Aquimarina aquimarinae sp. nov. and *Aquimarina spinosulus* sp. nov., new bacterial species with versatile natural product biosynthesis potential

Joana F. Couceiro[1,2]

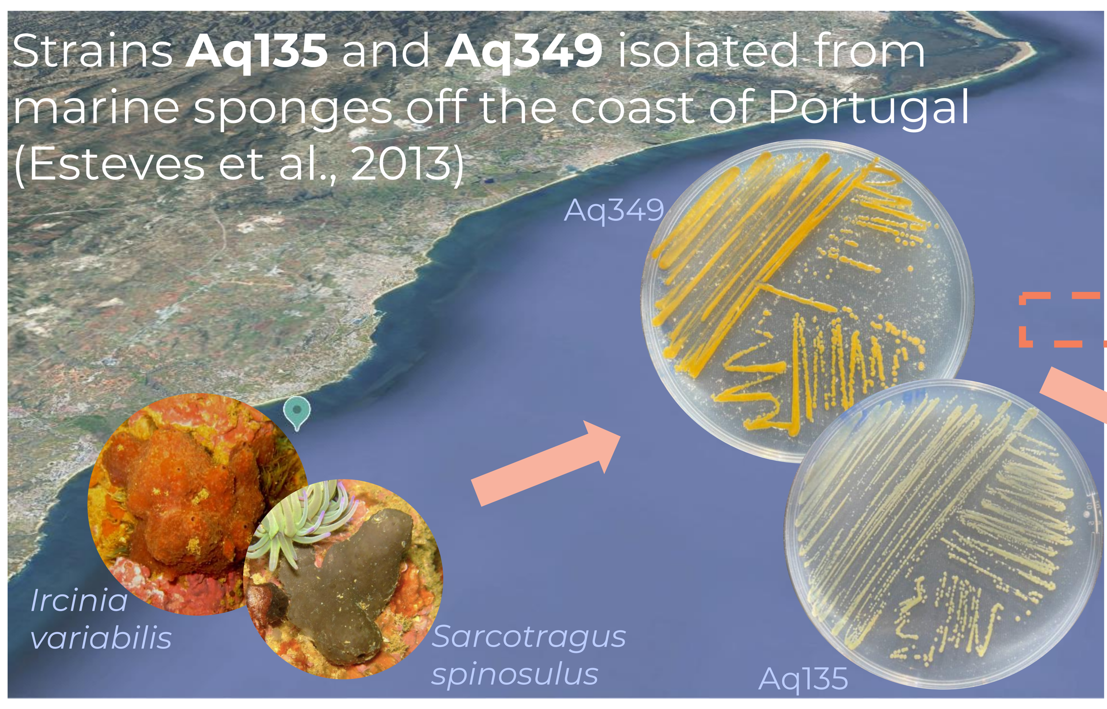
joana.couceiro@tecnico.ulisboa.pt

Supervisor(s): Tina Keller-Costa[1,2], Rodrigo Costa[1,2]

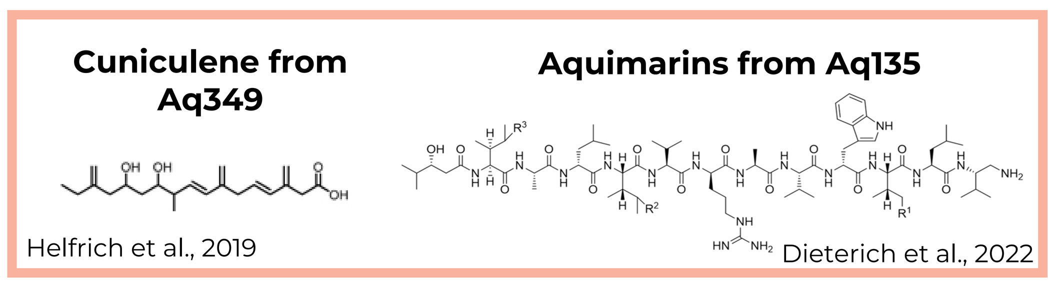
1. IBB—Institute for Bioengineering and Biosciences and i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisbon, Portugal
2. Bioengeneering Department, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisbon, Portugal

MIT Portugal
2022 Annual Conference

Introduction



We here describe two novel species in the *Aquimarina* genus *A. aquimarinae* and *A. spinosulus*, known to produce the secondary metabolites aquimarins and cuniculene, respectively



