

**ANTIMICROBIAL-PRODUCING
BACTERIAL SYMBIONTS FROM
ABALONE *Haliotis asinina***

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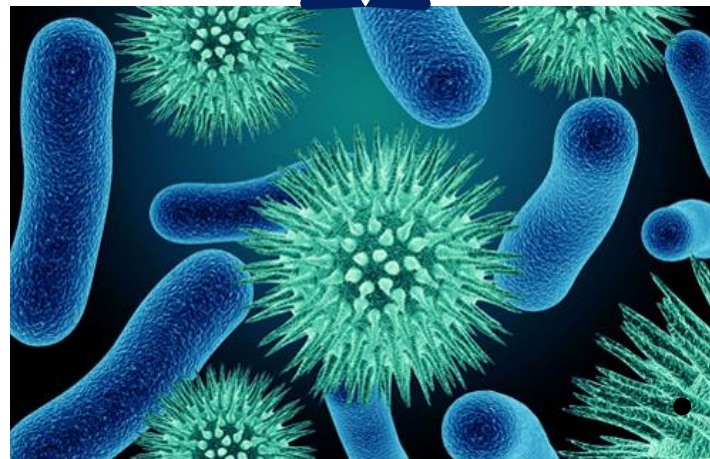
Introduction

With the issues...



- Climate change

- Overuse, misuse of drugs



- Antibiotic resistance

- Abalone is a flagship organism of the Western Philippines University
- Highly priced commodity
- Develop culture system suitable in Palawan, Philippines setting
- Including health and nutritional management





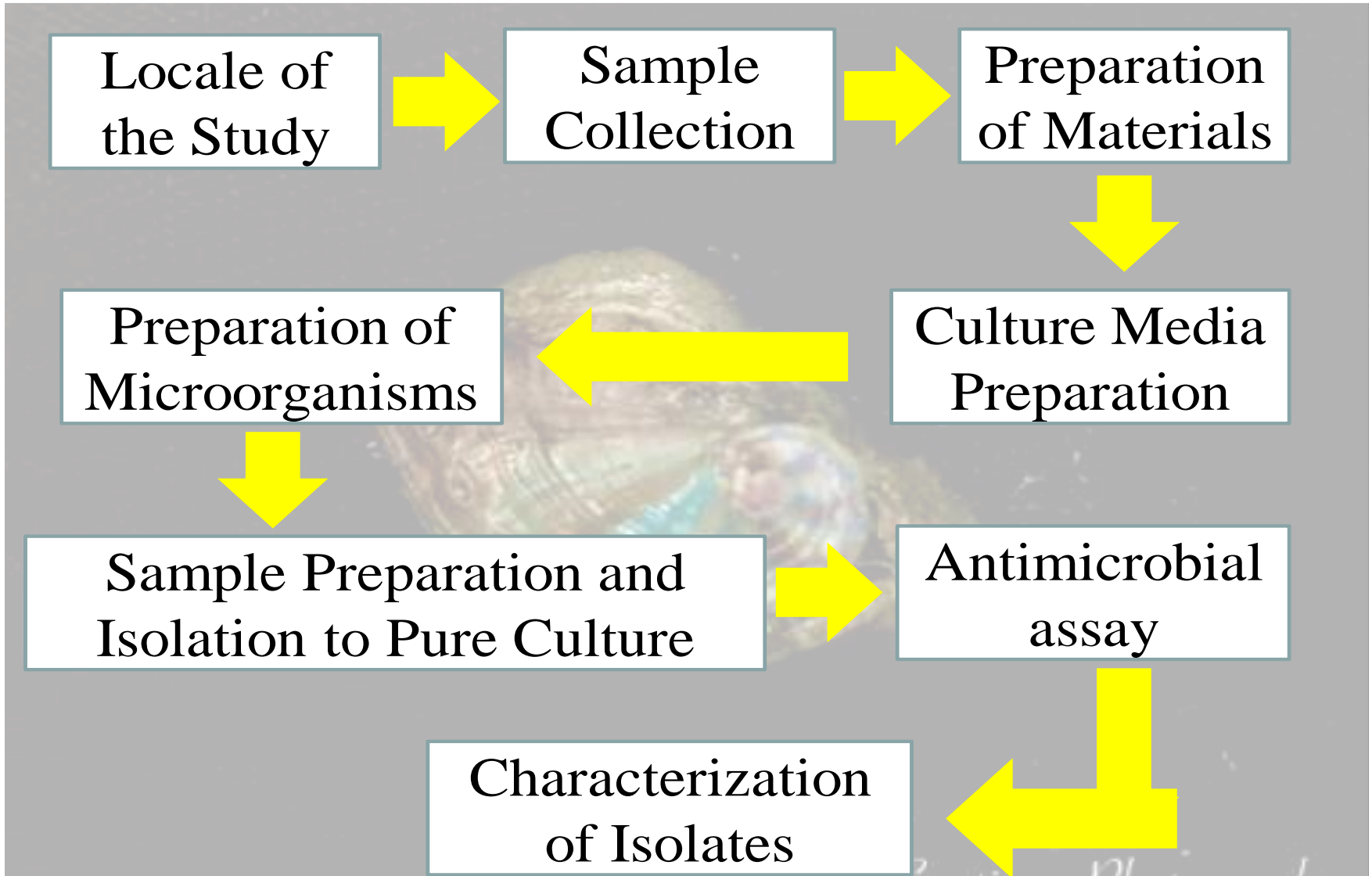
The hepatopancreas of abalone contains microbial symbionts that produce secondary metabolites serving as chemical defenses and aid in the digestion of food

