

Chemodiversity and bioactive natural products from deep-sea-sourced *Streptomyces* (*Actinomycetota*, *Kitasatosporales*, *Streptomycetaceae*) strains

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INTRODUCTION & AIM

Marine environments harbour a wealth of diverse and underexplored microbiota, including species of the actinobacterial genus *Streptomyces* (*Actinomycetota*)—remarkably prolific producers of diverse natural products with unique bioactivities and high potential for drug discovery and other applications.

In our study, we explored liquid culture supernatants from *Streptomyces* strains sourced from deep-sea environments, corresponding to different species, aiming to uncover their chemical diversity and potentially identify valuable natural products. We followed a multi-step extraction procedure involving liquid–liquid and sorbent-assisted extraction steps. Leveraging a high-throughput UPLC–HRMS dereplication workflow, we employed feature-based molecular networking (FBMN) and cheminformatic approaches to obtain insights from complex spectral datasets. Our aim was to identify the chemical classes of compounds through chemical dereplication, emphasizing both known and potentially novel metabolites in the extracts. Additionally, we sought to visualise the differences in chemical profiles among the various strains/species. We fractionated the extracts, performed general and targeted TLC assays, and structurally elucidated compounds via HRMS and NMR spectroscopy, guided by the molecular networking-based dereplication.

METHOD

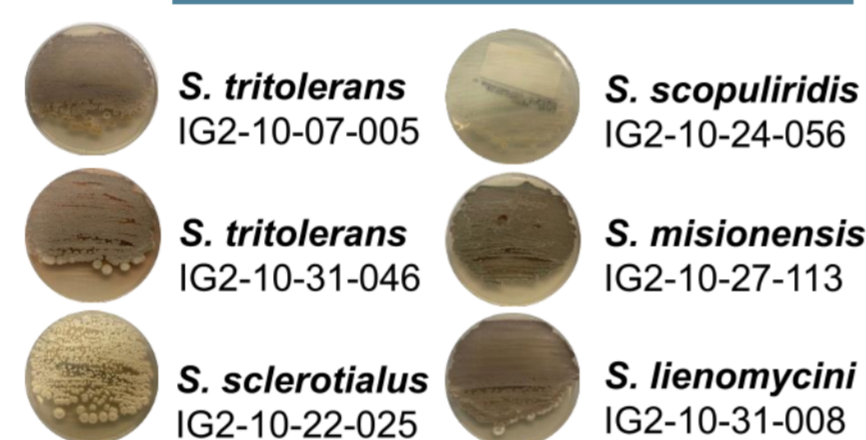
Supernatants from scaled-up liquid cultures (900 mL) of the deep-sea-derived *Streptomyces* strains, with the corresponding controls (culture media), were provided by PharmaMar*.

The strains chosen to be presented here are shown on the right.

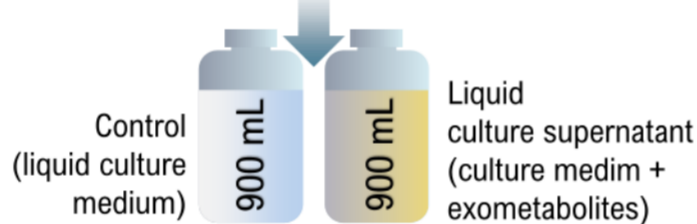
*PharmaMar undertook the isolation and identification of deep-sea bacteria from marine sediments collected at depths between 500 and 3000 meters in 2010.

This sampling was conducted by the Instituto Geológico y Minero de España – Consejo Superior de Investigaciones Científicas (IGME – CSIC), a Spanish governmental organization, during an exploration of submarine volcanoes in the Canary Islands and the Gulf of Cadiz.

