

BIOACTIVITY OF A MARINE SPONGE EPIBIONT BACTERIUM FROM SOLOMON ISLANDS WATERS.

Wayne Kavora¹, Katy Soapi¹, Klaus Feussner¹ and Samson Viulu¹

1. Institute of Applied Sciences, The University of the South Pacific, Fiji.
2. Solomon Islands National University, Solomon Islands

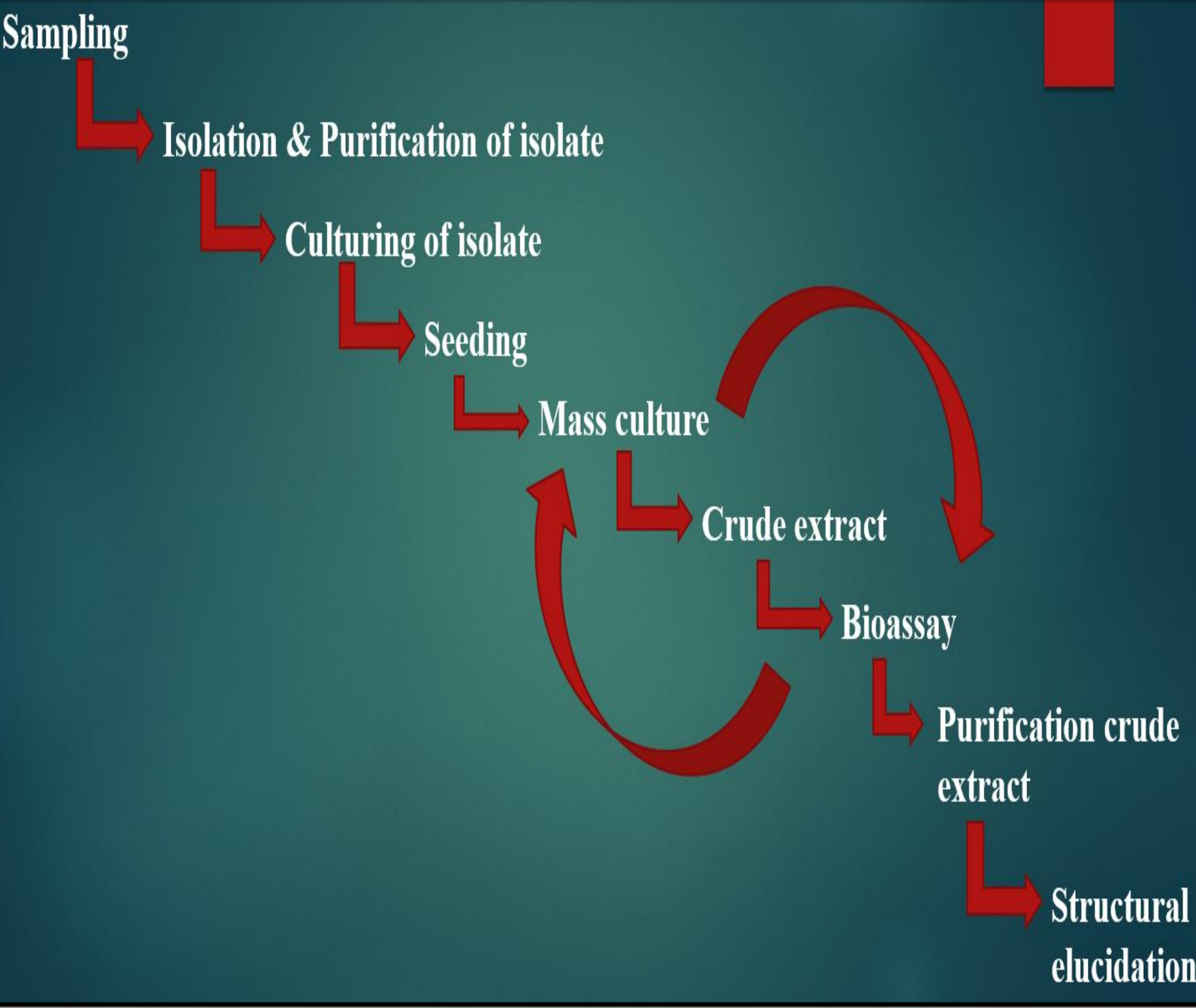
ABSTRACT & INTRODUCTION

Marine microbes are known treasure troves for biochemical compounds with potential uses in the pharmaceutical, agrochemical and cosmetic industries. A marine bacterium strain S-011 was isolated from the sponge *Haliclona fascigera*, collected in the Solomon Islands sea waters. 16S rRNA gene sequencing of the epibiont strain S-011 and NCBI online BLASTN search showed that it is 99% similar to *Bacillus aquimaris*. Strain S-011 is a Gram positive bacterium and grows best in seawater media compared to deionized distilled water (DDW) media. A compound identified by NMR