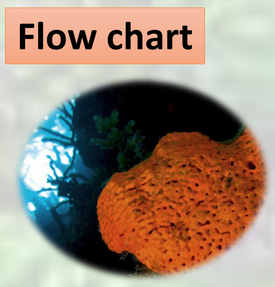
The analysis of secondary metabolite from symbiotic bacteria of *Agelas nemoechinata, Vibrio tubiashii*.

洪茂軒 (Mao-Xuan Hung)#，廖志中 (Chih-Chuang Liaw)*
Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung, Taiwan,
Doctor Degree Program in Marine Biotechnology, National Sun Yat-sen University Academia Sinica, Kaohsiung, Taiwan

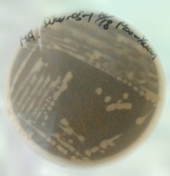
Abtract

In the past study, the EA extract of *Agelas nemoechinata* has inhibitory activity against *Staphylococcus aureus* and *S. epidermids.* This time, we isolated some bacteria from the sponge, *A. nemoechinata,* one of which is *Vibrio tubiashii* DJW05-1 by 16S rRNA sequencing analysis. EA extract of *V. tubiashii* DJW05-1 was screened its antibacterial activity. The result showed it had inhibitory activity against bacterial indicators. Thus, herein, an interesting question is who the producers of the bioactive compounds in *A. nemoechinata* are, sponge? or symbionts living with the sponge? For clarifying the question, I cultured *V. tubiashii* DJW05-1 on MA for three days. And, its EA extract showed apparent antibacterial activity. By the way, *Vibrio tubiashii* is a kind of facultative anaerobic bacterial. Thus, I also cultured *V. tubiashii* DJW05-1 in anaerobic chamber for comparison with that in aerobic condition. The result showed that the antibacterial activity of that cultured in anaerobic condition disappeared. So far, I isolated 6 compounds from the activity fractions of the EA extract in aerobic condition. One of them, andrimid, was identified by consulting Joy Clardy's paper in 2006[1]. Others also have very similar spectral patterns as that of andrimid. We hope that they can have potential to development a new type antibiotic in the future.

Flow chart



Agelas nemoechinata Hoshino,1985



DJW-05-1 → Aerobic / Anaerobic → EA extract → Partition with MeOH and Hex → Hex layer / MeOH layer / MeOH layer / Hex layer → MeOH layer → MPLC → DJWF1 / DJWF2 / DJWF3 / DJWF4 / DJWF5 / DJWF6

MPLC

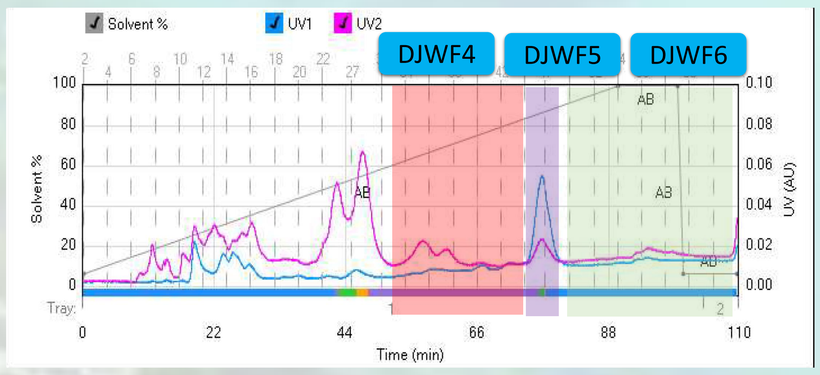
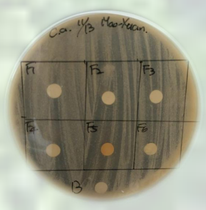
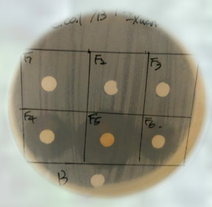


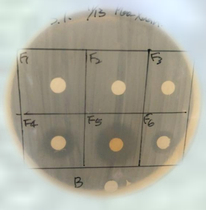
Figure 1. is MPLC profile, and I referred its peaks to separate six fracitions roughly. Then, I test each of fracitions of antibacterial activity. The results of experiment show only fracition 4-6 have activity against *Escherichia coli, Salmonella typhimurium, Acinetobacter baumannii, Staphylococcus aureus*.(like below)



