Exploring the potential of co-cultures to enhance secondary metabolites production in the marine bacterium *Aquimarina* sp. Aq135

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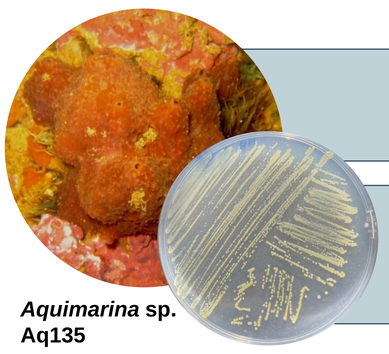
Background & Motivation

Marine sponge host *Ircinia variabilis*



***Aquimarina* sp. Aq135**

Marine sponges and their microbiomes are one of the most prolific sources of bioactive natural products.

However, standard laboratory cultivation conditions fail to harness the full potential of these microbial secondary metabolite producers.

The **OSMAC** ("**One Strain Many Compounds**") approach states that one microorganism can produce a great variety of compounds if manifold culture conditions are used.

- Co-cultivation can mimic possible ecological interactions (e.g., competition, cross-feeding) and promote the production of compounds not present in single culture:
  - ***Aquimarina* sp. Aq135**, isolated from the marine sponge *Ircinia variabilis,* is a known producer of peptide antibiotics (aquimarins) and other secondary metabolites - but yield is very low.
  - ***Vibrio* sp. EL41,** isolated from the octocoral *Eunicella labiata,* is a close relative of *Vibrio breoganii*. a non-pathogenic species associated with marine eukaryotes (e.g., macroalga).

Objective: Elucidate the main differences between the metabolome of *Aquimarina* sp. Aq135 in single culture and grown in co-culture with a *Vibrio* sp.

Results & Discussion

Desynchronised inoculation of *Aquimarina* and *Vibrio*

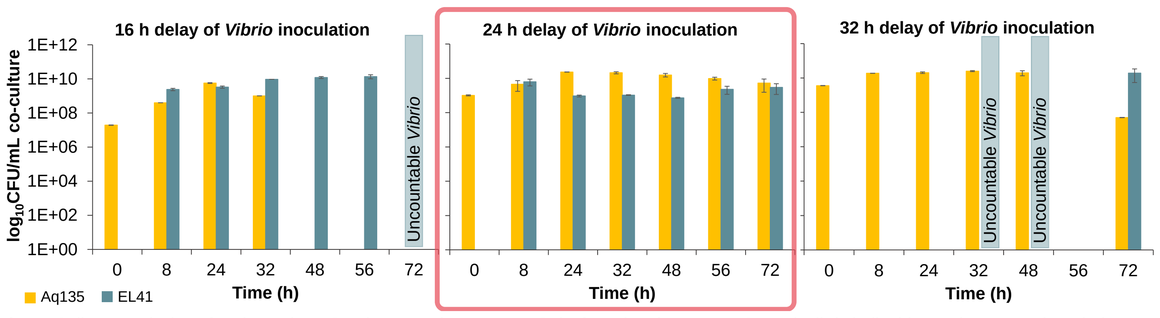


Fig. 1: Cell counts (colony forming units – CFU/mL co-culture; mean±SE) of *Aquimarina* sp. Aq135 and *Vibrio* in liquid co-culture over time during assays with a delayed inoculation of *Vibrio* (16, 24, or 32 hours of delay, respectively). t= 0 hours (x-axis) corresponds to the moment of *Vibrio* inoculation.

Vibrio shows strong presence but does not dominate the co-culture when **inoculated 24 hours after** *Aquimarina* sp. Aq135 was inoculated.

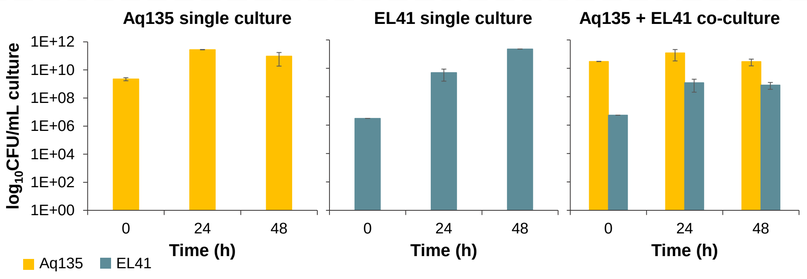


Fig. 2: Cell counts (colony forming units – CFU/mL co-culture; mean±SE) of *Aquimarina* sp. Aq135 and *Vibrio* sp. EL41 in liquid single culture and co-culture over time during an assay with inoculation of *Vibrio* sp. EL41 24 hours after Aq135 inoculation. t= 0 hours (x-axis) corresponds to the moment of *Vibrio* inoculation in all cultures (equals 24 hours of *Aquimarina* sp. Aq135 growth).

A slight decrease in *Vibrio* CFU counts may indicate *Aquimarina* sp. Aq135 antagonistic activity.