A. MICROBIAL MATERIAL

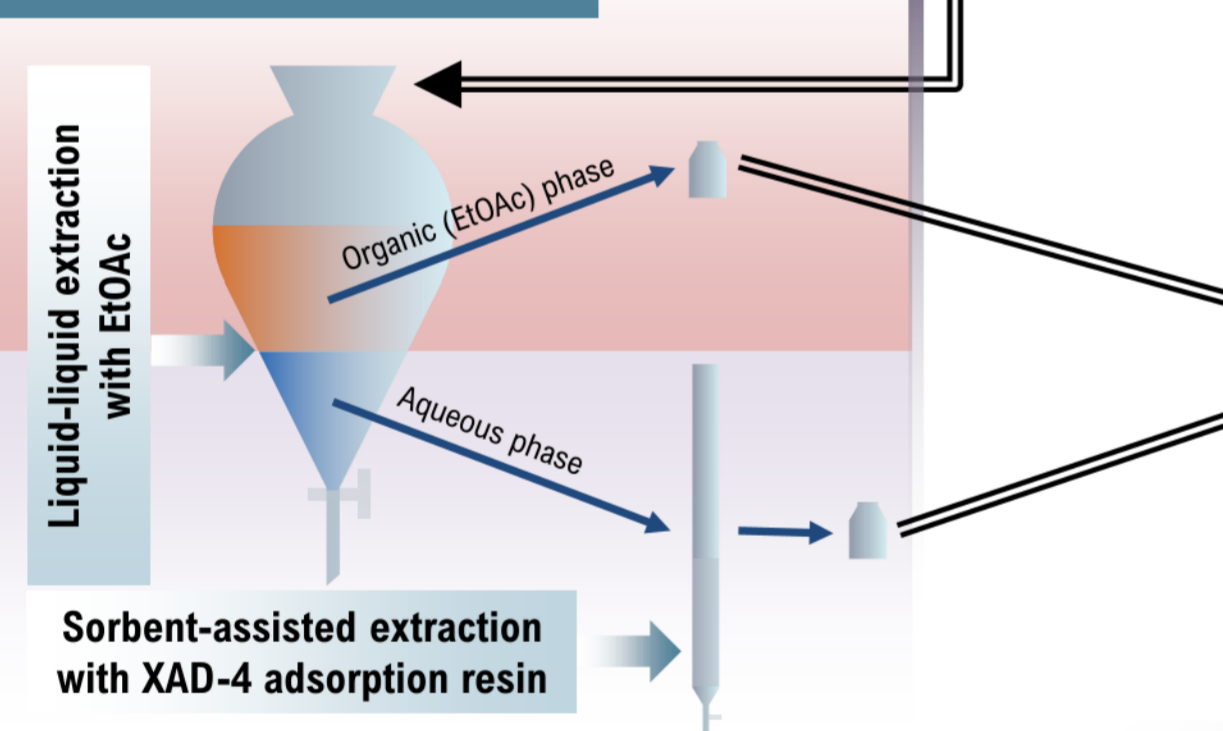


Scaled-up liquid cultures