27 different *Aquimarina* species are already described

Free-living (FL): Seawater, Marine Sediments, Saltpans

Host-Associated: Red Algae, Mussels, Sea Urchins, Pacific White Shrimp, Marine Sponges

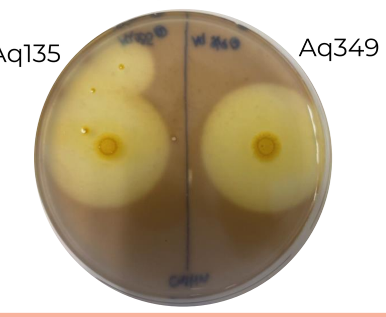
Characterization of *A. aquimarinae* Aq135 and *A. spinosulus* Aq349

Functional (Pfam-based) genome annotation highlights features of the *Aquimarina* genus

	Aquimarina aquimarinae Aq135	*Aquimarina spinosulus* Aq349	*Aquimarina macrocephali* JAMB N27[T]	*Aquimarina megaterium* XH134[T]	*Aquimarina sediminis* w01[T]
Glycosyl hydrolases					
GH1, GH2, GH3 (8 Pfams) β-glucosidases and β-galactosidases	7	13	11	15	17
GH5, GH6*, GH9, GH18, GH48 (5 Pfams) cellulases and chitinases	8	11	8	14	6
GH16 β-agarases and κ-carrageenases	1	0	0	0	0
GH20 (2 Pfams) N-acetylglucosaminidases	4	6	6	6	12
GH25 Lysozymes	1	1	1	1	1
GH31, GH63, GH97 (5 Pfams) α-glucosidases and α-galactosidases	2	6	6	6	5
GH43 arabinases and xylosidases	0	0	1	1	1
GH53 galactanases	0	0	0	1	0
GH65 (3 Pfams) phosphorylases	3	3	3	3	4
GH76, GH92, GH99 (4 Pfams) α-mannanases and α-mannosidases	5	13	14	13	13
GH115 α-glucuronidases	1	0	0	0	0
Chitin					
Chitin synthase	1	1	1	1	0
Chitinase class I	2	1	1	1	1
Compound resistance					
Bacitracin resistance protein BacA	1	1	1	1	1
CrcB-like protein, Camphor Resistance (CrcB)	1	1	1	1	1
Erythromycin esterase	1	1	0	1	0
Glyoxalase/Bleomycin resistance protein/Dioxygenase superfamily (2 Pfams)	14	17	15	20	20
MerC mercury resistance protein	1	2	2	1	1
Tellurite resistance (2 Pfams)	7	7	7	7	7
Tetracyclin repressor-like, C-terminal domain (4 Pfams)	8	4	5	4	3
Copper resistance protein D	0	0	0	0	1
Putative small multi-drug export protein	1	1	1	1	1

Versatile carbon metabolism
For example, cellulase, agarase, N-acetylglucosaminidase and chitinase encoding genes

A. aquimarinae and ***A. spinosulus*** possess **chitinase genes** and are both able to **degrade chitin**, a key feature governing the cycling of C and N in marine ecosystems.



Compound resistance
For instance, potential resistance to tellurite (a trait usually encoded in plasmids), and other heavy metals as well as antibiotics

Biochemical characterization – traits that distinguish ***A. aquimarinae*** and ***A. spinosulus***

	Aquimarina aquimarinae Aq135	*Aquimarina spinosulus* Aq349	*Aquimarina macrocephali* JAMB N27[T]	*Aquimarina megaterium* XH134[T]	*Aquimarina sediminis* w01[T]
Colony colour	yellow	yellow-orange	orange	orange	orange
Growth Temperature Range (°C)	4 - 30	4 - 30	8 - 30	8 - 37	4 - 37
Growth with NaCl range (%)	1 - 5	1 - 5	1 - 4	2 - 4	2 - 5
Flexirubin-type pigments	+	+	NA	+	-
Biofilm formation	+	+	NA	NA	+
Gliding motility	+	+	+	+	+
Nitrate reduction	-	-	-	+	+
Enzymes					
Catalase	+	-	+	+	+
β-galactosidase	-	+	+	-	NA
Utilization of:					
Starch	+	+	+	+	+
Mannose	-	-	+	-	NA
Maltose	-	-	+	NA	NA
Citrate	-	-	NA	+	NA
Antibiotic Resistance					
Ampicillin	S	S	R	R	R
Gentamicin	R	R	R	R	R
Tetracycline	S	S	R	R	R
Vancomycin	S	S	S	S	S
Erythromycin	S	S	S	S	S
Kanamycin	R	R	R	R	R
Chloramphenicol	S	S	R	S	S