as 2-aminobenzoic acid was isolated from strain S-011 and active against the antibiotic resistant *Bacillus oceanisediminis* CNY-977. Crude extracts from the isolate also exhibited inhibition against CNY-977 and amphotericin resistant *Candida albicans* (ARCA). To the best of our knowledge this is the first report on the intergeneric bioactivity of strain S-011 against CNY-977, both belonging to the same genus *Bacillus*.

AIM & OBJECTIVES

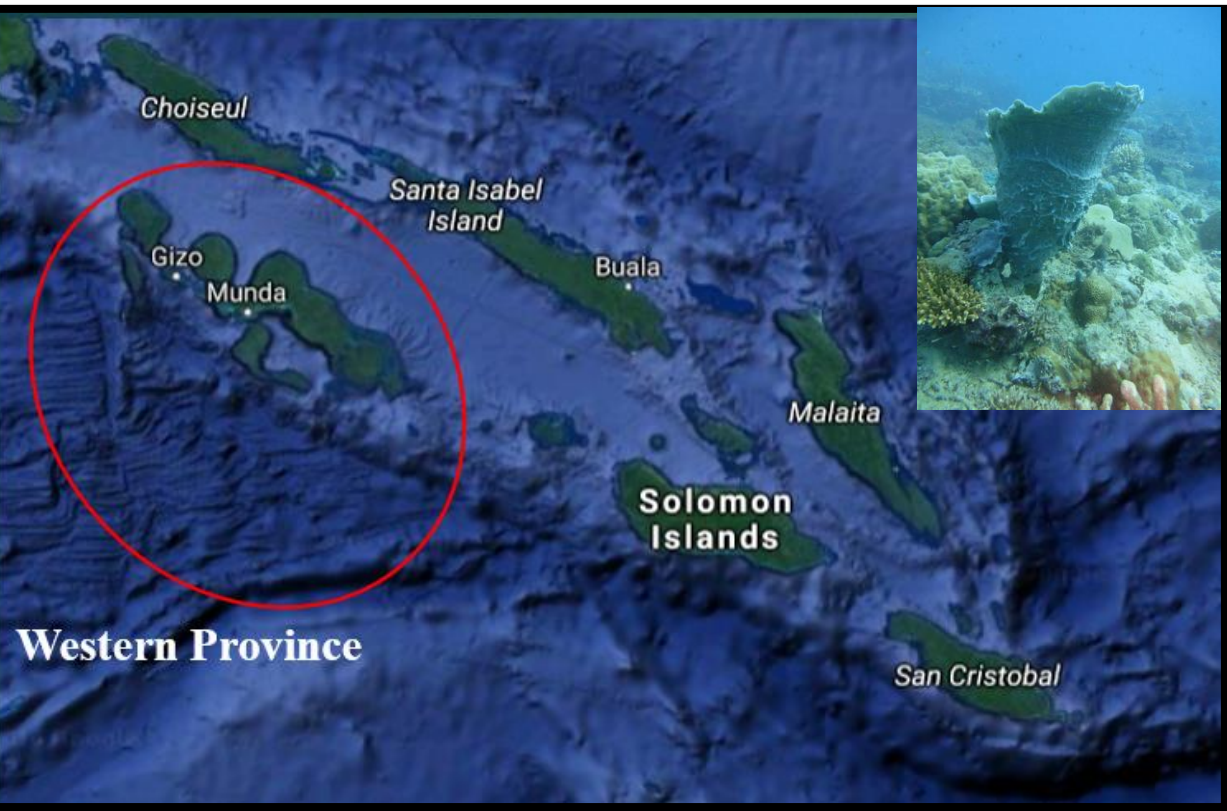
- Isolation and purification of microbe (*Haliclona Poseidon* sponge).
- Mass culture of microbe
- Extraction of secondary metabolites.
- Determination of bioactivity through bioassays (antibacterial, antifungal and antiviral (hopefully) as well as cytotoxicity test by brine shrimp assay (BSA)
- Bioassay guided purification of bioactive compounds
- Structural elucidation of bioactive compound(s) through spectroscopic analysis.

