

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

Sanjida Akter Shanta, Joseph Taylor, Kaunain Fatima Syeda. Mentor: Lalitha Jayant

Science Department, Borough of Manhattan Community College, The City University of New York
199 Chambers Street, New York, NY

ABSTRACT

This study aims to identify and characterize bacterial flora that coexists on the surface and test off he sea urchin, *Lytechinus variegatus*. Identifying unknown marine bacteria helps in studying the diversity of marine ecosystem and discovering new chemicals that may be helpful in medicine or biotechnology. Several bacterial strains were isolated from sea urchin *Lytechinus variegatus* by gently swabbing their external surfaces and plating them onto marine agar plates. Individual colonies were streaked repeatedly to ensure that all bacteria used in our studies were derived from single colonies. All of them were stored in glycerol cultures. Among the stored bacteria, 14 were chosen for identification and characterization. Gram staining of these chosen bacteria revealed all were gram negative rods. Additionally, bacteria were tested for antibiotic resistance and plasmid presence. A few of them were resistant to common antibiotics and some harbored plasmids. Results also indicated that many bacteria are amylase positive, however, only one of them is mildly cellulase positive. Chromosomal DNA of all the 14 bacteria will be isolated, and the conserved 500 bp of the 16S rRNA gene will be amplified by PCR. Samples will be sequenced using Thermal Fisher MicroSEQ kit and subjected to phylogenetic analysis using NCBI website and MicroSEQ software. They will also be tested for the presence of bioactive compounds and other enzymes.