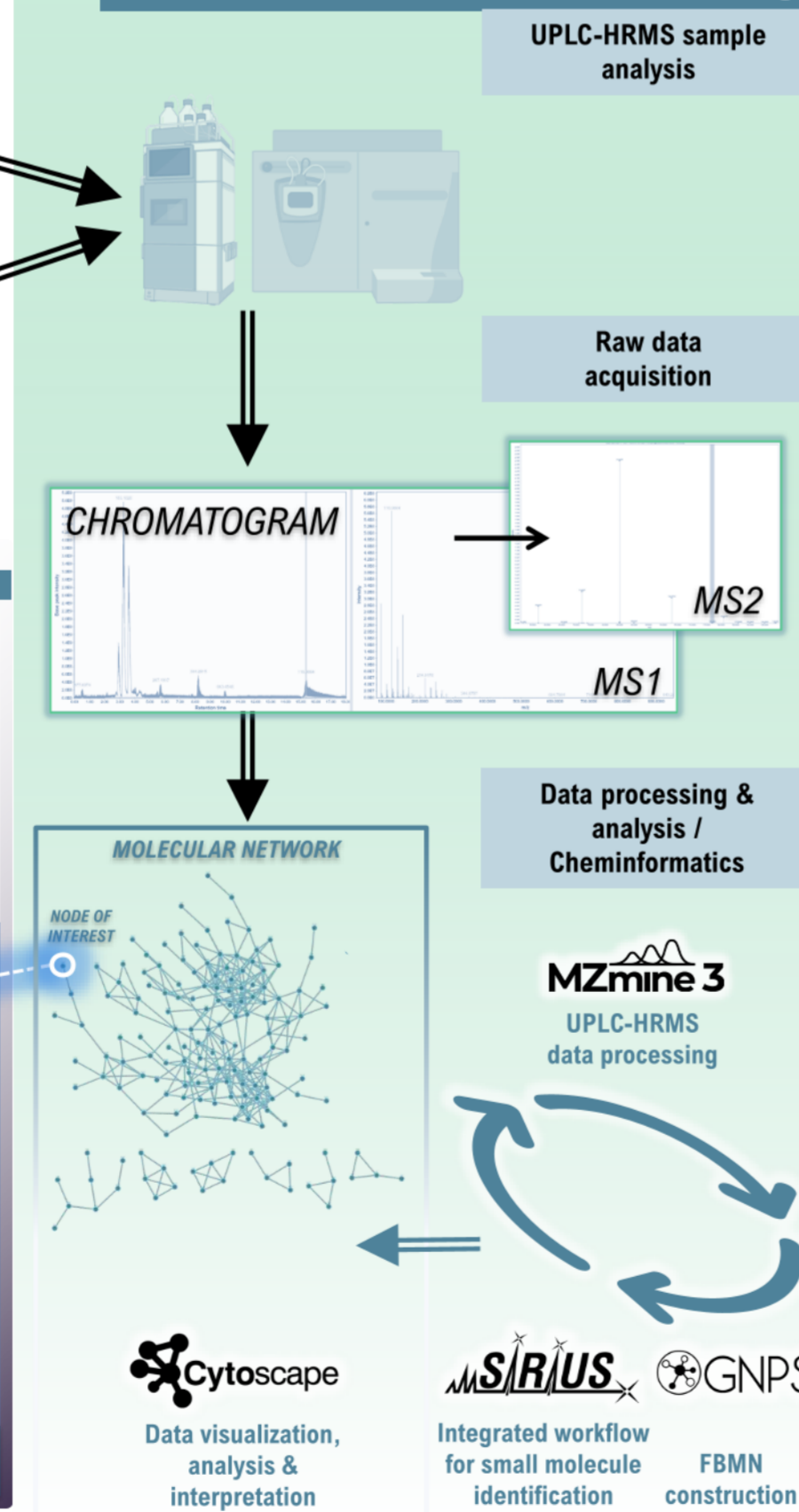


B. ANALYTICAL PART

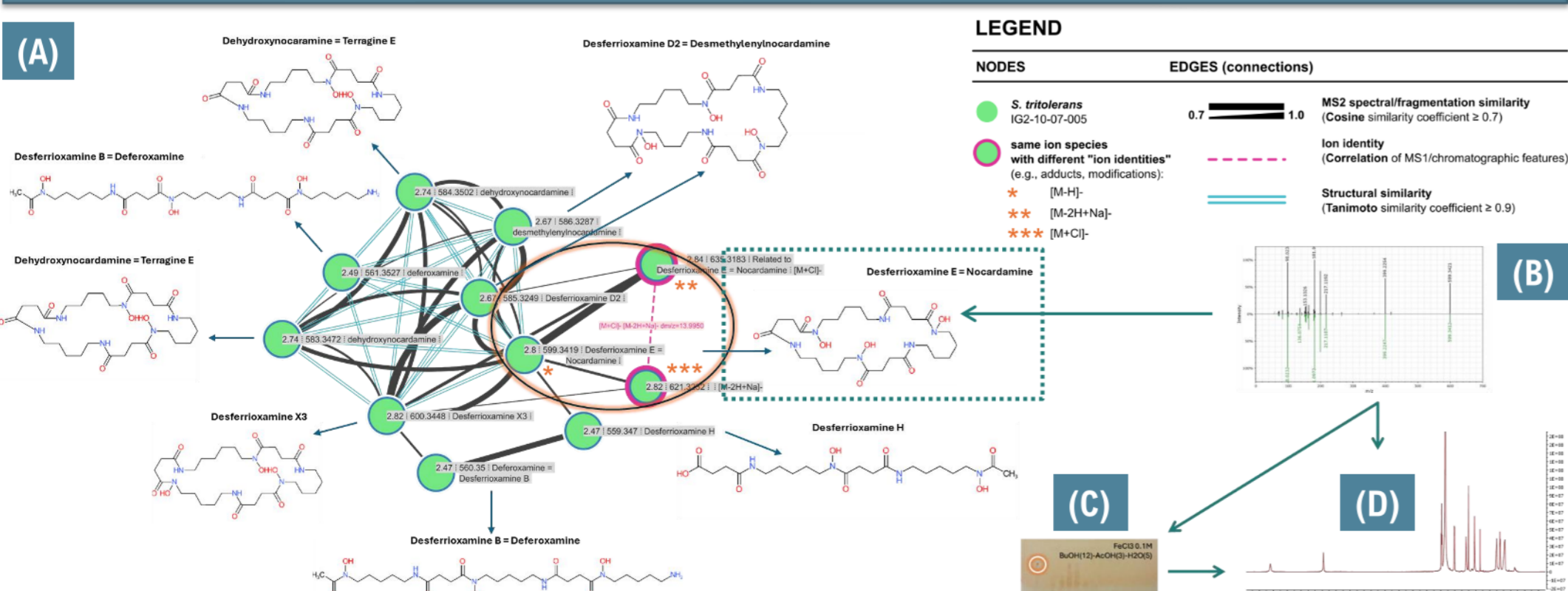