A. aquimarinae* & *A. spinosulus display **gliding motility**, which is indeed a common feature of *Aquimarina spp.*

The **new species, *A. aquimarinae*** and ***A. spinosulus,*** are distinguished from closely related *Aquimarina* species by the:

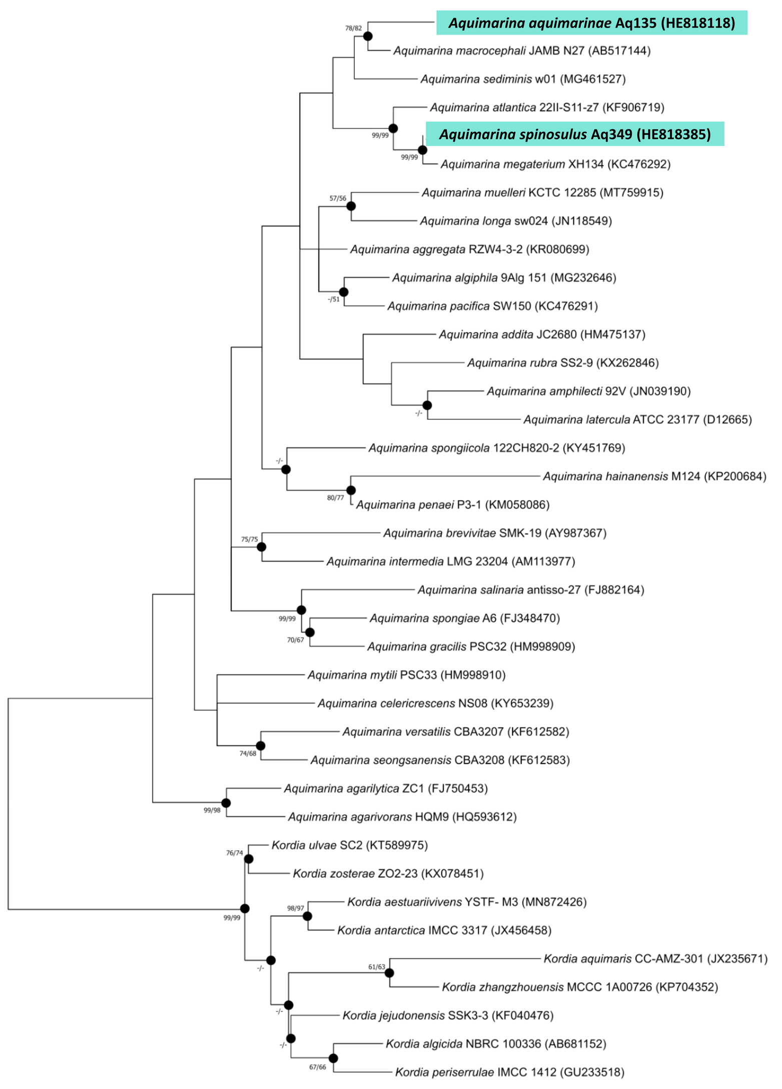
i) **inability to reduce nitrate**,

ii) **absence of catalase and β-galactosidase activity** in Aq349 and Aq135, respectively;

iii) **susceptibility to ampicillin and tetracycline;**

iv) **different temperature and salinity ranges for growth**.

Phylogenetic (based on 16S rRNA genes) and **Phylogenomic inferences** and genome-wide **Average Nucleotide Identity** (ANI) values

