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On the spore formation process in actinomycetes

I. Morphology and development of *Streptoverticillium* species as examined by scanning electron microscopy*

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Abstract. Details of *Streptoverticillium* structures as detected by scanning electron microscopy are illustrated.
The development of species belonging to the genus has been followed from germination to spore production. Sporulating features of pseudoverticillate cultures have also been observed.
The peculiar characteristics of spore formation, which includes an initial twisting of the sporulating hypha, are documented.

In a recent paper (Locci et al. 1969) a taxonomic study of the species belonging to the genus *Streptoverticillium* was carried out. On that occasion a full documentation, both by light and transmission electron microscopy, of morphological structures peculiar to the genus was included.

Having experienced the advantages offered by scanning electron microscopy (SEM) in the study of fungal features (Locci 1969a, 1969b, 1969c), this technique was applied to the study of morphological characteristics of verticillate actinomycetes.

SEM has already been employed in the examination of actinomycetes (Williams & Davies 1967, Dietz & Mathews 1969).

However to the best of our knowledge there are no reports in the literature of its use in species of the genus *Streptoverticillium.* Due to their tridimensional structures an investigation using SEM on these organisms appeared to be of particular interest.

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Materials and methods. *Streptoverticillium* species, selected from among the most representative of the genus (Locci et al. 1969), were chosen for SEM investigation.

Some strains of pseudoverticillate actinomycetes were also examined for comparison. All the above cultures and their origin are referred to in Locci et al. (1969).

In addition a culture of *Streptomyces caespitosus,* IPV 2085, received from A. Seino, Japan, as strain KCC S-0438 was examined.

Strains were cultivated on the media best for the expression of morphological characteristics. As shown by Locci et al. (1969) these are not always the ones supporting the most luxuriant growth. In particular potato dextrose agar, starch agar, Czapek and carbon utilization media were employed.

Selected strains were grown on Petri dishes. Either 10 mm diameter cylinders cut from the agar surface or fragments of coverslips embedded in the medium (Kawato & Shinobu 1959) were collected at regular intervals.

Agar cylinders were reduced to 1 mm depth by cutting off the lower surface. This was done in order to reduce shrinkage of the samples during coating.

Except for the study of initial stages of development, agar cylinders are more useful as growth is more abundant and a larger amount of material is available for observations.

Both samples were glued to aluminium holders, coated with a gold-platinum film under vacuum as already described (Locci 1969a, 1969b, 1969c) and examined with a Stereoscan electron microscope (Cambridge Inst. Co., Mark 2A). Pictures enclosed were taken with a Kodak Tri-X 35 mm film.

Results

a) Verticil structure. A clear idea of the verticil appearance can be had from the enclosed documentation (Pl. I, figs. 1 to 6).

The sporophore structure of *Streptoverticillium* species consists of a main axis having at regular intervals sets of three or more side branches