I. Extraction



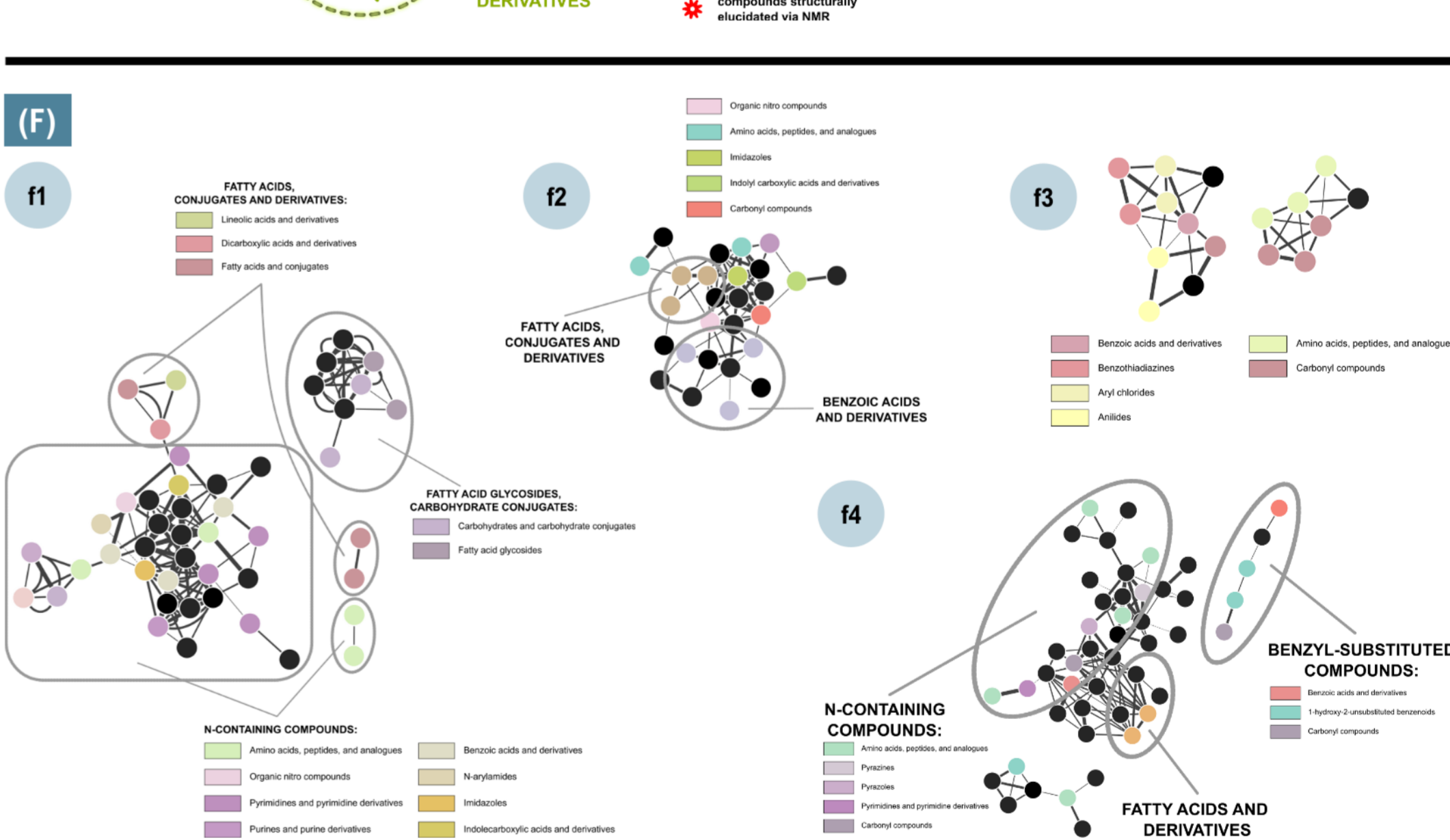