Antimicrobial activity of co-culture extracts

Table 1: Growth inhibition halo dimensions (mm) of disc diffusion assays performed with microbiological discs containing 2 µg chloramphenicol (Chlor2) as positive control, 1:1 methanol-water solution (MeOH) as negative (solvent) control, and SPE extracts from culture broth supernatants obtained from *Aquimarina* sp. Aq135 single culture (Aq135). or co-culture of Aq135 with *Vibrio* sp. EL41 (Aq135+EL41) against human and aquaculture (fish) pathogens ***Streptococcus iniae*** and ***Vibrio parahaemolyticus***.

	S. iniae	*V. parahaemolyticus*
MeOH (-)	0	0
Chlor2 (+)	3.75±0.25	5.5±0.5
Aq135	4.75±0.25	0.5
Aq135+EL41	4.75±0.25	1

Solid Phase Extraction (SPE) extracts of supernatants of single cultures (Aq135) and co-cultures (Aq135+EL41) show **antimicrobial activity against both pathogens tested** here.

SPE extracts perform better against ***Streptococcus iniae***, a Gram-positive bacterium, than against the Gram-negative *Vibrio parahaemolyticus*.

Identity of metabolites present in SPE extracts?

Effects of co-culture on metabolite production?

Metabolomics

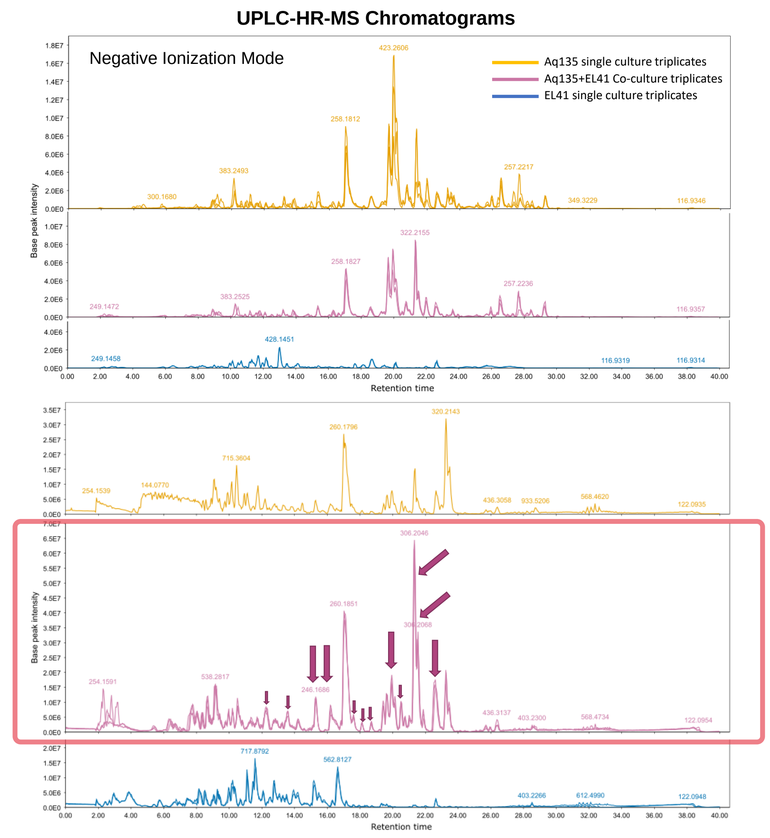


Fig. 3: UPLC-HR-MS chromatograms of culture extracts obtained in negative and positive ionization modes. Metabolomes of Aq135. EL41 and co-culture are displayed in the same chromatogram for each ion mode.

UPLC-HR-MS chromatograms show consistency between replicates as well as visible differences in peak profiles and peak intensities between different groups of samples for both negative and positive ionization modes.

Table 2: Examples of masses (compounds) detected in the untargeted UPLC-HR-MS metabolome profiling experiment that display positive fold-changes in peak area in co-culture opposed to both pure cultures. Aq135 and EL41.

Ionization Mode	Calc. MW	m/z	RT [min]	Area (Max.)	Reference Ion	Ratio: (Co-cult.) / (Aq135)	Ratio: (Co-cult.) / (EL41)	Log_2 Fold Change: (Co-cult.) / (Aq135)	Log_2 Fold Change: (Co-cult.) / (El41)
positive	305.1982	306.2054	21.539	306415537.5	[M+H]+1	10502.07	32245.987	13.36	14.98
positive	315.2383	316.2456	22.621	255059894.5	[M+H]+1	9282.898	13295.268	13.18	13.7
positive	259.176	260.1832	17.631	73861161.07	[M+H]+1	2658.277	1166.619	11.38	10.19
negative	323.2237	322.2165	21.33	83512335.78	[M-H]-1	4295.5	2732.897	12.07	11.42
negative	384.2399	383.2326	21.524	45086979.55	[M-H]-1	2665.517	19479.659	11.38	14.25
negative	286.2634	285.2561	29.671	1149934.47	[M-H]-1	1052.42	13.393	10.04	3.74
positive	1104.558	553.2863	9.21	162681774.29	[M+2H]+2	2488.322	2561.279	11.28	11.32
negative	2192.99	1095.488	12.075	546232.76	[M-2H]-2	57.728	59.835	5.85	5.9
positive	2485.128	829.3832	10.956	27003405.83	[M+3H]+3	832.424	89.621	9.7	6.49