RESEARCH PLAN



MATERIAL & PROCEDURE

1. SAMPLING SITE



2. ISOLATION & PURIFICATION



3. MASS FERMENTATION



4. ANTIMICROBIAL ASSAYS



5. ISOLATION & PURIFICATION OF CRUDE



6. NMR SPECTROSCOPY



CONCLUSION

A bacterial isolate strain S-011 was isolated from *H. fascigera* sponge collected in the Solomon Islands seawaters at approximately 15 meters depth. S-011 is a marine obligate and requires seawater for its growth. It is a Gram positive bacterium with single colonies appearing orange in color, soft and regular or round in shape. BLASTN search of its partial 16 rRNA gene sequences showed that strain S-011 is 99% similar to *B. aquimaris*, supporting the similar morphological descriptions they share in common. Crude EtOAc extracts from the fermentation cultures of strain S-011 showed bactericidal property against the antibiotic resistant CNY-977 and fungicidal activity against ARCA and the wild type *Candida albicans*, with an inhibition zone of 12 mm at 10 mg/mL. Fractionation by TLC, VLC, LH20 and ISCO flash and purification by HPLC yielded 2-aminobenzoic acid, according to NMR spectra analyses. The 2-aminobenzoic acid obtained in this study showed bactericidal activity against the antibiotic resistant CNY-977, with an inhibition zone of 13 mm at 10 mg/mL.

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ACKNOWLEDGEMENT & CONTACT

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Contact

Email: - wayne.kavora@sinu.edu.sb
Phone contact (Mobile): (679) 7214794

RESULT & DISCUSSION

1. STRUCTURAL ELUCIDATION

The chemical structure of the bioactive compound (Figure 2) purified from strain S-011 was determined to be 2-aminobenzoic acid by 1H NMR, 13C NMR, HMBC and HSQC analyses (Table 1).

Position	¹³ C		¹ H		J (Hz)	COSY (¹ H- ¹ H)	HMBC (¹ H- ¹³ C)
	δ _C (ppm)	mult.	δ _H (ppm)	mult.			
1	112.7	C					
2	152.9	C					
3	117.3	CH	6.73	d	10	4	1, 5, 7
4	134.6	CH	7.22	td	10, 5	5, 3	3, 6, 2
5	115.4	CH	6.56	t	10	6, 4	1, 3, 6, 4, 2
6	132.3	CH	7.8	dd	10, 5	5	4, 2, 7
7	171.2	C					

Table 1. 13C (125 MHz) and 1H (500 MHz) NMR data of 2-aminobenzoic acid

Peak	Peak identification	Mass (mg)	Inhibition zone (<i>Bacillus</i> CNY977) (mm)
P1	S-011V1L3V3V1F2P2P1	3.1	13
	Control (vancomycin)		24