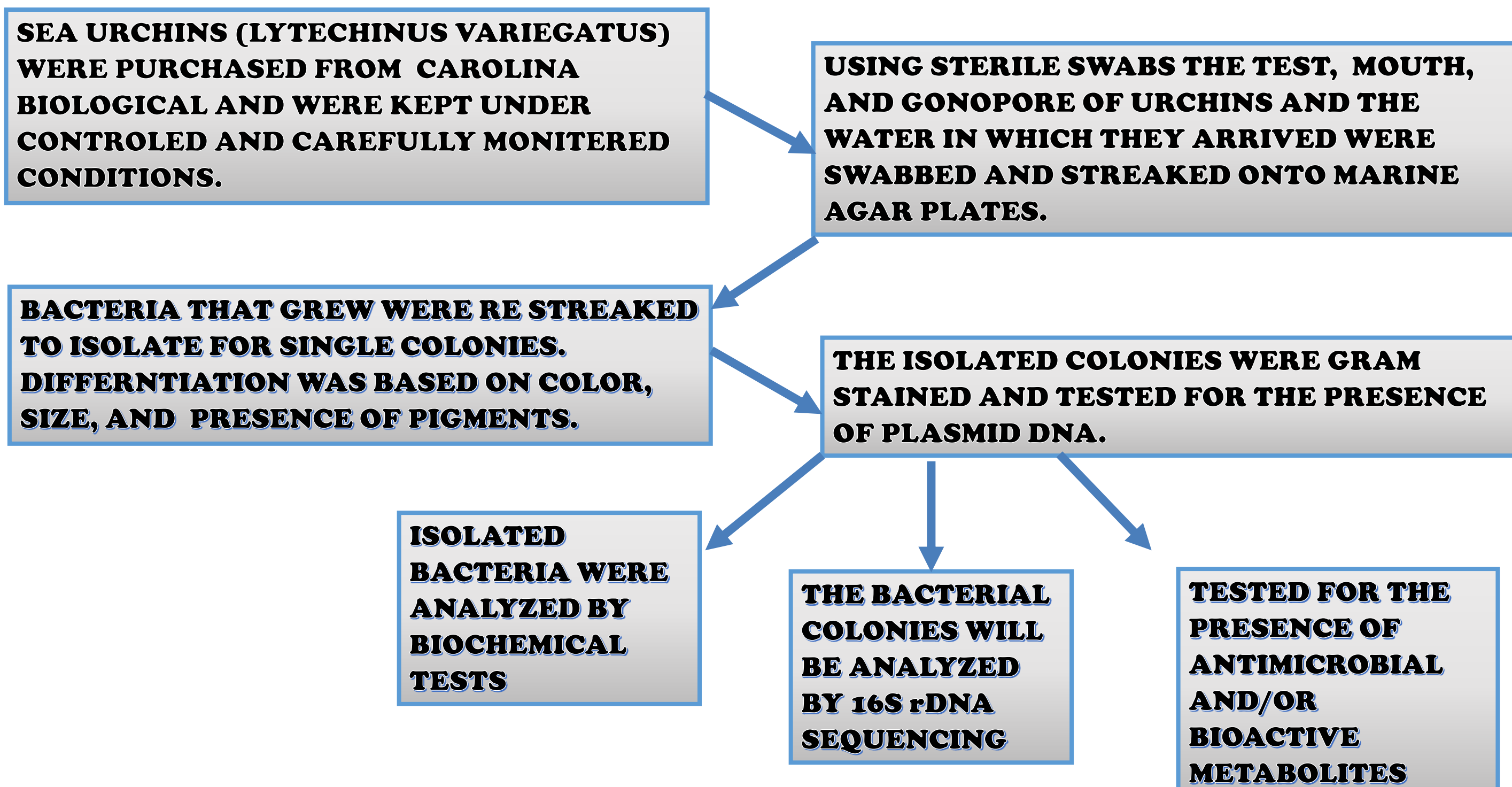
INTRODUCTION

Marine invertebrates, particularly sessile ones, are rich sources of unusual metabolites (1). 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 (2). 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 (3). 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



Figure 1 Sea Urchin *Lytechinus variegatus* maintained in the lab with Instant Ocean