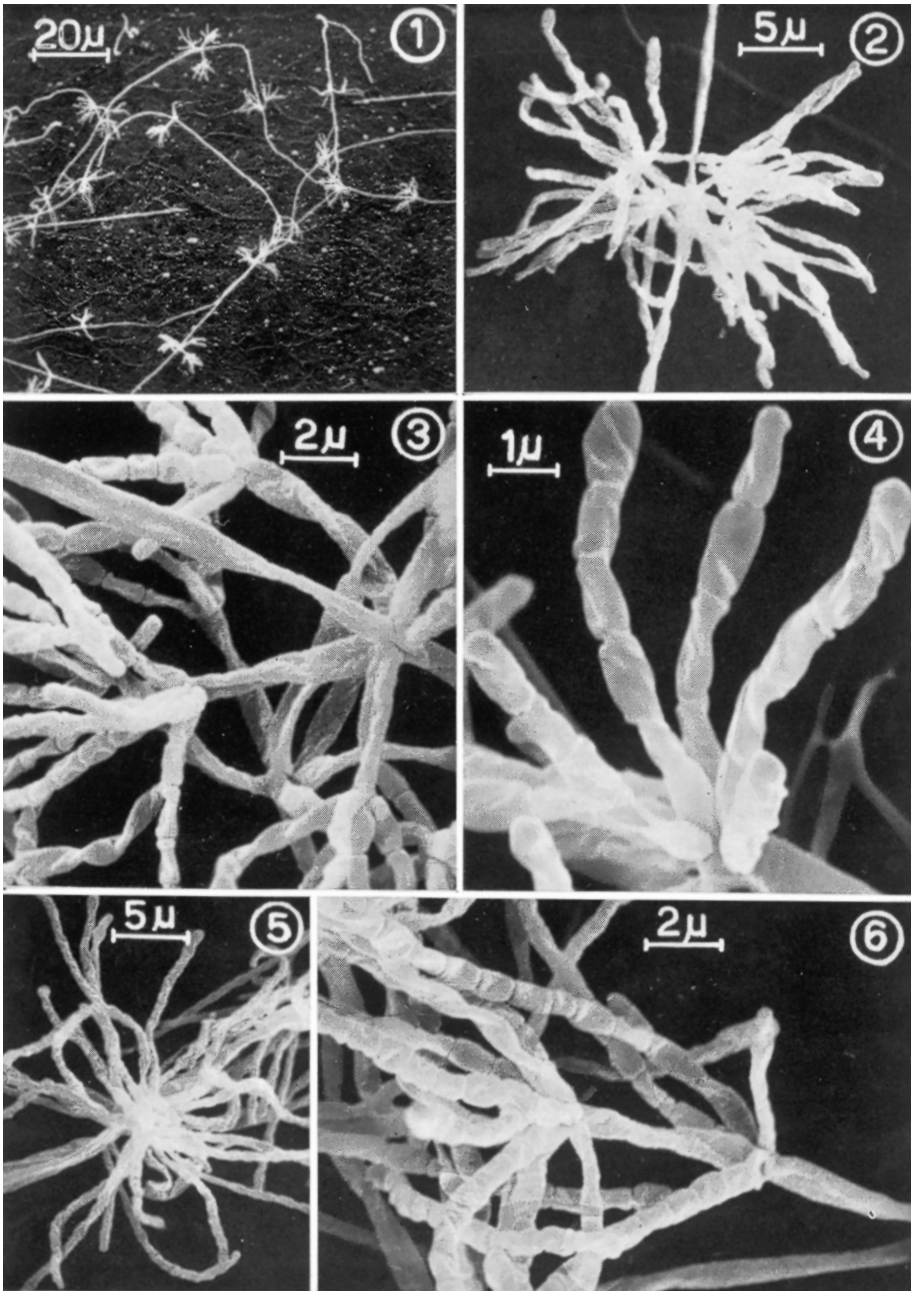

Plate 1.