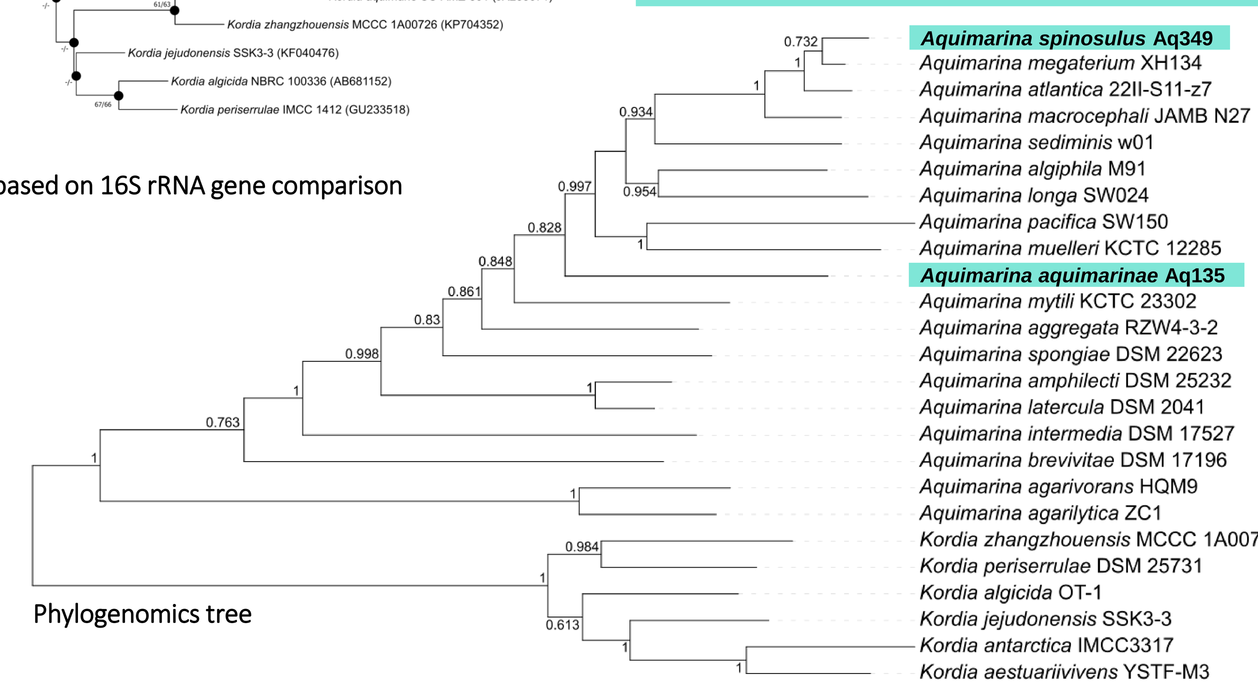


Phylogenetic tree based on 16S rRNA gene comparison

Query genome	Reference genome	DGGC* DDH**	DGGC* Prob. DDH >= 70%	DGGC* ΔG+C [%]	FastANI (KBase) ANI [%]	FastANI (KBase) AF [%]
A. aquimarinae Aq135	*Aquimarina* sp. Aq349	22.5	0	0.52	79.8736	48.12
A. aquimarinae Aq135	*Aquimarina macrocephali* JAMB_N27[T]	22.6	0	0.46	79.826	45.73
A. aquimarinae Aq135	*Aquimarina megaterium* XH134[T]	22.5	0	0.46	79.8365	47.25
A. aquimarinae Aq135	*Aquimarina sediminis* w01[T]	21.6	0	0.81	79.3314	42.09
A. spinosulus Aq349	*Aquimarina atlantica* 22II-S11-z7[T]	19.90	0	1.02	91.4312	78.15
A. spinosulus Aq349	*Aquimarina macrocephali* JAMB_N27[T]	41.00	3.5	0.05	90.4194	76.52
A. spinosulus Aq349	*Aquimarina megaterium* XH134[T]	53.90	31.23	0.05	93.6519	81.09