PRELIMINARY 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.

U6T	U2Ta	U9Ga	U1M6	U6G	U13G	U5Mb
U13T	U9T	U7G	U8T	LU80	U10Wagl	U5Mc

2. GRAM STAINING

Gram staining of isolated bacterial samples indicated that they were all gram negative rods.

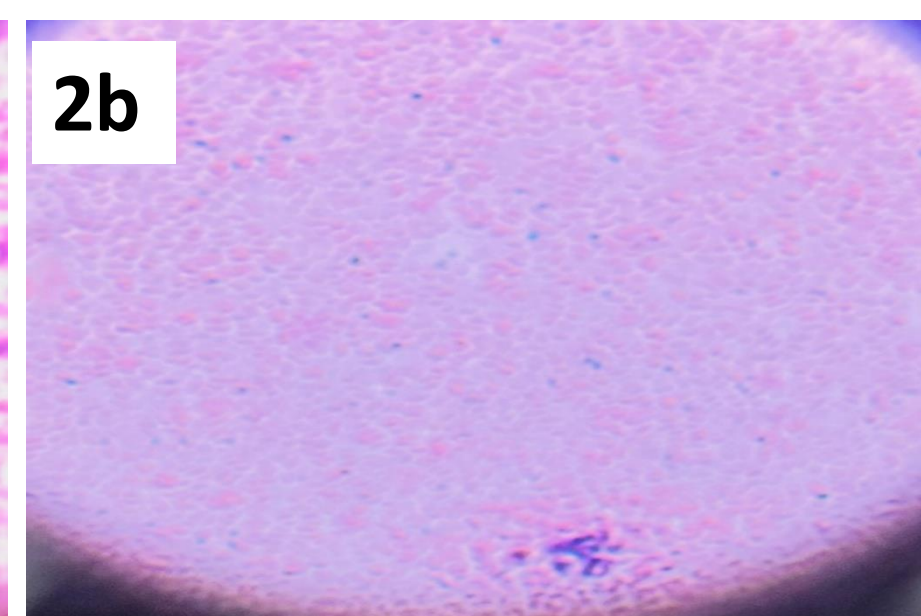
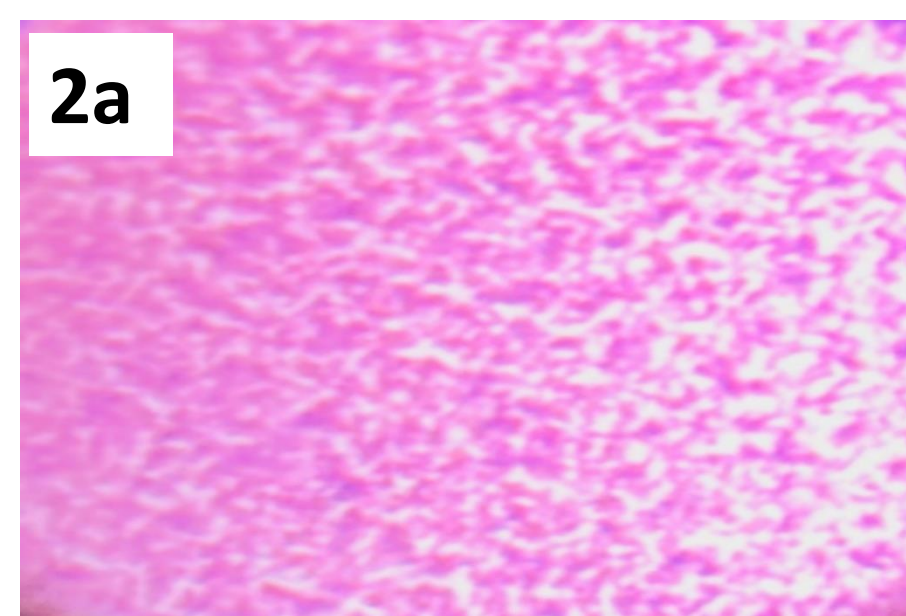