Microbial symbionts are associated with marine natural products (MNP) acknowledged as the “blue gold” in the quest for therapeutic agents.

Objectives

- This study aimed to isolate bacterial symbionts with antimicrobial-producing potential from hepatopancreas of *Haliotis asinina*.
 - to enumerate and isolate bacteria from the hepatopancreas of cage-cultured and wild-caught abalone
 - to test the antimicrobial potential of the isolated microorganisms against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*
 - to characterize and identify the isolated microorganisms that would exhibit antimicrobial potential up to lowest possible taxa

MATERIALS AND METHODS



Sample Collection

Cage-cultured abalone samples were bought from abalone farm in Pamantolon, Taytay, Palawan while wild-caught samples were bought from a local trader in Tagbueros, Honda Bay, Puerto Princesa City, Palawan, Philippines



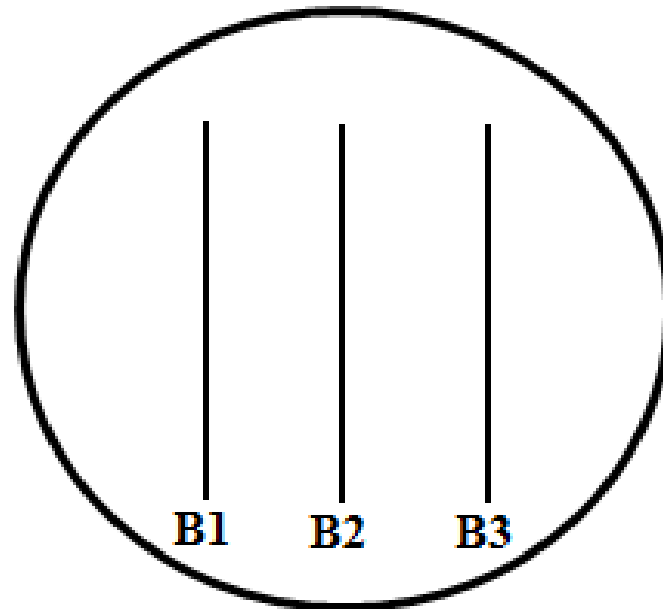
Culture Media Used

- Nutrient agar/broth – general bacteria
- Egg albumin medium – Gram + bacteria
- Egg albumin + crystal violet – Gram – negative
- Dextrose nitrate medium – Actinomycetes

Test Microorganisms

- 24-hour culture of :
 - *Escherichia coli*
 - *Staphylococcus aureus*
 - *Bacillus subtilis*
 - *Bacillus cereus*
 - *Pseudomonas aeruginosa*
 - *Bacillus megaterium*
 - *Aspergillus flavus*
 - *Aspergillus niger*
 - *Candida albicans*

Antimicrobial assay



Test Organism Laden Plate

Characterization of Isolates

- Following standard methods
 - Cultural, morphological and physiological test (Brown 2005)
- Bergey's Manual of Systematic/Determinative Bacteriology
 - Identification of the isolate

RESULTS and DISCUSSION

Table 1. Presence (+) or absence (-) of zones of inhibition of isolates from cage-cultured and wild-caught samples against *Staphylococcus aureus* and *Escherichia coli*.