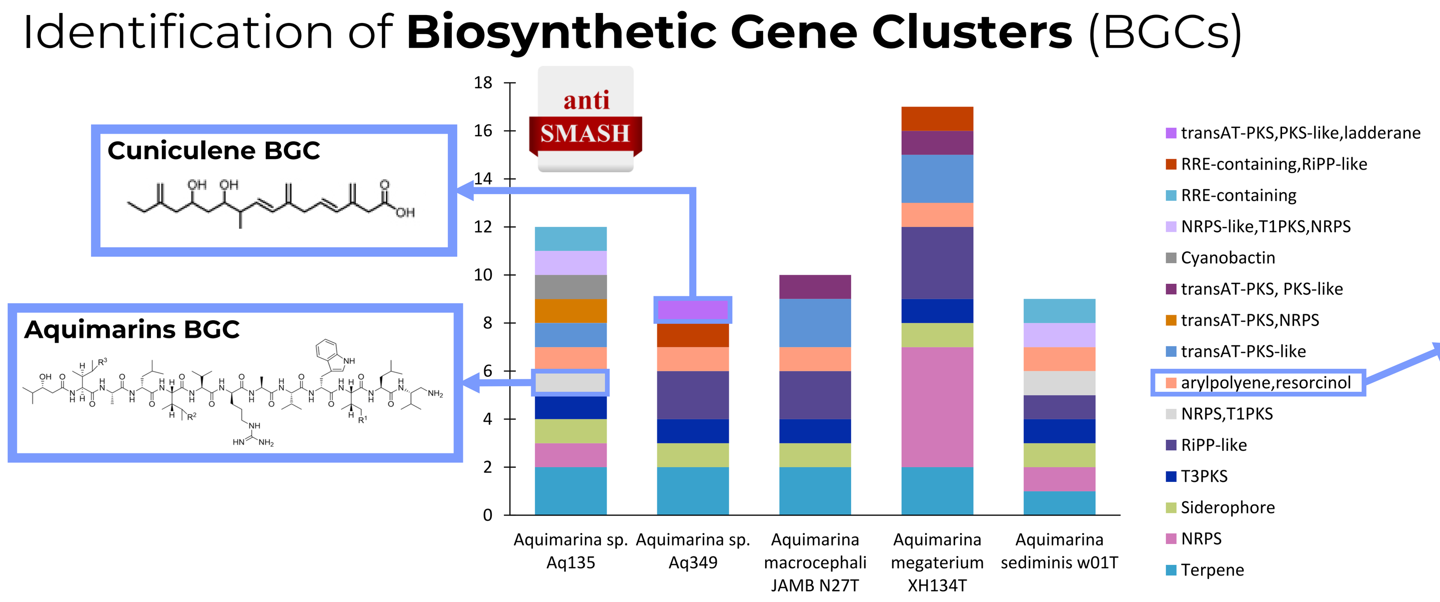
*DGGC- Genome-to-Genome Distance Calculator | **DDH - digital DNA-DNA hybridization

16S rRNA gene phylogeny showed that the closest type strains *A. aquimarinae* Aq135 are ***A. macrocephali* (97.84%)** and ***A. sediminis* (97.05%)** whereas *A. spinosulus* Aq349 is closely related to ***A. atlantica* (98.06%)** and ***A. megaterium* (99.21%)**.
While 16S rRNA gene similarity values were above the species-level threshold, genome-wide analysis revealed that both *A. aquimarinae* Aq135 and *A. spinosulus* Aq349 exhibit **ANI scores below the species level cut-off (95%)** for all described *Aquimarina* type species.

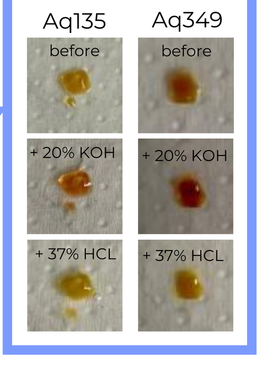


Phylogenomics tree

Identification of **Biosynthetic Gene Clusters** (BGCs)



Flexirubin BGC
Lab tests show: Both species produce flexirubin pigments



References:
1. Esteves AI, Hardoim CC, Xavier JR, Gonçalves JM, Costa R. Molecular richness and biotechnological potential of bacteria cultured from Irciniidae sponges in the north-east Atlantic. FEMS Microbiol Ecol. 2013 Sep;85(3):519-36. doi: 10.1111/1574-6941.12140. Epub 2013 May 20. PMID: 23621863.
2. Dieterich CL, Probst SI, Ueoka R, Sandu I, Schäfle D, Molin MD, Minas HA, Costa R, Oxenius A, Sander P, Piel J. Aquimarins, Peptide Antibiotics with Amino-Modified C-Termini from a Sponge-Derived Aquimarina sp. Bacterium. Angew Chem Int Ed Engl. 2022 Feb 14;61(8):e202115802. doi: 10.1002/anie.202115802. Epub 2021 Dec 28. PMID: 34918870.
3. Helfrich EJN, Ueoka R, Dolev A, et al. Automated structure prediction of trans-acyltransferase polyketide synthase products. Nature Chemical Biology. 2019 Aug;15(8):813-821. DOI: 10.1038/s41589-019-0313-7. PMID: 31308532; PMCID: PMC6642696.

Funded by:

FCT Fundação para a Ciência e a Tecnologia | MIT Portugal | fundoazul | bsrg biological sciences research group | iBB Institute for Bioengineering and Biosciences | TÉCNICO LISBOA

under the Doctoral Grant PRT/BD/153503/2021 | Earth Systems: Oceans to Near Space