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Unexpected Properties of Micromonosporae from Marine Origin

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Abstract

Members of the genus *Micromonospora* show a complex life cycle which normally involves the presence of substrate or vegetative mycelia and sporulation with single spores born on the vegetative hyphae followed by the synthesis of a dark extracellular polysaccharide. Bergey's Manual states that micromonosporae rarely produces aerial mycelia (AM) and if so, is considered "*sterile*". During the characterisation of novel micromonosporae from the Sea of Cortes, it was observed that AM is produced reproducibly in the presence of certain carbon and/or nitrogen sources. Micromanipulation of the AM subcultured onto fresh media produced colonies; hence, this structure should not be called "*sterile*". TEM of the AM producing isolates suggests that the spores also show activity as reported for bacilli of marine origin. This would be the first report of the presence of "inducible" AM in micromonosporae of marine sources and that the spores of this genus have a role other than just dispersal.

Keywords

Actinobacteria, Actinomycetes, Aerial Mycelia, *Micromonospora*, Spores

The genus *Micromonospora* is the type genus of the family *Micromonosporaceae* and, after *Streptomyces*, is still regarded as a good source of bioactive compounds [1] [2]. Among the *Actinobacteria*, that is Gram Positive bacteria with a high GC content, *Micromonospora* are ubiquitous in aquatic environments but their distribution and specific role is poorly understood. Because of their economic importance, the best studied life cycle is that of the genus *Streptomyces*. In streptomycetes, a single spore will germinate and give rise to a colony which will

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produce two but related complex structures: (a) the substrate or vegetative and (b) the aerial mycelia (AM), the latter which will undergo further differentiation into spores [3]. For the genus *Micromonospora*, there is only one report dealing with the formation of AM [4] but taxonomic references mention this as "*rare*" and even "*sterile*", notably because the "single spores" will be attached to the substrate or vegetative mycelia [5]. Description of species within *Micromonospora* is usually based on a polyphasic approach employing chemotaxonomic markers, geno- and phenoypic properties but this is usually cumbersome because of the limited and taxonomic value of the physiological properties of micromonosporae. *Micromonospora* colonies will start as yellow or light orange, then become darkorange and end with a black mucoid exopolysaccharide which completely covers the colony and indicates sporulation [6]. In this work, micromonosporae recovered from sediments of the Sea of Cortes (aka Gulf of California; [7]) were characterised using a polyphasic taxonomic approach including both geno- and phenotypic properties. During the phenotypical characterization of the isolates growing on the **I**nternational ***S****treptomyces* **P**roject media (**ISP**; 30 carbon sources and 15 nitrogen sources; [8]) it was noticed that the strains reproducibly developed AM on media supplemented with several (a) carbon, (b) nitrogen sources or (c) different NaCl concentrations (0% to 5% on Glucose Yeast Extract agar -GYEA-; [9]). The strains were confirmed to belong to the genus *Micromonospora* by using the genus specific primers of Qiu *et al.* [10] after DNA extraction by using standard methods employed previously [7]. Seven micromonosporae out of fifty (codes 14, 45, 64, 67, 81, BL-1, and BL-9) were found to produce AM after 14 to 21 days of incubation in ISP media supplemented with L-glutamic acid (acid), D(+) cellobiose (disaccharide), D-mannitol (poli-alcohol), D(+) mannose (monosaccharide), D(+) melibiose, L(+) arabinose (monosaccharide) or different NaCl concentrations ranging from 2% to 4% (**Figure 1**). To our knowledge, this is the first communication where the presence of AM is reported for micromonosporae of marine origin growing in ISP media hence suggesting it may be an inducible property of some strains. Together with our isolates, one validly described species of the genus *Micromonospora*, namely *Micromonospora chersina* DSM 44151^{T}, also exhibited AM when growth in the same conditions as the AM-producing isolatesof marine origin though recent descriptions of novel species of micromonosporae indicate mixed results.Micromanipulation of the observed AM onto fresh media (either GYM—**G**lucose **Y**east Extract **M**alt Extract- or ISP2—**I**nternational ***S****treptomyces* **P**roject media number **2**-) taken from our isolates produced single colonies also suggesting that the AM should not be called "*sterile*" as taxonomically reported and this assessment should perhaps be revisited. Although Suarez and Hardisson [4] working on *Micromonospora chalcea* ATCC 12452^{T} (the type strain of the genus) stated that "*at least in M. chalcea, there are aerial mycelia* [*and*] *it does have a reproductive function*" ours would be the first report to extent such view but on micromonosporae of marine source. In addition, of the 32 validly described species mentioned in the latest version of Bergey's Manual only 3 of those (*i.e. M. chersina*, *M. echinospora* and *M. rosaria*) are reported to produce AM and none of those species are of aquatic or marine origin. If *M. chalcea* is included with the previous 3 on the basis of the results provided by Suarez and Hardisson [4], this only accounts for 12.5% of all the validly described species of *Micromonospora*.