Table 2: Bioactivity of compound A (2-aminobenzoic acid)

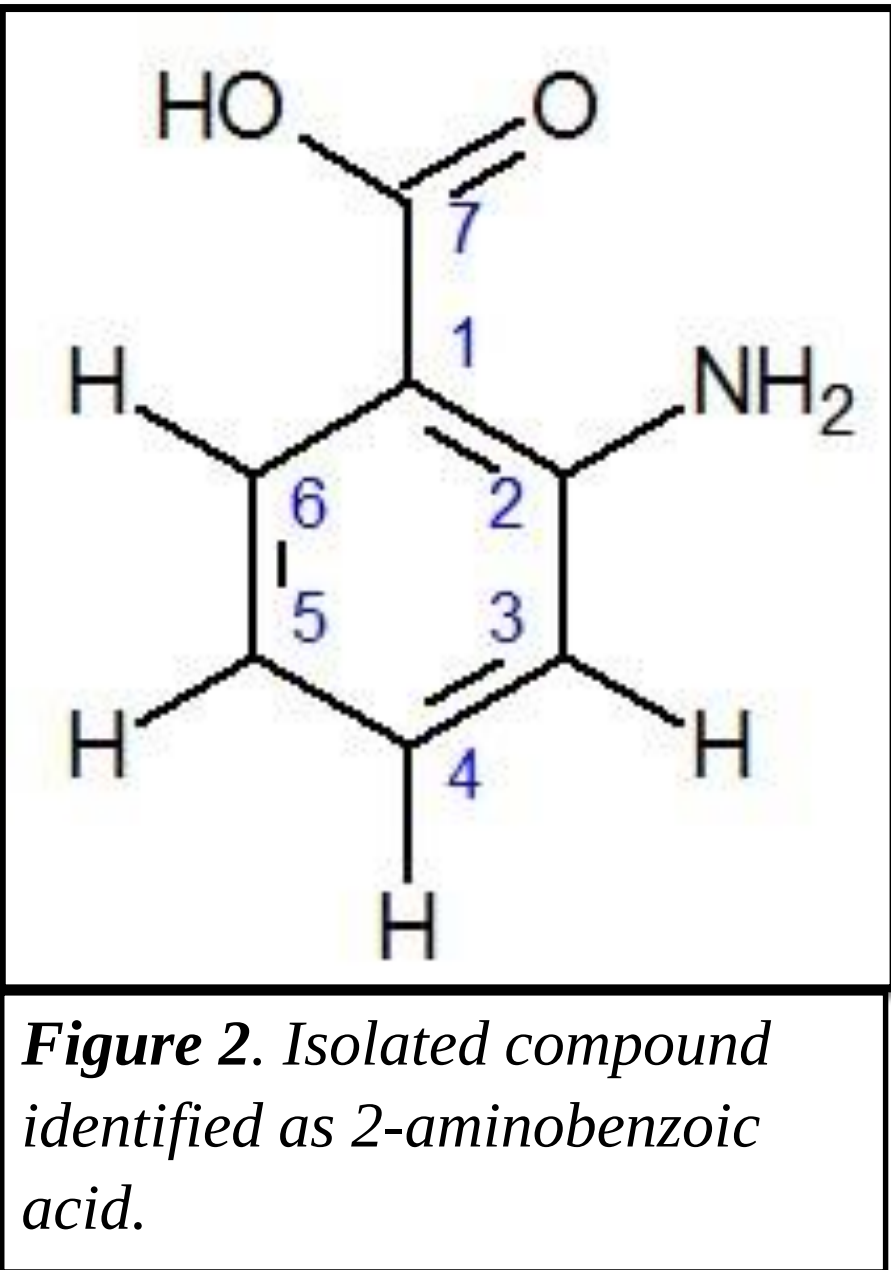


Figure 2. Isolated compound identified as 2-aminobenzoic acid.