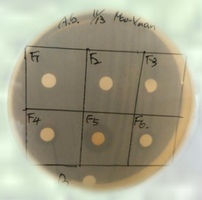
Candida albicans



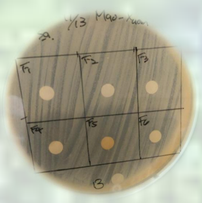
Escherichia coli



Salmonella typhimurium

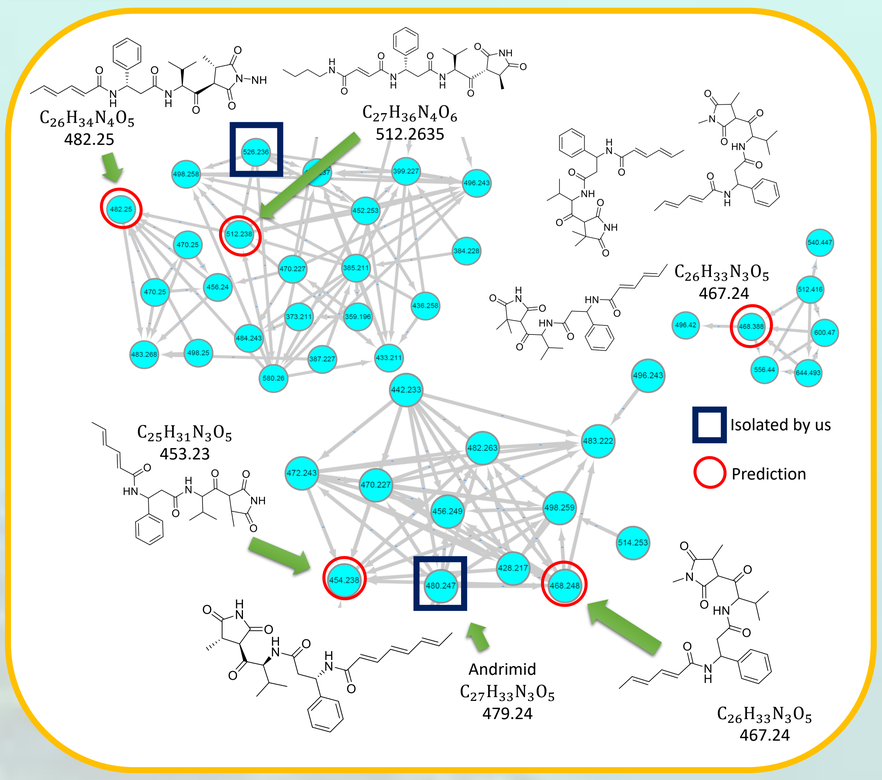


Acinetobacter baumannii



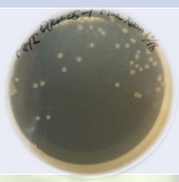
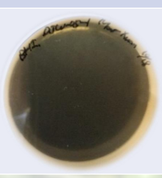
Staphylococcus aureus