Further studies on the AM-producing strains included both SEM and TEM by using standard and well-reported procedures with another unusual feature coming out: crystals or zones of mineralization around and near the colony as shown in **Figure 2**. This suggests that there is AM of reproductive function in micromonosporae and that the spore is not a dormant structure. Thus, it does seem likely that the spore may have additional active roles for the microorganism in its ecosystem. Similar mineralization structures or precipitates have only been reported in isolates of the genus *Bacillus*, interestingly those of aquatic origin and notably those capable of oxidazing manganese(II) as reported by Tebo *et al.* 1997 [11]. Although it is clear that further studies are required to assess the precise role of the AM and the spores on this microbial group of Actinobacteria, this would be the first report dealing with: (a) AM as an inducible property in *Micromonospora*, (b) AM on micromonosporae from marine origin is certainly not "*sterile*" and (c) there is an active role of the spore other than just dissemination of the microorganism in its environment. One ecological implication would be as follows: *Micromonospora* employ the spore to continue generating inorganic compounds of use (perhaps as a source of energy for the colony?) whereas the presence of AM contributes to dissemination. Additionally, both the AM and the spore are structures of dissemination to facilitate the colonization of other niches within its environment, whether the microorganism is a resident or a tourist of such microniche. On the other hand, which could be the biotechnological role of these observations?

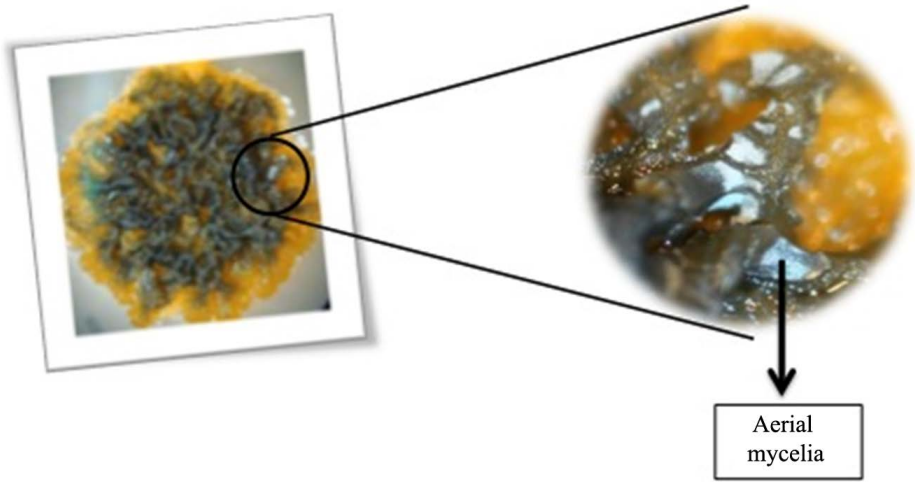

Figure 1. Aerial mycelia (AM) on isolate BL-1 growing in GYM medium with 2% of NaCl. The picture was taken after 14 days of incubation at 30˚C.

