

### **A question concerning the identity of *Streptomyces* sp. MSU-2110**

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We appreciate the comments of Cavaletti & Monciardini (2004) relating to our report on the coronamycins from *Streptomyces* sp. MSU-2110 that appeared in the April issue of *Microbiology* (Ezra *et al.*, 2004). These workers suggest that we had mistakenly identified our coronamycin-producer as a streptomycete based on morphological observations and 16S rRNA gene sequence data. However, not noted in our original article is the fact that the organism (MSU-2110) is Gram-positive. Furthermore, attempts to find nuclei in MSU-2110 were inconclusive when nuclear staining procedures were used in conjunction with fluorescence microscopy (see Footnote). Such staining procedures were successfully applied to a fungus – *Penicillium* sp. These facts alone suggest that we should dismiss the classification of MSU-2110 as a possible eukaryote as might be exemplified by one of the verticillated fungi, e.g. *Verticillium*, *Gliocladium* or *Stachylidium*.

The isolate, MSU-2110, has multiple organized chains of spores arising from verticillated hyphae, as shown in the original *Microbiology* article (Ezra *et al.*, 2004). It does turn out that lemon-shaped spores, in chains, are known from streptomycetes including *Streptomyces* sp. strain SF 1293 and '*Streptomyces routienii*' (Miyadoh, 1997). It is also apparent that while most *Streptomyces* species do have spores in the range of  $1 \times 1.5 \mu\text{m}$ , as mentioned in the comments of Cavaletti & Monciardini (2004), there are some reported to have spores in the range of 2–3  $\mu\text{m}$  in length, e.g. *Streptomyces hygroscopicus* (Miyadoh, 1997). Also, the spore size of strain MSU-2110 in the environmental scanning electron micrographs are more in the range of 2  $\mu\text{m}$  while in the regular scanning electron micrographs the spores may appear larger due to the gold coating that was used on the specimens (Ezra *et al.*, 2004).

A question as to the shape of the verticles of strain MSU-2110 was also raised. It is to be noted that the overall shape of these structures, as viewed by the non-destructive environmental electron microscopy method, revealed more of an even cylindrical outline in contrast to the images in regular scanning electron microscopy in which some distortion resulting from chemical fixation may have arisen. This can readily be seen by comparing the environmental scanning electron micrographs to the regular scanning electron micrographs in Fig. 2 in the report (Ezra *et al.* 2004).

With regard to the partial 16S rRNA gene sequence of strain MSU-2110, there was sequence similarity at the 94 % level to *Streptomyces caelestis*, which had been described previously as a *Streptovercillium* sp., and this was even lower at the 90 % level when aligned to the overall sequence of this one organism. However, it should be pointed out that when PCR was performed using ITS1 and ITS4 rRNA gene primers, designed especially for fungi and other eukaryotes, there was no detectable PCR product. This fact also was not mentioned in the original report (Ezra *et al.*, 2004). Cavaletti & Monciardini (2004) point out that there was greater similarity to the 16S rRNA gene sequences of members of the genera *Kocuria*, *Rothia*

and *Micrococcus* and that one or more of these may have existed as a cultural contaminant. We greatly appreciate this suggestion and have re-checked our cultures of MSU-2110 by plating and scanning electron microscopy techniques and have not found any evidence of other micro-organisms. The lack of high similarity of MSU-2110 to other streptomycetes is probably related to the well-recognized divergence in *Actinomycetes* between whole genome DNA–DNA homologies and similarity to 16S rRNA (Ward & Goodfellow, 2004).

Ultimately, unlike the hundreds of other streptomycetes originating from soil samples that have been so thoroughly studied over the years, we need to recognize that strain MSU-2110 is an endophytic micro-organism. We also need to vehemently stress that strain MSU-2110 was obtained from a very remote region in the upper reaches of the Peruvian Amazon. It is perhaps to be expected that micro-organisms with structural and genetic uniqueness do exist in such locations. Furthermore, endophytes existing within the living tissues of plants may have undergone lateral gene transfer as they have co-evolved with the host plant and perhaps other micro-organisms (Strobel & Daisy, 2003; Ward & Goodfellow, 2004). Strong evidence for lateral gene transfer between a microbe and a plant has recently been presented by Davis & Wurdack (2004). The implications of this have an impact on the outcomes of studies on the genetics of micro-organisms that are associated with higher plants. Most recently, for instance, is the discovery of a strain of *Gliocladium* sp. existing as an endophyte in *Eucryphia cordifolia*, whose closest related fungus, by 18S rRNA gene similarity studies, is *Chloroscypha enterochroma* (Stinson *et al.*, 2003). Morphologically, *Gliocladium* sp., a verticillated fungus, is most closely related to *Trichoderma* spp.

Overall, in spite of all of these morphological and molecular biological observations, we recognize that there is a much more work to be done on this interesting and promising endophytic microbe, but we still prefer to describe strain MSU-2110 as a verticillated streptomycete. Finally, since anomalous

16S and 18S rRNA gene sequence results keep appearing with plant-associated endophytic microbes, perhaps this fact alone warrants further investigation, as does a complete re-examination of the taxonomic issues surrounding the verticillated streptomycetes?

#### Footnote

The staining procedure was that described by Molecular Probes Co. (Eugene, OR, USA) and it uses Hoechst 33342 trihydrochloride trihydrate at the level of  $1 \mu\text{g ml}^{-1}$  and an exposure time of 15–20 min prior to viewing under a fluorescence microscope with an activation wavelength of 352 nm and an emission wavelength of 461 nm.

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