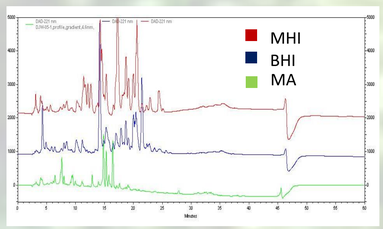
Net-working

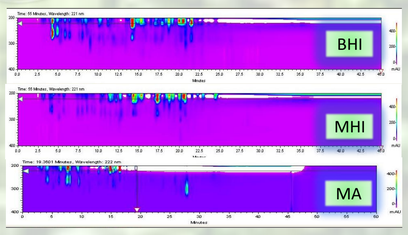


Cultured

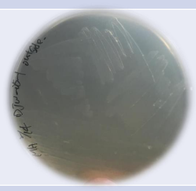
Aerobic condition

MA	MHI	BHI
		

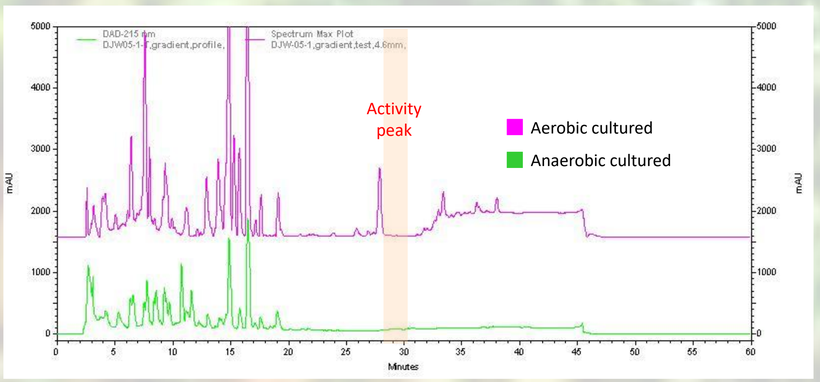




Anaerobic condition

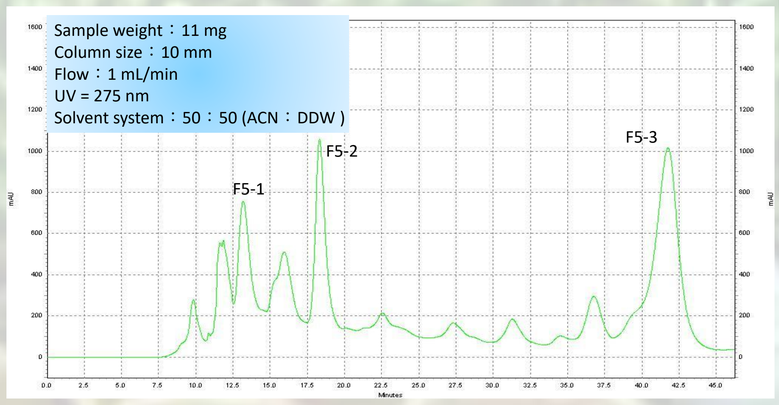
MA	MA + $C_6H_{12}O_6$+ Vit 12
	
MA+DMSO + Vit 12	MA+TMAO
	

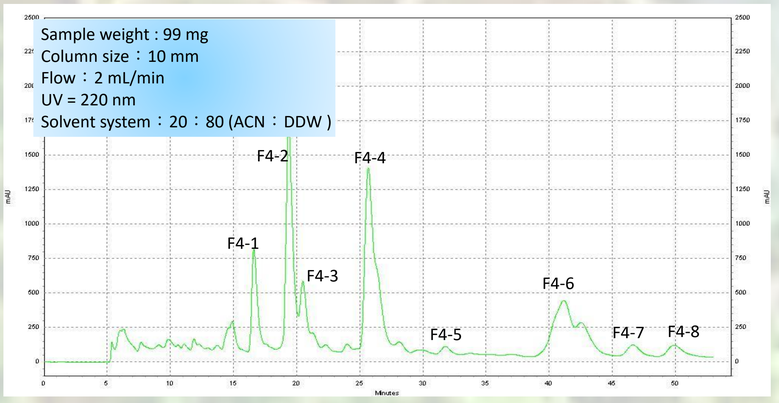
Compared with the aerobic



In the study of Lita M. Proctor, *Anaerobic respiratory growth of Vibrio harveyi, Vibrio fischeri and Photobacterium leiognathi with trimethylamine N-oxide, nitrate and fumarate: ecological Implications,* on 2000[2], show *Vibrio. sp.* would use trimethylamine N-oxide (TMAO) and dimethylsulphoxide (DMSO) as their electron acceptors when they grow in anaerobic environment. Thus, I choiced the both TMAO and DMSO as my test condition in anaerobic cultured to compared with aerobic condition.