CODE	<i>S. aureus</i>	<i>E. coli</i>	CODE	<i>S. aureus</i>	<i>E. coli</i>
C1	-	-	W1	-	-
C2	-	-	W2	+	-
C3	-	-	W3	-	-
C4	-	-	W4	-	-
C5	-	-	W5	-	-
C6	-	-	W6	-	-
C7	-	-	W7	-	-
C8	-	-	W8	-	-
C9	-	-	W9	-	-
C10	-	-	W10	-	+
C11	-	-	W11	-	+
C12	-	-	W12	-	+
C13	-	-	W13	-	-
C14	-	-	W14	-	-
C15	-	-	W15	-	-
C16	-	-	W16	-	-
C17	-	-	W17	-	-
C18	-	+	W18	-	+
C19	-	-	W19	-	-
C20	-	-	W20	-	-
C21	-	-	W21	-	-
C22	-	-	W22	-	-
C23	+	+	W23	-	-
C24	-	-	W24	-	-
C25	-	+	W25	-	-
C26	-	-	W26	+	-
C27	-	-	W27	-	-
C28	++	++	W28	-	-
C29	+	+	W29	-	-

Sample of Isolates with Zones of Inhibition against *S. aureus* and *E. coli*

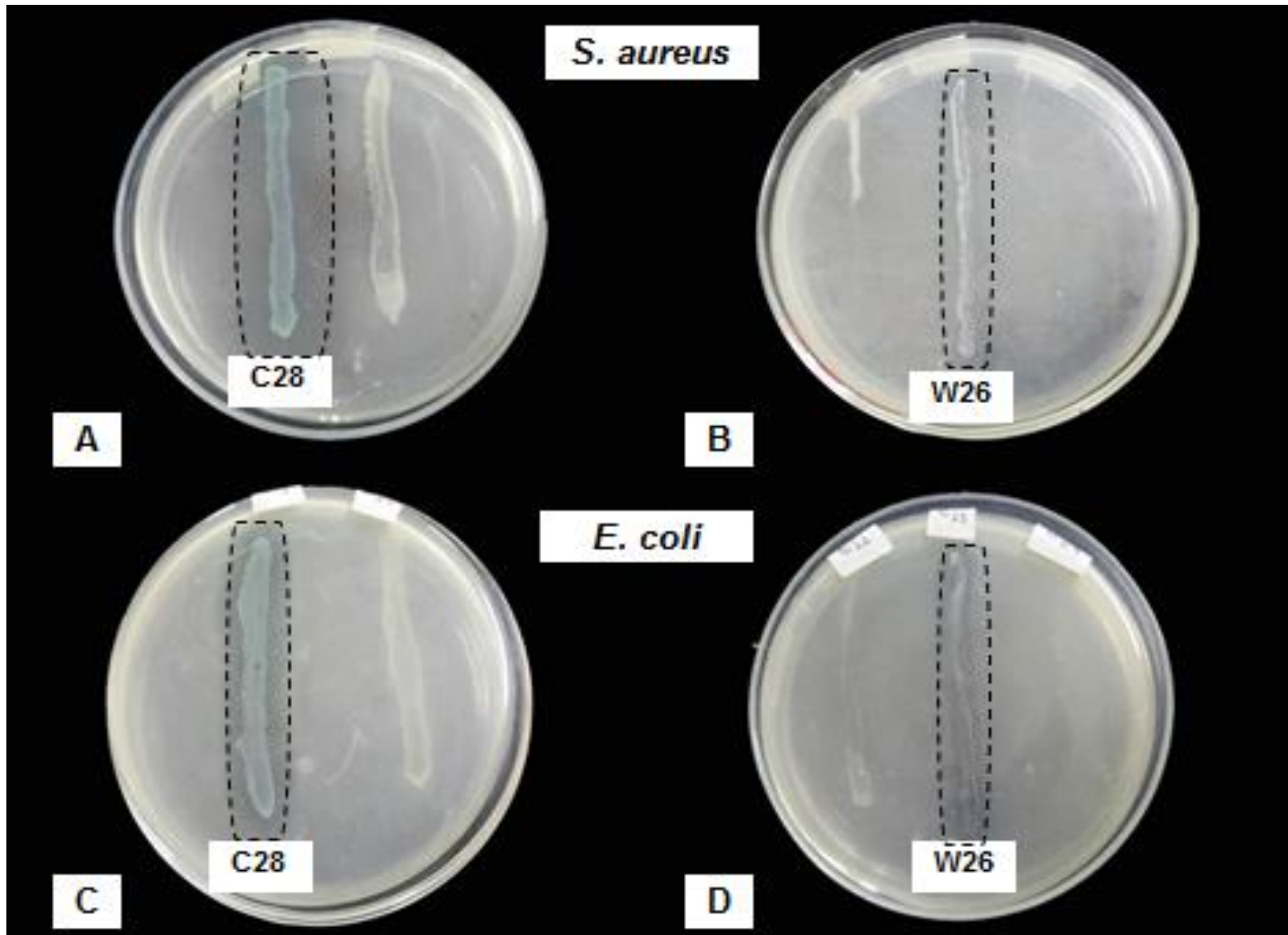


Table 2. Presence (+) or absence (-) of zones of inhibition against *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis* and *Pseudomonas aeruginosa* of isolates from cage-cultured and wild-caught samples.

CODE	<i>B. cereus</i>	<i>B. megaterium</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
C18	+	+	+	-
C23	-	-	-	-
C25	+	-	+	-
C28	++	+++	++	+
C29	-	+	+	+
W2	+	+	-	-
W10	+	-	-	-
W11	-	-	-	-
W12	+	+	+	-
W18	-	-	-	-
W26	+	+	+	-

Some Isolates with Zones of Inhibition against *B. cereus*, *B. megaterium*, *B. subtilis* and *P. aeruginosa*