Figure 2 Gram staining of bacterial isolates
◆ Figure 2a
U8T (obtained from the Test urchin #8)
◆ Figure 2b
U5Mc (obtained from the test of urchin # 5).

RESULTS

1. BIOCHEMICAL TEST

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

- a. Cellulase activity Test : Congo red interacts with cellulose to give a red color.
Clear Yellow Halo: Positive Cellulase Activity
Uniform Red: The bacteria did not produce effective cellulase

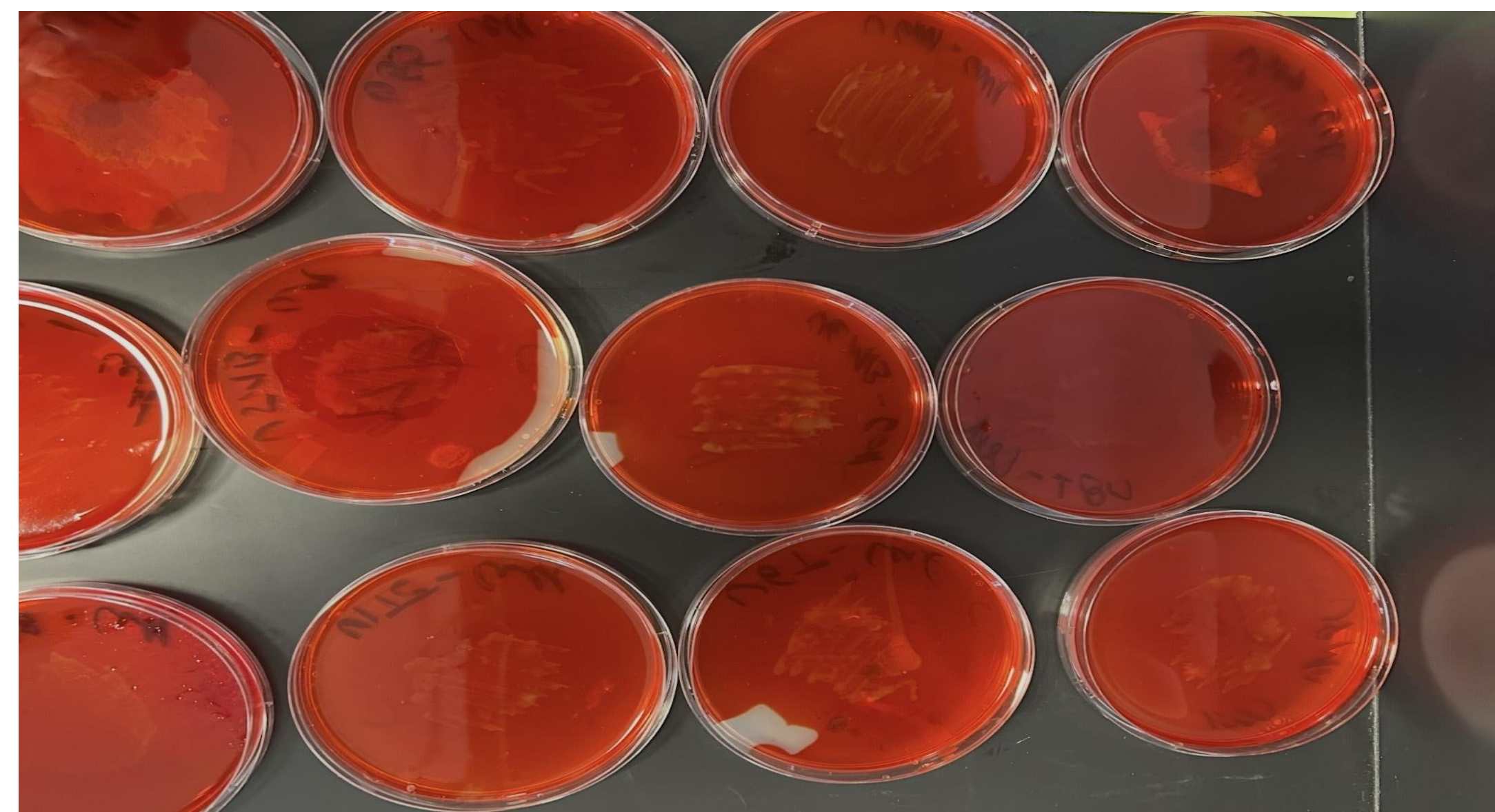


Figure 3: Cellulase Activity Test (Bacteria showing cellulase activity – U10Wagl)

- b. Amylase Activity Amylase reacts with starch to give bluish black color.
Clear zone surrounding the growth: The bacteria produce and secret amylase which breakdown starch.
Black color up to the edge of the growth: The bacteria did not produce amylase. The starch remains intact and bind with iodine.