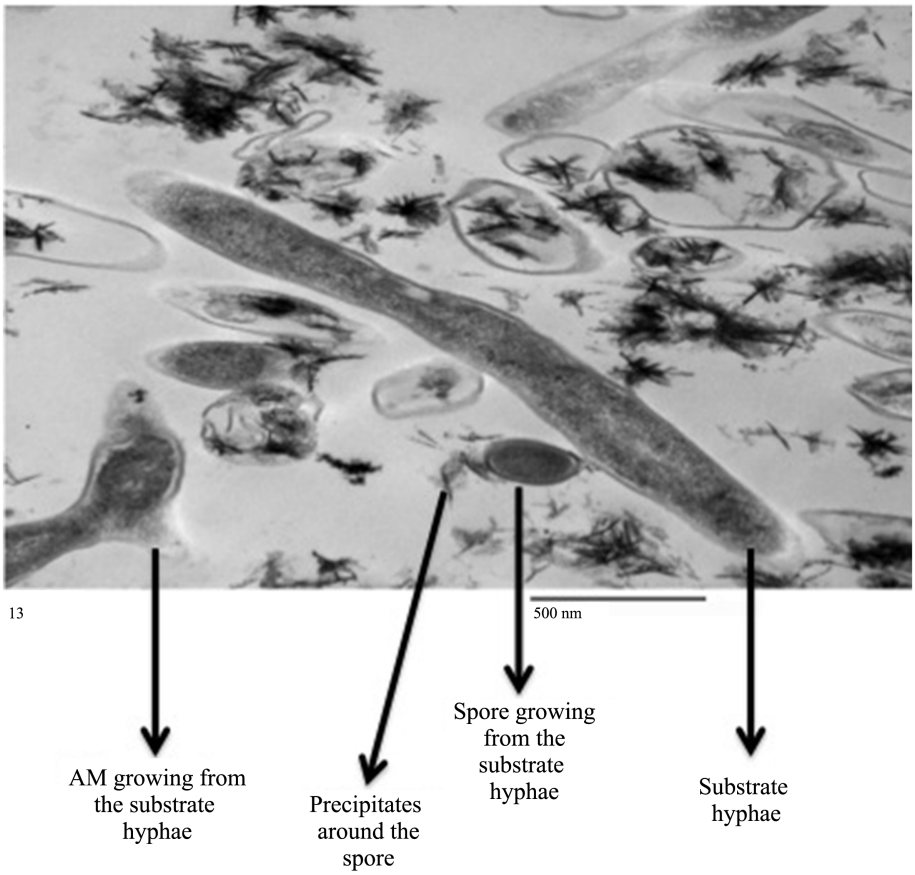

Figure 2. Transmission electron micrograph of an AM-producing isolate with Precipitates around the structures, namely aerial, substrate hyphae and spores. The strain was grown in GYM medium with 2% NaCl and the picture taken after 14 days of incubation at 30˚C.

The three species reported to produce AM according to Bergey's Manual synthesize secondary metabolites: (a) dynemicin by *M. chersina*, (b) compounds of the gentamicin complex by *M. echinospora* and (c) rosaramicin by

M. rosaria. The model organism for the production of secondary metabolites (*i.e. Streptomyces*) undergoes a life cycle in which the presence of AM is related to the production of bioactive compounds. Could specifically AM in *Micromonospora* have such role as well? The genus *Salinispora* Maldonado *et al.* 2005 [12], a marine obligate actinobacteria closely related to *Micromonospora* does not produce AM but is a good source of novel biologically active compounds [13]. The recent description of a novel species of *Micromonospora* (*i.e. M. jinlongensis*) also proposed to emend the genus [14]. Undoubtedly, the observations shown here also leave further room for improvement on the taxonomic properties of the genus not only from a biotechnological perspective but also from an ecological one. For some actinobacteria of marine origin, the production of bioactive compounds is only present when the microorganism is grown under media supplemented with seawater [15]. This secondary metabolite production may also represent an adaptation of the microorganism to its marine ecosystem and for micromonosporae this could also be related to the AM absence or presence. An ongoing study to explore the presence of AM in our collection of nearly 250 micromonosporae is expected to expand the observations detailed on this communication and to expand the current view on the complex and fascinating life cycle of this group of *Actinobacteria*.

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Conflict of Interest

The authors declare no conflict of interest.

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