genus pseudoalteromonas.

Future Work

1.All the 14 bacterial isolates will be phylogenetically identified by 16SrDNA sequencing.
2.They will be tested for the presence of any anti-microbial and bioactive metabolites or products.

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