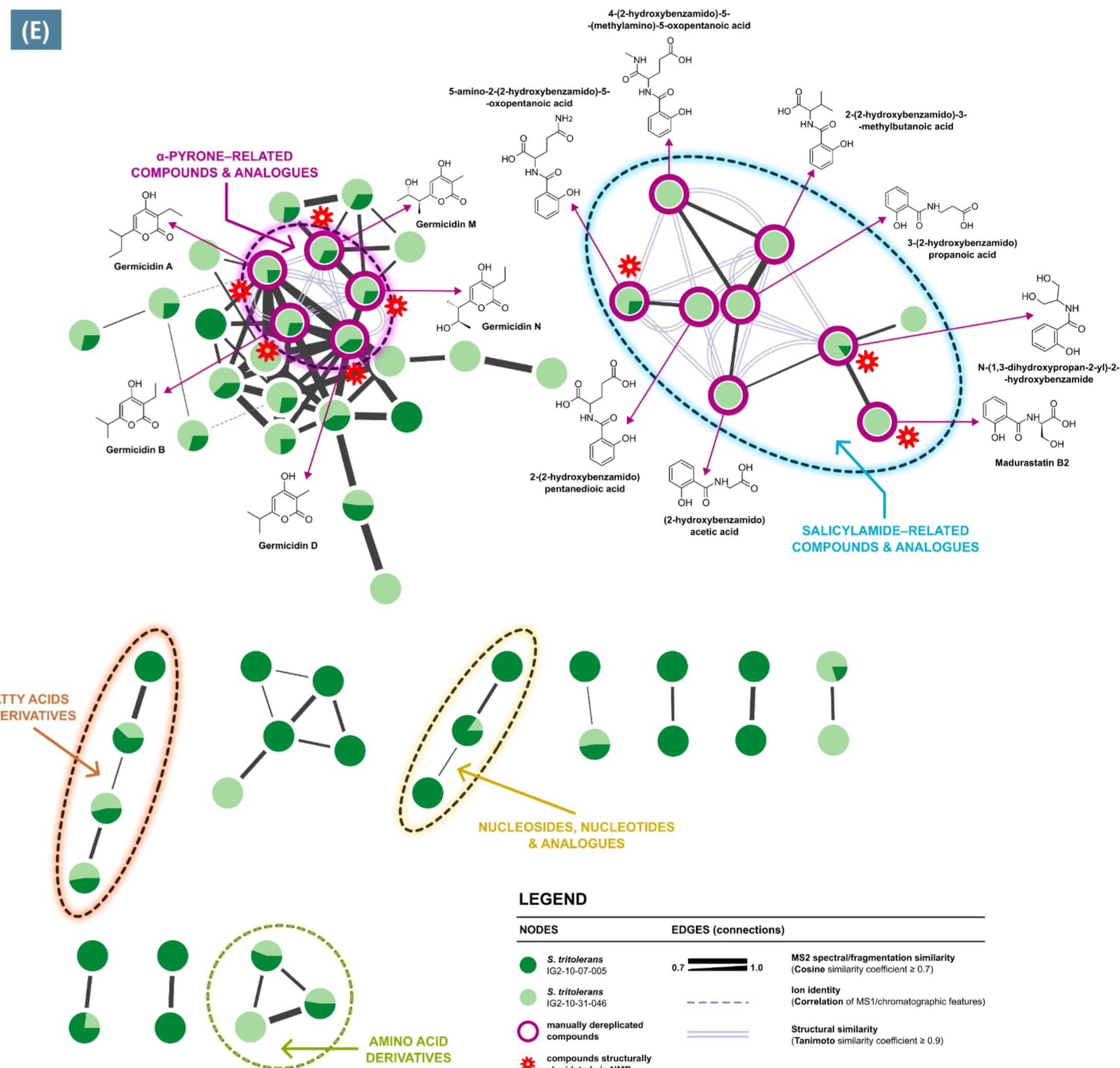
II. Cheminformatics / Dereplication / Molecular networking