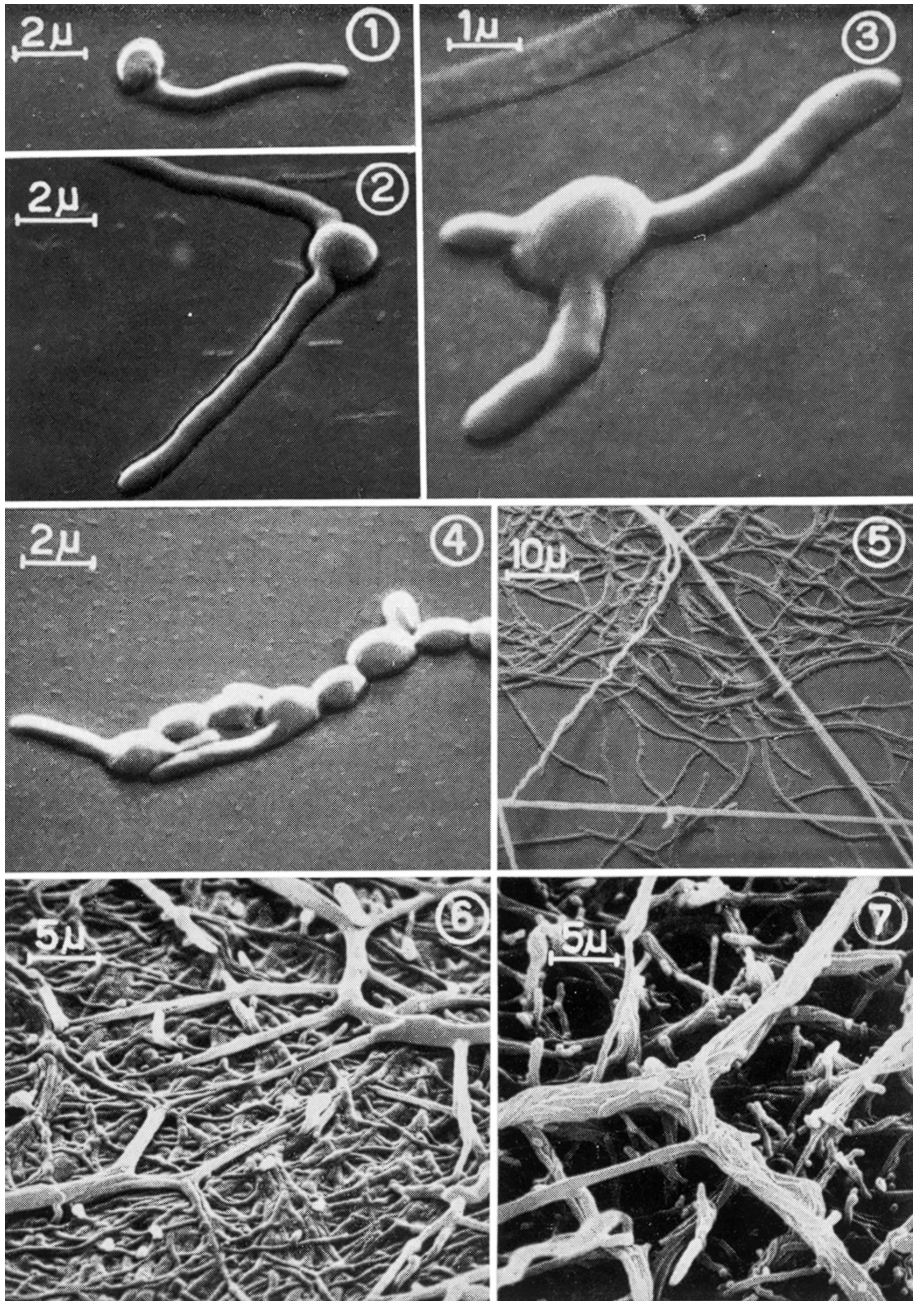

Plate 2.

(PI. I, fig. 1). These in turn bear (Pl. I, figs. 2, 3 and 4) terminal umbels of spore chains (Baldacci et al. 1966). Such formations should be interpreted as being umbellate monoverticillate and not biverticillate. That is the so called «secondary verticil» is actually an umbel, being single and terminal. Although rare, biverticillate structures do exist (Pl. I, fig. 6), as shown by Locci et al. (1969). This term must be restricted to forms where a true second series of verticils, and not just a single apical umbel, appears. In short streptoverticillia are not characterized by having verticils of spore chains but more correctly verticils of sporophore hyphae bearing terminal umbels of spore chains.

Therefore it is essential that spores should have been found before a correct definition of sporophore structure could be attempted. Too many descriptions of so called simple monoverticillate forms, that is forming verticils of spore chains on the main axis, have been based on observations at low microscope power or on non-sporulated cultures.

The twisting of hyphae, as described below, could also lead to misinterpretations relative to the true presence of spores. As previously described by Locci et al. (1969), the following morphological differences can be found among *Streptoverticillium* species: interval between verticils along the main axis, number of branches forming the verticil, length of branches, presence of true biverticils, type and number of spore chains forming the umbel, characteristics of spore surface.

Some examples of the above features can be recognized in the photographs enclosed.

A special comment on spore surface in *Streptoverticillium* species will be made in regard to hyphal twisting as observed by SEM.

b) Development stages in Streptoverticillium *species.* The successive stages of development of *Streptoverticillium* were followed beginning from germination. The latter can be seen in Pl. II, figs. 1 to 4. Spore germination can take place by means of one (PI. II, fig. 1), two (PI. II, fig. 2) or three (PI. II, fig. 3) germ tubes. The process can occur even when they are still joined together in chains (PI. II, fig. 4).

The primary mycelium does not bear any spores. Secondary mycelium hyphae can arise from any point of the primary growth and due to their relative positions the two types of hyphae are clearly distinguish-

able (Pl. II, fig. 5). The secondary mycelium consists of a network of interwoven hyphae and is often characterized by the development of long straight filaments.

Verticil formation can take place on these hyphae. In other cases sporulation occurs in a culturally differentiated «tertiary» (Farina & Locci 1966) mycelium. Such formations can partly explain the development in height typical of some *Streptoverticillium* species and the cottony consistency of the same. Also some *Streptomyces* species can have a build up of aerial hyphae and hence a cottony appearance in contrast to the flat and dusty aspect of species characterized by direct sporulation on the hyphae emerging from the primary mycelium. In *Streptoverticillium* on media with less readily available nutrients a strong aerial development is lacking. However even in these cases, when sporulation is more readily achieved, the development of a basal secondary mycelium can be recognized.

