Title
Antibiotic Drug Discovery from the New Marine Actinomycete Genus Marinomyces

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Publication Date
2007-07-19

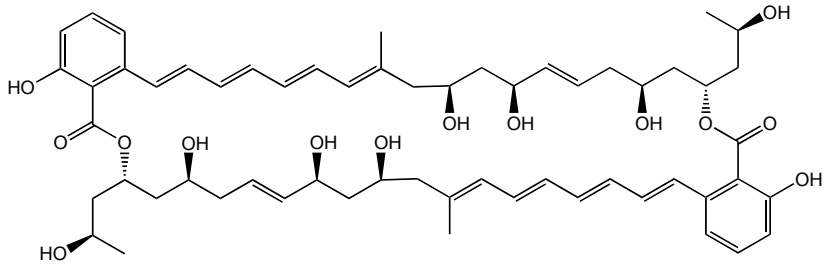
Post Award Narrative

Actinomycetes are high G+C content Gram-positive bacteria with an unparalleled ability to produce diverse secondary metabolites. These bacteria, which are best known from soils, have been studied extensively by the pharmaceutical industry and account for a disproportionately large amount of the $25.3 billion annual global sales of microbially derived pharmaceuticals. In recent years however, the yield of new lead compounds from common soil-derived actinomycetes has diminished significantly, thus providing incentive to broaden the search for new metabolites to include actinomycetes that occur in the sea. The goals of this proposal were to gain a better understanding of actinomycete diversity in the ocean and their ability to produce unique secondary metabolites. The approach taken was to develop cultivation techniques specific for marine actinomycetes and to assess the diversity of cultured strains using molecular systematics. Representatives of various taxonomic groups were then cultured, extracted, and the extracts assessed for biological activity and the presence of novel secondary metabolites. Special efforts were made to focus on a unigue group of chemically prolific marine actinomycetes that we discovered and called MAR2 or "*Marinospora*".

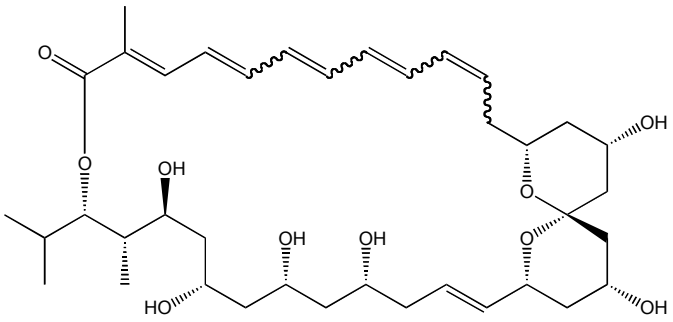
Much of the research performed as part of this program centered on an expedition to the islands of Palau in 2004. This trip led to the cultivation of 1624 Gram-positive bacteria of which more than 80% were actinomycetes. These strains spanned 22 Families and included at least 78 operational taxonomic units of which 29 appear to be new taxa. The results of this study were recently published (Gontang et al., 2007) and represent the foundation of the thesis of the Sea Grant trainee funded by this project. It is clear from this work that the ocean's harbor considerable new actinomycete diversity and that these bacteria have tremendous potential as a source of novel secondary metabolites.

Among the bacteria cultured from Palau include three MAR2 strains. Seventeen additional MAR2 strains were cultured from other samples raising the total in the collection from seven at the start of the grant to 26. These strains are the subject of ongoing chemical investigations that have already led to the publication of a significant new group of polyene antibiotics, the marinomycins (Kwon et al., 2006). In addition, a manuscript has been submitted describing a second new group of metabolites from MAR2 strains called the marinosporolides (Kwon et al.,

submitted). The structures of these compounds are presented in figure 1.



Marinomycin A



Marinosporolide

Figure 1. Novel secondary metabolites isolated from MAR2 "*Marinospora*" strains.