RESULTS & DISCUSSION



RESULTS & DISCUSSION (cont.)



▲ FIGURE 2. FBMN of EtOAc extracts derived from liquid culture supernatants of *Streptomyces* strains/species; mass spectral data were acquired in HRMS-ESI(–) mode. **(E)** Comparative FBMN of two different *S. tritolerans* strains, grown in the same culture medium. **(F)** FBMN of different *Streptomyces* species, showing varied chemical profiles: **f1**: *S. sclerotialis*; **f2**: *S. scopuliridis*; **f3**: *S. misionensis*; **f4**: *S. lienomycini*.

The annotated chemical categories shown were predicted using the robust SIRIUS environment, by employing CANPUS algorithm. For clarity only significant clusters and annotations are presented (with CANOPUS chemical subclass probability > 0.7).

◀ FIGURE 1. Chemical dereplication and structural elucidation workflow—proof of concept. **(A)** FBMN of *S. tritolerans* IG2-10-07-005 XAD-4-derived extract, showing a cluster of linear and cyclic hydroxamate siderophores (hydroxamic acid derivatives). **(B)** Spectral library match of Desferrioxamine E (Nocardamine); cosine score > 0.95. **(C)** TLC positive FeCl₃ reaction for hydroxamate siderophores. **(D)** ¹H NMR spectrum of Desferrioxamine E (Nocardamine) from a fraction of *S. tritolerans* IG2-10-07-005 extract.

CONCLUSION

- The marine-derived *Streptomyces* strains exhibited diverse chemical profiles, both within and among species.
- Key identified compounds included derivatives of hydroxamic acids, salicylamides, and α-pyrones, all with various bioactivities, the former two categories being siderophores. Other potentially bioactive compounds were also annotated.
- Our analytical workflow, enhanced by cheminformatics, provided insights into the chemical diversity of the extracts and highlighted promising compounds.

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