Investigating the metabolic potential of the rare actinomycete genus *Actinospica*

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Introduction

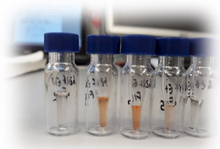
Natural products derived from micro-organisms represent an incomparable source of chemical novelty, and the structural and chemical diversity which can be obtained is greater when genetically diverse strains are considered. Our collection of 45.000 actinomycetes is enriched in uncommon genera, and in this work we focused on the genus *Actinospica*, which we described in 2006[1]. Our early finding of NRPS and PKS genes in *Actinospica* strains[2] and the presence in the genome of *A. robiniae* (NZ_KI632511.1) of >20 genetic clusters related to secondary metabolism suggest a potential to produce bioactive metabolites. We therefore investigated the diversity and metabolic potential of 22 isolates derived from our strain library.

Workflow

1. Strains grown on plates

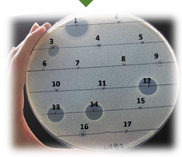
2. Flask cultures

3. EtOH (mycelium) or SPE (cleared broth) extracts



4a. LC/MS analysis

4b. Bioassay

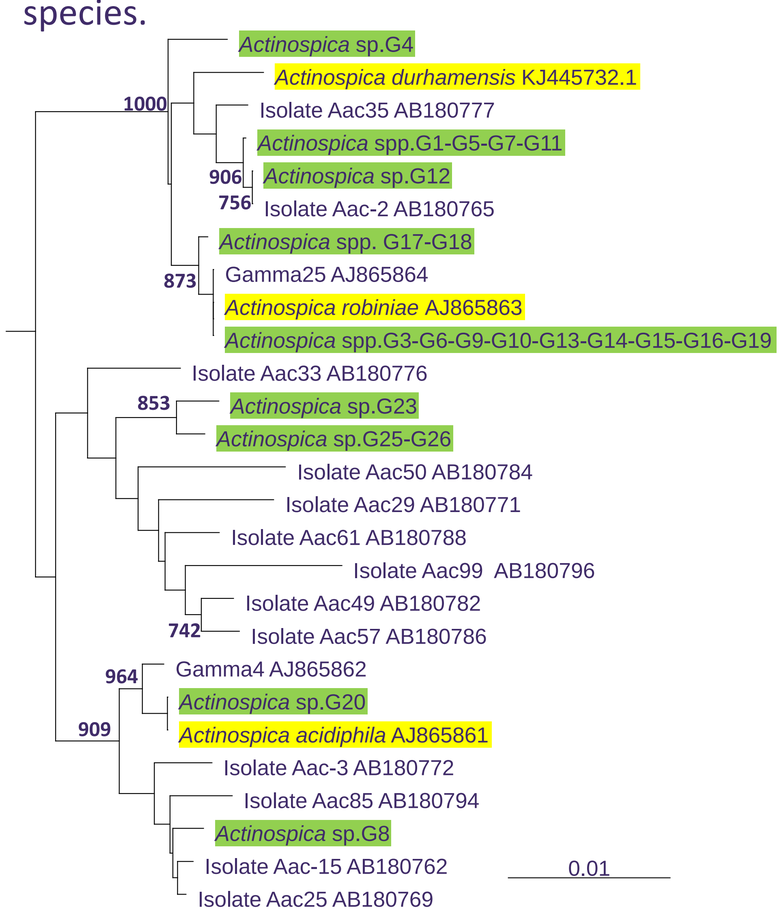


GNPS

5. Metabolite clusterization based on MS/MS data[3]

Strain Diversity

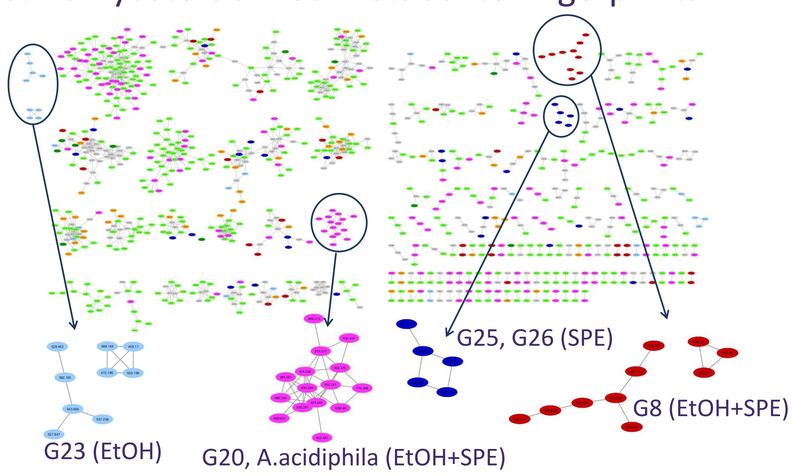
Based on 16S phylogeny, the isolates represent different lines of descent within *Actinospica*, including some distantly related to described species.