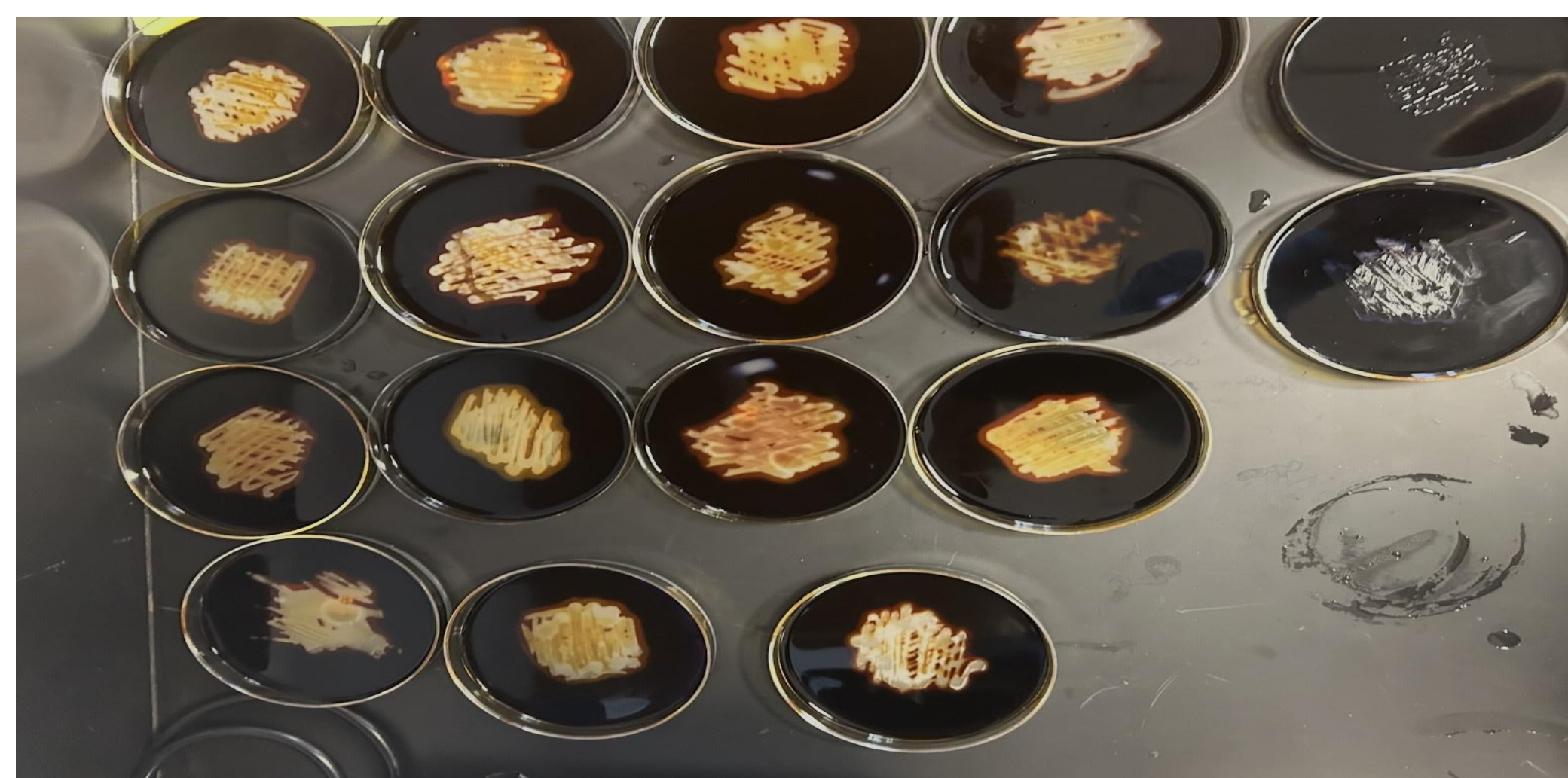


Figure 4. Lactose fermentation test of all the 14 bacteria. Most of them were amylase positive

RESULTS

2. ANTIBIOTIC RESISTANCE

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

Table 1 Antibiotic resistance shown by the 14 bacterial isolates

Bacteria Antibiotic	Lu80	U2 Ta	U9 Ga	U1 M6	U6 G	U2 Ta	U10W agl	U13 G	U5M b	U5M c	U8T	U7G	U9T	U13T
Bacitracin	+	-	+	+	-	-	-	-	+	+	+	-	+	-
Ciprofloxacin	-	+	-	-	+	-	+	+	-	-	-	+	+	+
Oxytetracycline	+	-	+	+	-	-	-	-	-	+	+	-	+	-
Erythromycin	-	+	-	-	+	-	-	+	-	-	-	+	-	+
Trimethoprim	+	-	-	+	-	+	+	+	-	-	+	-	-	-
Vancomycin	+	+	-	-	-	+	+	+	-	-	-	-	-	+
Kanamycin	-	+	-	-	-	+	+	+	-	-	+	+	+	+
Streptomycin	-	+	-	-	-	-	+	+	-	-	+	+	+	+