Under particular conditions aerial hyphae tend to join into strands which can fuse together again to form larger ones (Pl. II, figs. 6 and 7). Some sort of connecting material seems to be present between the hyphae (Pl. III, fig. 1). Successive fusions, which appear to be almost complete, give origin to a synnema-like structure (Pl. III, fig. 2).

The initiation of verticil branches (Pl. III, fig. 3) is not simultaneous in each verticil as also happens in umbel and spore chain formation. The successive development of the verticil branches can be followed in figs. 4 to 9 (Pl. III) and 1 and 2 (Pl. IV).

In extreme cases one branch may be well developed and even have started umbel formation while the others are still in the initial phase. The reasons for such a phenomenon are not yet clear, however if due to some unfavourable condition growth is halted at this stage or if a localized inhibition stops further development of these branches, these structures could be regarded as irregularities. In fact they are nothing but developmental malformations. In some species an enlargement of the main hypha at the point of insertion of the verticil can be observed (Pl. IV, fig. 3).

Similarly the formation of umbels is not simultaneous in all branches of the verticil.

The same can also be said of sporulation in the umbel hyphae. Completely sporulated and not sporulated hyphae can be found in the same umbel (Pl. VI, figs. 4 and 5).

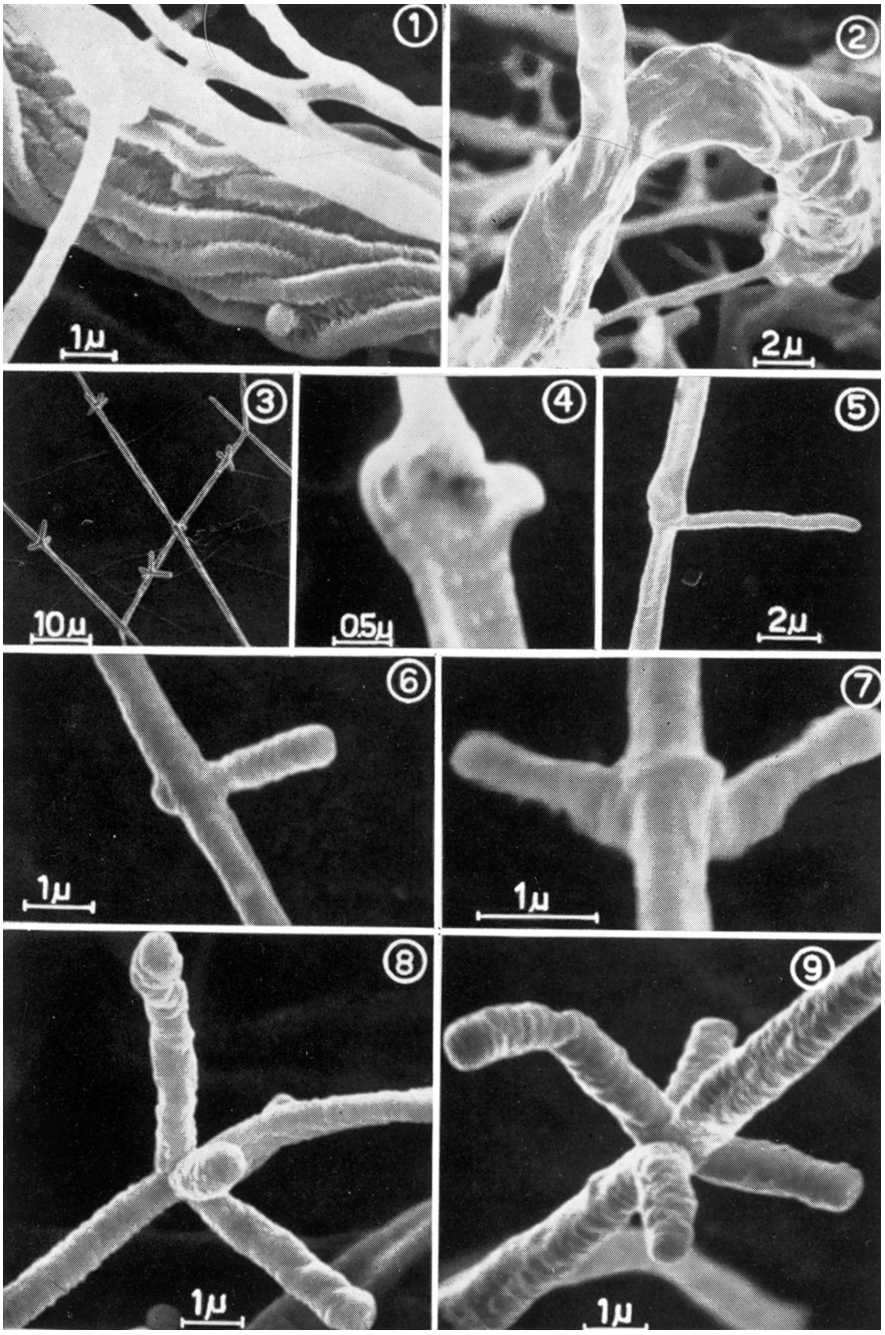

Plate 3.