Neighbor-Joining tree based on 16S phylogeny showing the placement of the investigated strains (green) in comparison with described species (yellow) and with GenBank sequence entries deriving from undescribed isolates. Bootstrap values (1000 replicates) >70% are shown at nodes. Scale bar: 1 inferred substitution per 100 nt.

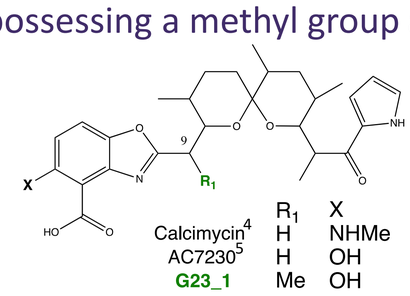
Molecular Networking

MS/MS based clustering coupled with 16S data can single out strain-specific metabolites (i.e., deriving from a single strain or strains with identical 16S sequence), absent in a reference database of actinomycete-derived metabolite fingerprints.



Metabolites from strain G23

Activity in the mycelium extract was due to the new Calcimycin-like metabolite **1** as the major component. Despite the numerous representatives of this class, metabolite **1** from *Actinospica* is the first one possessing a methyl group at position 9.



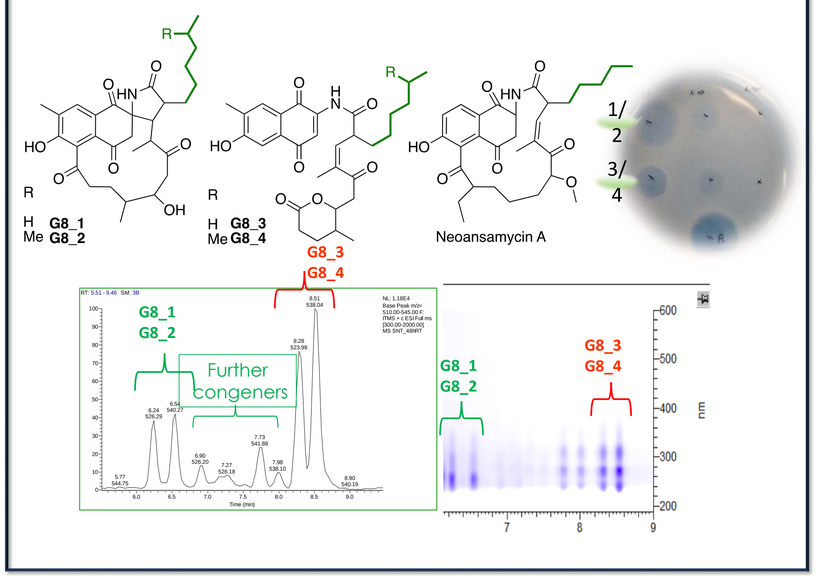
	R_1	X
Calcimycin[4]	H	NHMe
AC7230[5]	H	OH
G23_1	Me	OH

Bioactivity

Agar-diffusion tests showed that extracts from four strains (G8, G23, G25 and G26) were active *vs S.aureus*. The active extracts from strains G8 and G23 were also highlighted by GNPS analysis as harboring unique metabolites. Thus, the bioactive metabolites were purified and characterized.

Metabolites from strain G8

The bioactivity was associated to a complex of up to 16 related metabolites. Structure elucidation of the most stable and abundant congeners revealed new ansamycin-like metabolites characterized by a linear or branched lipophilic chain, longer than that observed so far in this class. Compounds **1**-**2** appear more active than **3**-**4**.



Conclusions

Molecular networking evidenced unique metabolites, which were confirmed to be structurally novel. With just a few analyzed strains, the genus *Actinospica* bears promise for novel metabolites.

Acknowledgements

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References

1. Cavaletti *et al.* (2006) *Int J Syst Evol Microbiol* 56, 1747-1753; 2. Busti *et al.*(2006) *Microbiology* 152, 675-683; 3. Wang *et al*. (2016) *Nature Biotechnol* 34, 828-837; 4. Wu *et al.* (2011) *Antimicrobial Ag Chemother* 55, 974-982; 5. Yaginuma *et al.* (1987) *J Antibiotics* 40, 239-241.