3. Presence of Plasmids in the isolated marine bacteria

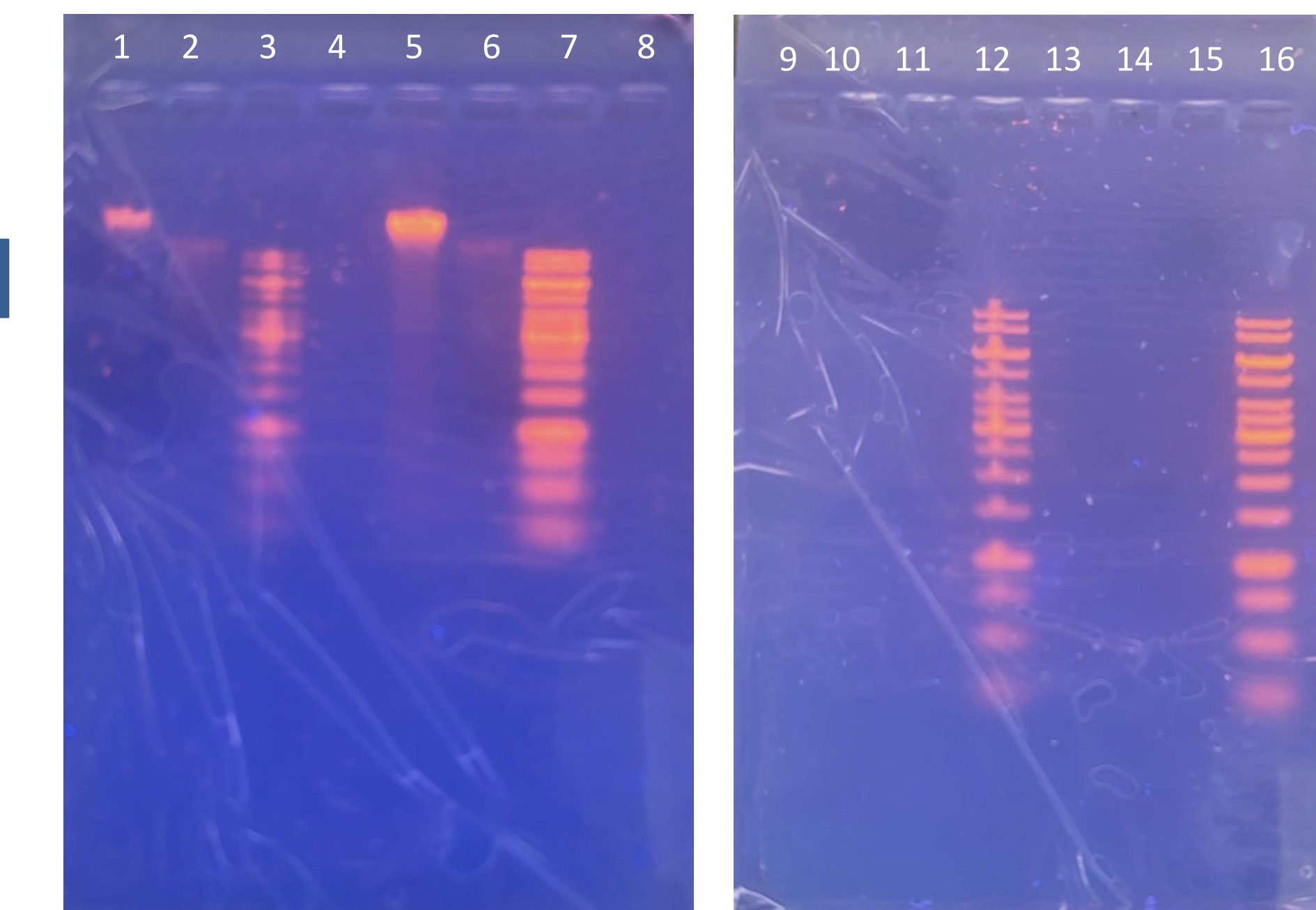


Figure 5: Bacteria in respective lanes are given in Table 2
Lanes 3, 7 12 and 16 are 500bp Molecular markers

Table 2 Order of Bacteria loaded in each lane.

Bacteria	U6T	U2Ta	U9Ga	U1MC	U6G	U13T	U9T	U7G	U8T	U13G	U5Mb	U1M6	U5Mc
Well	1	2	4	5	6	8	7	9	10	11	13	14	15

CONCLUSION AND DISCUSSION

- All bacterial isolates tested were gram negative rods
- Only one of them is mildly cellulase positive and many of them are amylase positive.
- They show resistance to one or more common antibiotics.

FUTURE WORK

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

ACKNOWLEDGEMENT

We appreciate to Professor. Lalitha Jayant for her support throughout this research project. This study was supported by The Foundation for City College grant from Alfred P. Sloan Foundation and BMCC C-STEP. We also thank the BMCC Office of Research for their support.

BIBLIOGRAPHY

- Romano G;Almeida M;Varela Coelho A;Cutignano A;Gonçalves LG;Hansen E;Khnykin D;Mass T;Ramşak A;Rocha MS;Silva TH;Sugni M;Ballarin L;Genevière AM; (n.d.). *Biomaterials and bioactive natural products from marine invertebrates: From basic research to innovative applications*. Marine drugs. <https://pubmed.ncbi.nlm.nih.gov/35447892/>
- S.; E. S. T. (n.d.). *Unlocking the diversity and biotechnological potential of marine surface associated microbial communities*. Current opinion in microbiology. <https://pubmed.ncbi.nlm.nih.gov/18524668/>
- Yao Q;Yu K;Liang J;Wang Y;Hu B;Huang X;Chen B;Qin Z; (n.d.). *The composition, diversity and predictive metabolic profiles of bacteria associated with the gut digesta of five sea urchins in Luhuitou Fringing Reef (northern South China Sea)*. Frontiers in microbiology. <https://pubmed.ncbi.nlm.nih.gov/31191489/>
- JF.; W. J. (n.d.). *Marine bacteria and fungi as promising source for new antibiotics*. Drug development research. <https://pubmed.ncbi.nlm.nih.gov/30370576/>