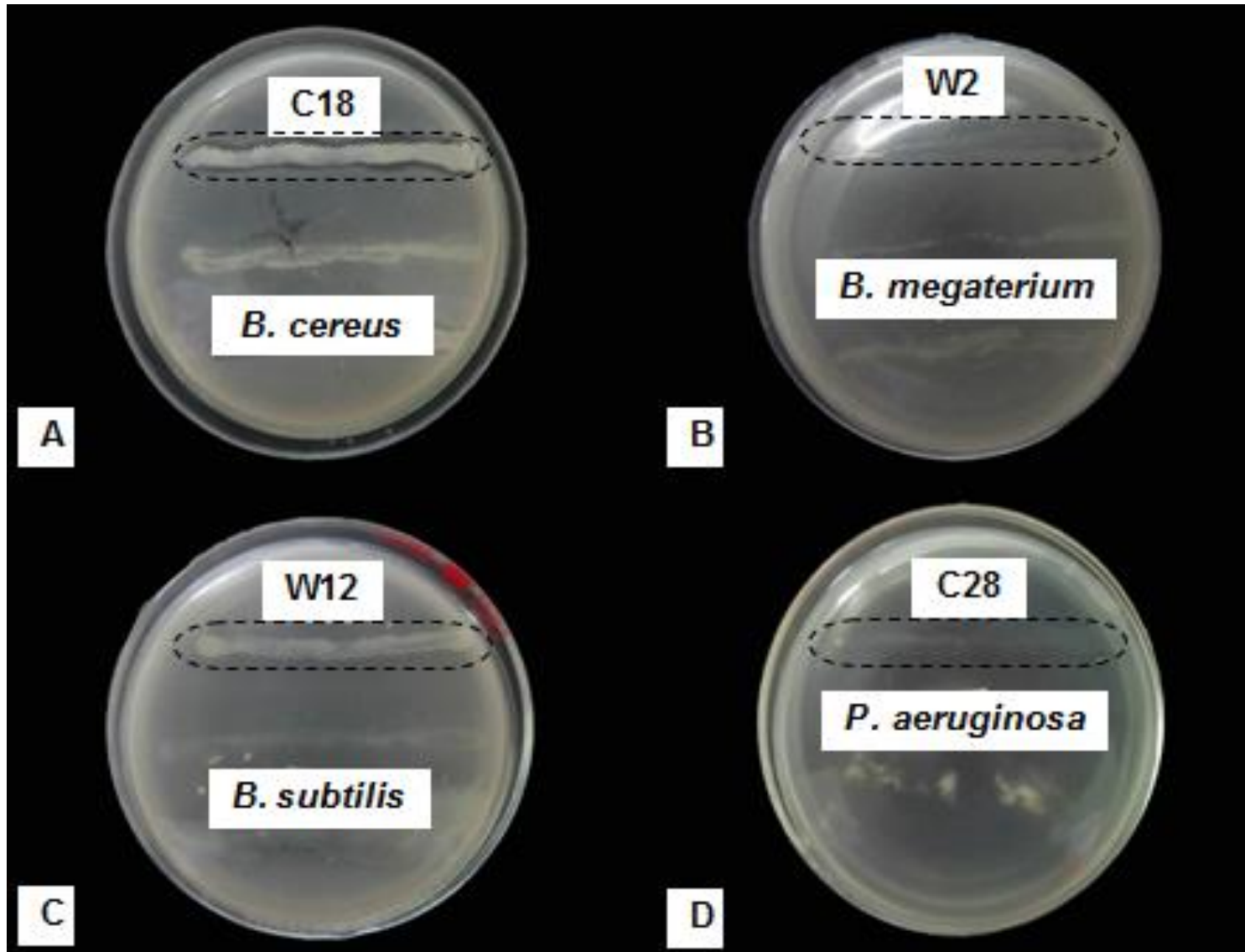


Table 3. Presence or absence of zones of inhibition of isolates from cage-cultured and wild-caught samples against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*.

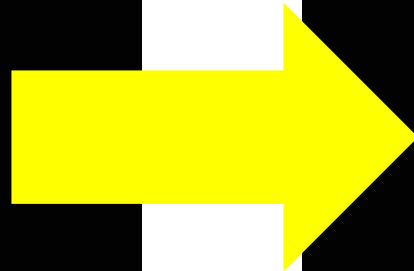
CODE	<i>C. albicans</i>	<i>A. niger</i>	<i>A. flavus</i>
C18	+	-	-
C23	-	-	-
C25	-	-	-
C28	++	-	-
C29	+	-	-
W2	-	-	-
W10	-	-	-
W11	-	-	-
W12	-	-	-
W18	+	-	-
W26	-	-	-

CHARACTERIZATION OF ISOLATES

Table 4. Descriptive chart for the characterization of isolate C28.

<p style="text-align: center;">Cultural Characteristics</p> <p>Nutrient Agar Plate Colonies</p> <p>Form: Circular Elevation: Raised Margin: Entire Size: 5mm – 15mm Opacity: Opaque Color: Milky white</p> <p>Agar Slant Growth</p> <p>Amount of growth: Moderate Form of growth: Filiform Luster: Glistening Chromogenesis: Chromogenic (green)</p> <p>Growth on Nutrient Broth</p> <p>Surface growth: Membranous Subsurface growth: Granular Amount of sediment: Moderate Type of sediment: Granular Odor: Absent Chromogenesis: Chromogenic (green)</p>	<p style="text-align: center;">Gelatin Stab Culture</p> <p>Liquefaction: No liquefaction Configuration: N/A Type of growth: Arborescent</p> <hr/> <p style="text-align: center;">Morphological Characteristics</p> <p>Cell shape: Rod-shaped Arrangement: Single bacillus Motility: Motile Flagella: Present</p> <hr/> <p style="text-align: center;">Physiological Characteristics</p> <p>Gram’s stain: Gram negative Catalase test: Positive Oxidase test: Positive Hydrolysis of starch: Negative Oxygen requirement: Aerobic Fermentation of carbohydrates: Negative Indole test: Negative Methyl red test: Negative Voges–Proskauer test: Negative Citrate: Positive.</p>
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Chromogenesis of C28



- Based on the cultural, morphological and physiological tests, C28 was found to be more closely related to the genus *Pseudomonas* which belongs to the family *Pseudomonadaceae*, order *Pseudomonadales*, and class *Gamma Proteobacteria*.

CONCLUSIONS

- Bacteria can be enumerated and isolated from the hepatopancreas of abalone species *Haliotis asinina*.
- These isolated bacteria can be a source of antibacterial and antifungal compounds.
- Bacteria closely related to the genus *Pseudomonas* was isolated from the hepatopancreas of abalone *Haliotis asinina*

Future direction

- C28 or this *Pseudomonas* isolate will undergo further studies for its suitability in any application such as probiotics, immunostimulant and natural antibiotics applications for the improvement of the culture system of abalone in Palawan, Philippines.

Acknowledgment

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Thank you for your attention