The results of this program have improved our understanding of the phylogenetic diversity of the MAR2 group. A tree is presented in figure 2 that includes nearly full-length 16S rRNA gene sequences for 22 of the 26 strains that have been cultured to date. This group includes considerable phylogenetic diversity and exemplifies the need for a taxonomic revision of the genus *Streptomyces*, which clearly is comprised of multiple genera. One particularly interesting observation is that phylogenetically similar strains produce the same chemistry regardless of their origin. For example, the three strains in the top of the figure all produce marinomycin A yet they originate from San Diego (CNQ-140) and Palau (CNJ-962 and CNJ-923). This follows a pattern we recently reported for *Salinispora* strains (Jensen et al., 2007) and reveals that phylogenetic novelty is more important than the geographic origin of the strain. Thus, efforts should be focused on developing new cultivation methods or sampling new niches as

opposed to traveling to remote sites to collect uniform sample types.

Finally, the strains cultured as part of the Palau diversity study have provided an opportunity to re-address the approaches we typically apply to natural product discovery. More specifically, we have developed a phylogenetic approach to assess the biosynthetic richness and novelty of individual strains. This is accomplished by performing phylogenetic analyses on PCR amplified keto-synthase domains from modular polyketide synthase complexes. Using this approach, it was possible to predict that one of the actinomycetes cultured from Palau (CNR-925) would produce the macrolide tetronomycin, as the KS domains from this strain clustered closely with those previously reported for this compound. The Sea Grant trainee working on this project was then able to experimentally confirm that strain CNR-925 produces this compound thus demonstrating that this method works and can be used to prescreen strains for the production of known compounds. The application of this technique has the potential to dramatically improve the rate with which new secondary metabolites, including antibiotics and anti-cancer agents, are discovered.

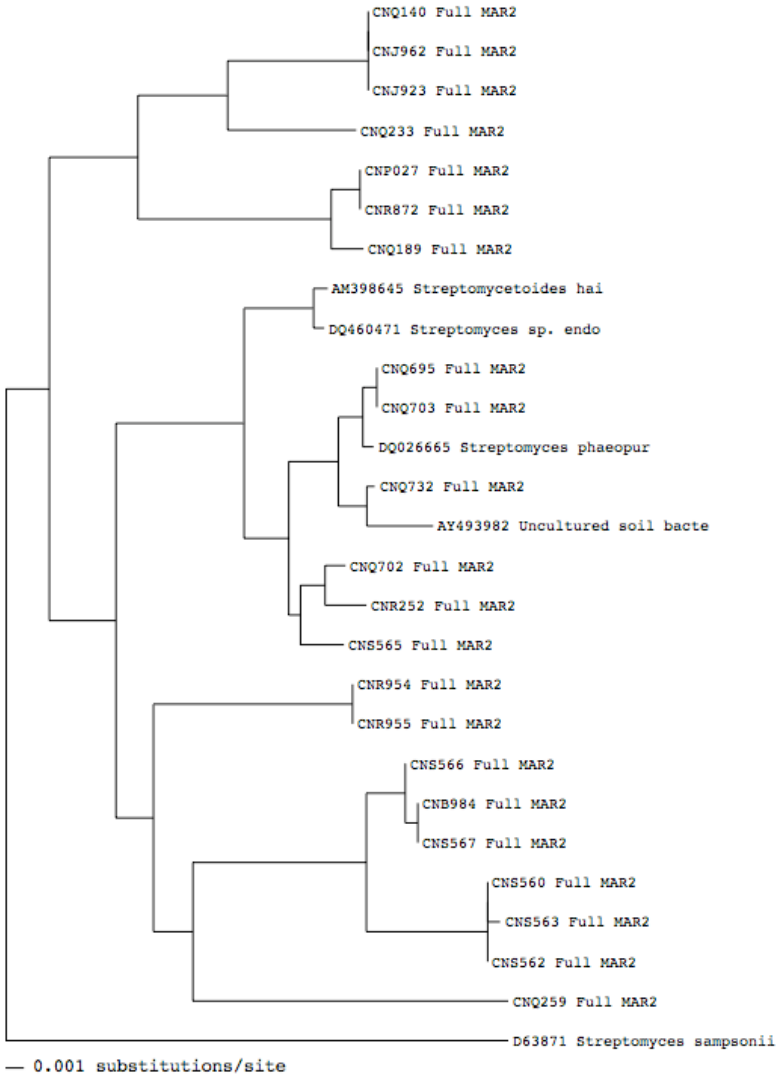


Figure 2. 16S rRNA gene phylogeny of the MAR2 strains (indicated by CN number).

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