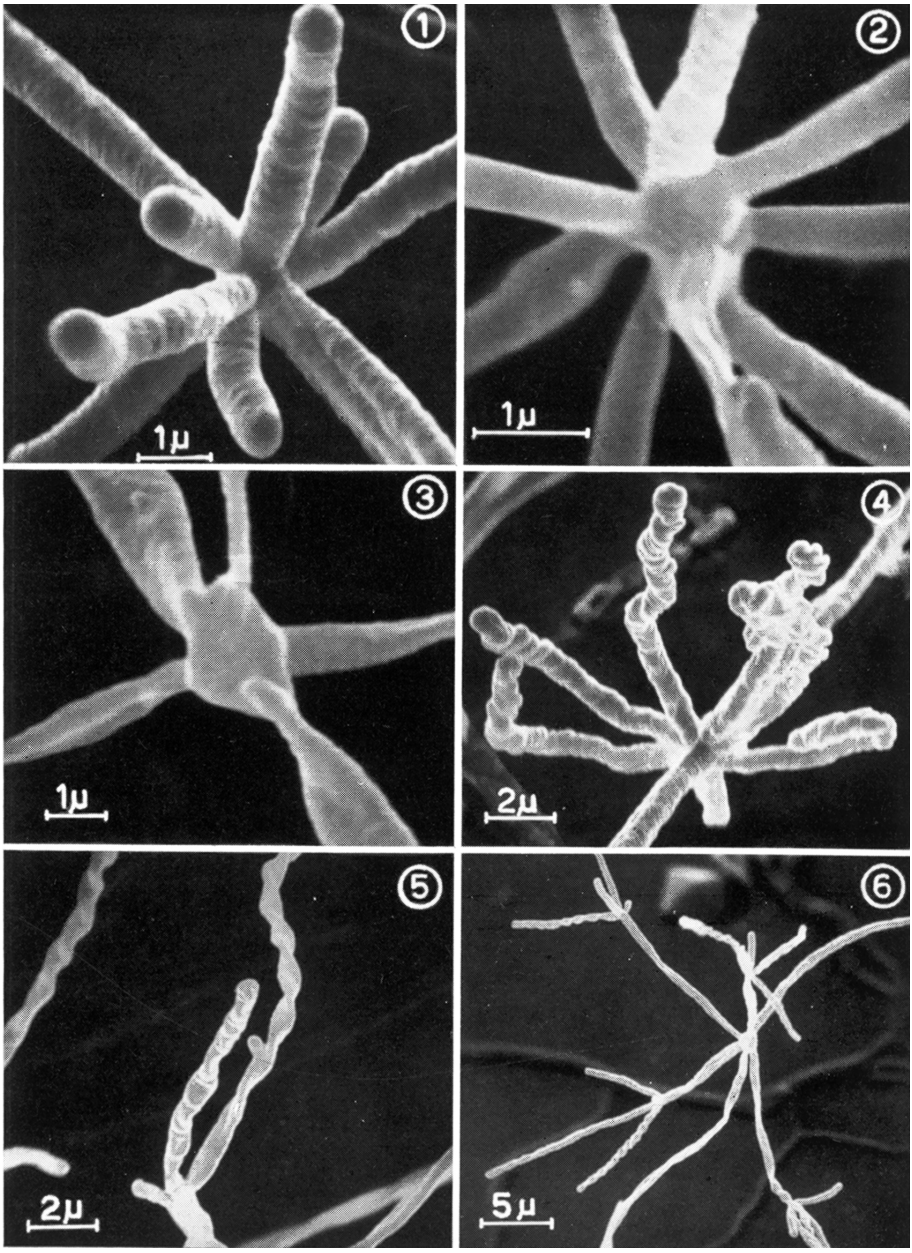

Plate 4.