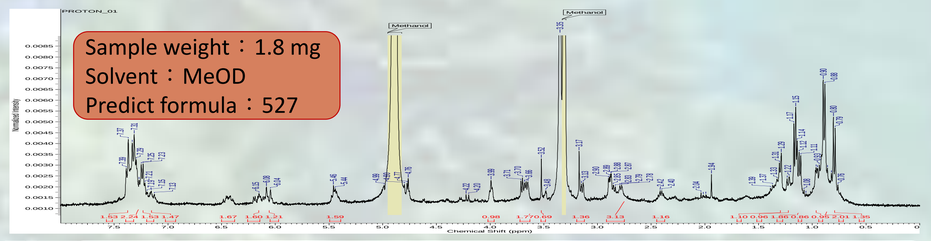
HPLC

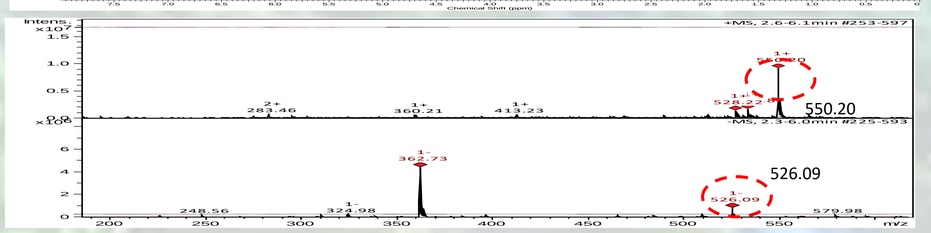


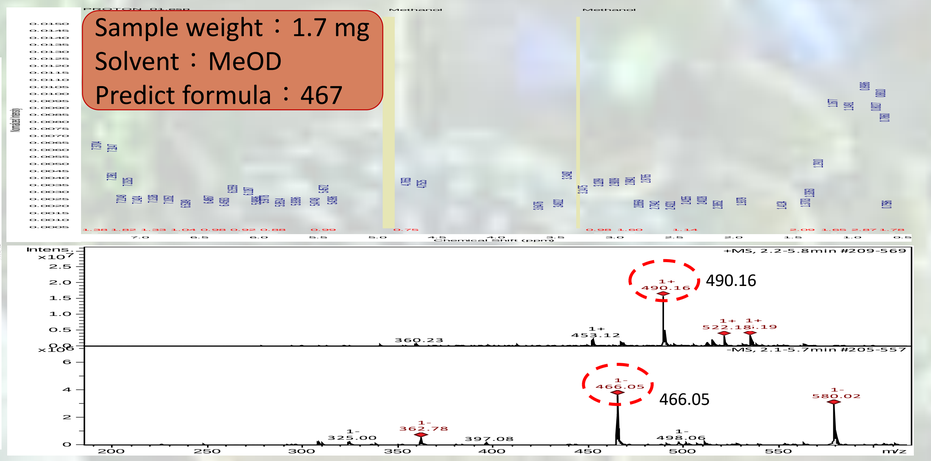


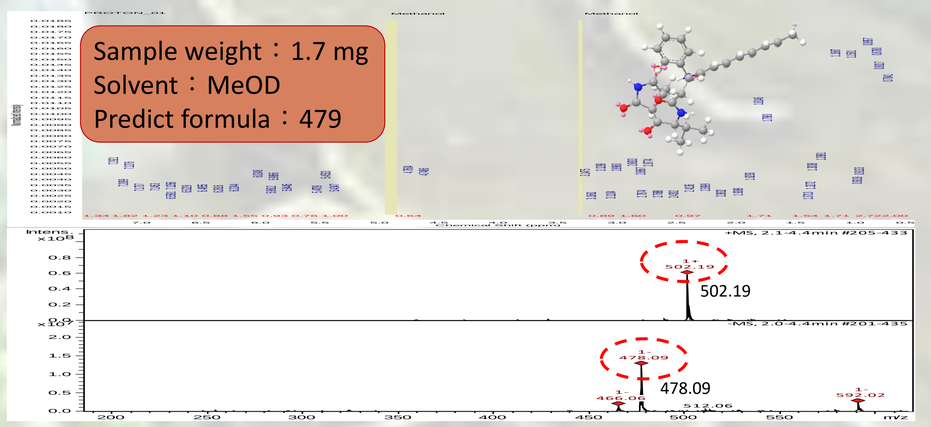
First, we isolated three major peaks, DJWF5-1 ~DJWF5-3, by HPLC, and elucidated their by the basis of spectroscopic methods (NMR, MS, UV, IR etc.) When I got the mass data of DJWF5-3, I compared with the net-working data to help me finding the structure type of my compounds. I found my structure type of my compounds in fractition 5 are belong to Pseudopeptide which potentiable to development a new type antibiotic in the future. Although the biosynthesis pathway have been elucidated, there were only four species can poduce this type of compounds so far. Since I think it is worth to study.

NMR spectrum









Reference

Jon Clardy, et al. **New antibiotics from bacterial natural products**, 12, November 2006, *J Am Chem Soc*. 2006 August 23; 128(33): 10660–10661 [1]

Proctor, L. M. et al. **Anaerobic respiratory growth of Vibrio harveyi, Vibrio fischeri and Photobacterium leiognathi with trimethylamine N-oxide, nitrate and fumarate: ecological implication,** 3 October 2001, *Environmental Microbiology* (2000) 2(4), 399±406 [2]