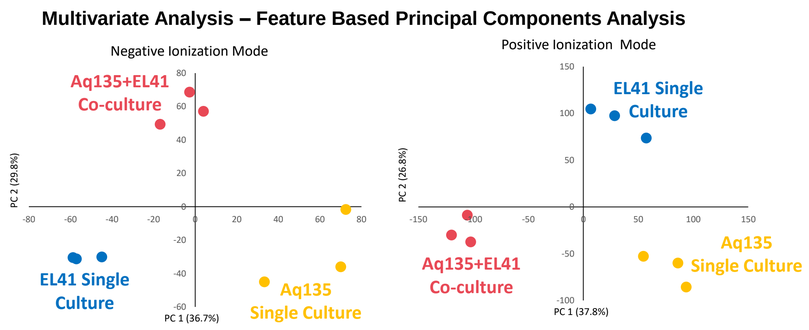


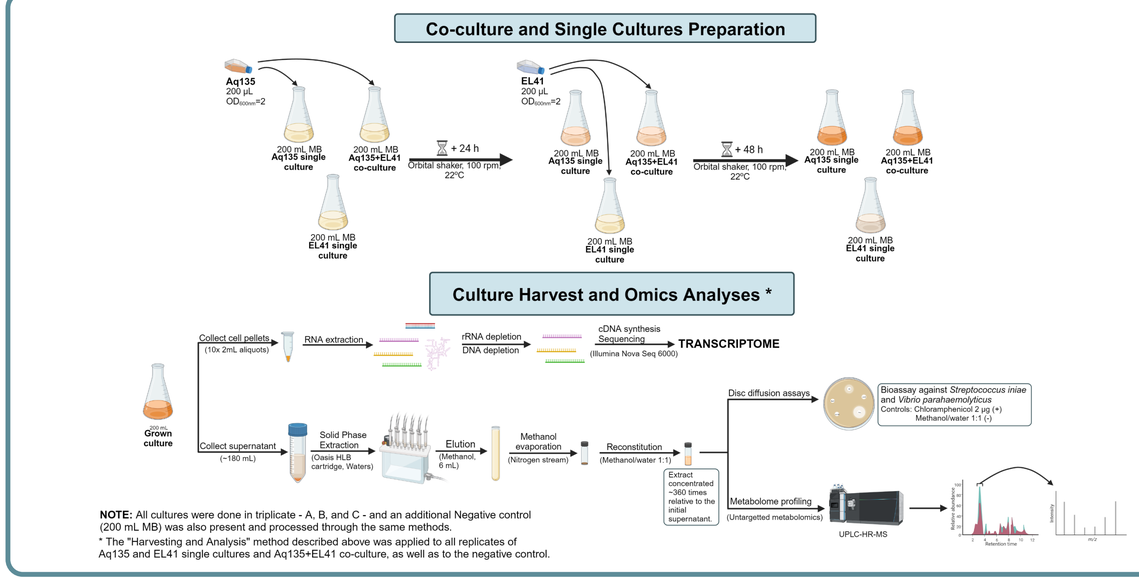
Fig. 4: Principal Components Analysis (PCA) of the metabolome profiles of the nine culture supernatant extracts, obtained in both ionization modes and based on peak area for all the features detected in the untargeted UPLC-HR-MS experiment.

Examples of masses (compounds, **Table 2**) with increased peak area in the co-cultures indicate increased production of certain, yet unidentified metabolites under co-culture conditions.

Future metabolomics analyses, such as molecular networking, will be employed to gain further insights on the relationship between features and compound classes present in different sample groups.

Principal components analysis based on all the chromatogram and mass features show separate clustering of sample groups, confirming a unique metabolome of the co-cultures.

Experimental Design



NOTE: All cultures were done in triplicate - A, B, and C - and an additional Negative control (200 mL MB) was also present and processed through the same methods.
* The "Harvesting and Analysis" method described above was applied to all replicates of Aq135 and EL41 single cultures and Aq135+EL41 co-culture, as well as to the negative control.

Conclusions

- Co-cultivation of marine *Aquimarina* sp. Aq135 with *Vibrio* sp. EL41 produces a metabolome different from the metabolomes of single cultures.
- This suggests that laboratory manipulation with naturally occurring bacterial competitors may induce activation of otherwise silent biosynthetic gene clusters in secondary metabolite-producing bacteria.
- Further metabolomic and transcriptomic analyses are ongoing to discern the differentially expressed genes and produced secondary metabolites of *Aquimarina* sp. under co-cultivation.

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