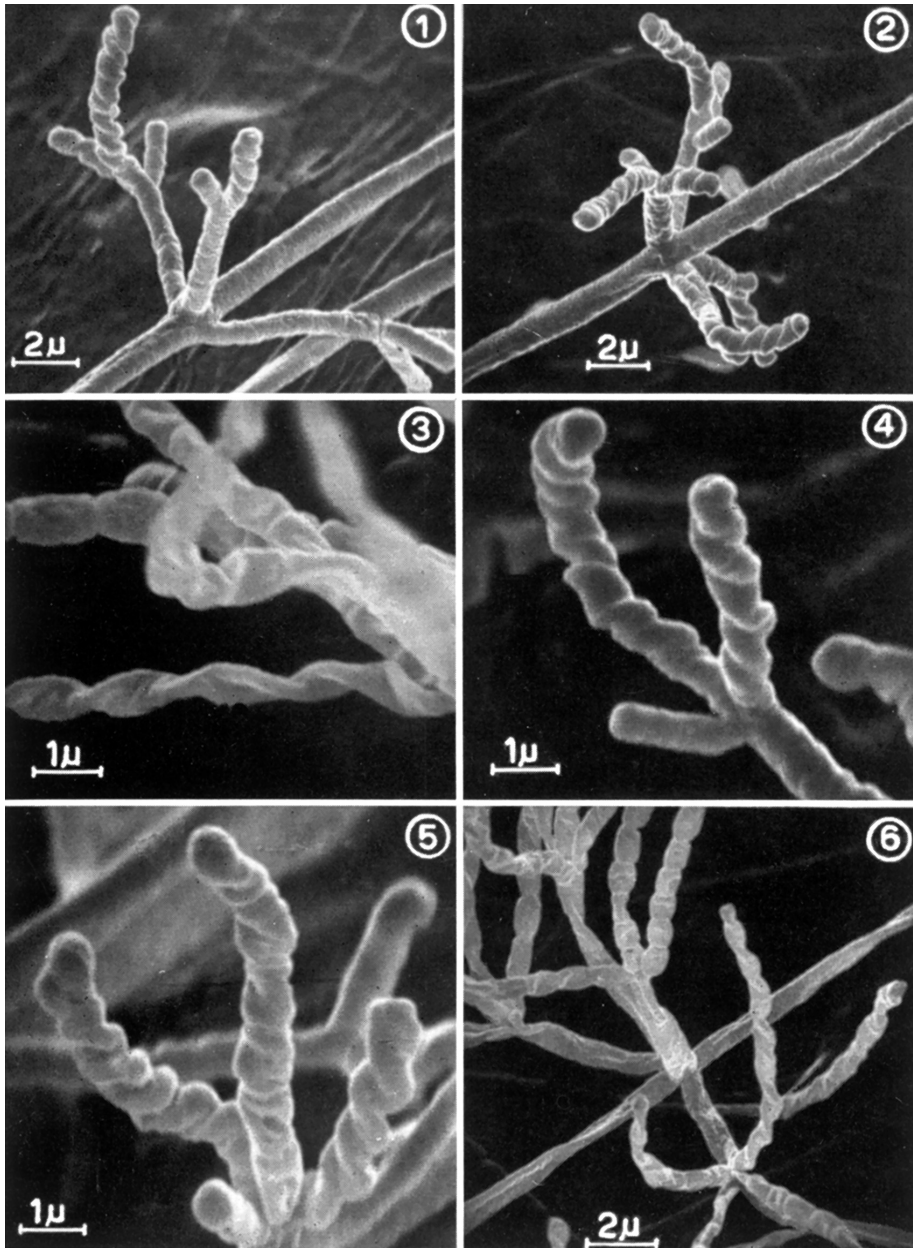

Plate 5.

Along the main axis development is acropetal, although the above mentioned malformations can partially alter the sequence which in summary is as follows: branching on the main axis and consequent appearance of the verticil, formation of umbel hyphae, actual sporulation by the latter.

A last set of observations was carried out on sporulation itself. In the species examined a peculiar phenomenon seems to occur prior to sporulation. In the few *Streptomyces* species examined nothing remotely similar could be observed, although we intend to carry out further work in order to investigate the spore formation process in this genus. As previously reported (Locci et al. 1969) none of the *Streptoverticillium* species shows a spiral arrangement of spore chains. At most some species show terminal hooks. Usually the relatively short spore chains are either straight or flexuous.

However what occurs in the umbel hyphae of the examined *Streptoverticillium* species can be summarized as follows. Before sporulation the umbel hyphae show a twisting along their main axis (Pl. IV, figs. 4 to 6; Pl. V, figs. 1 to 6). This is clearly visible since folds appear on the surface of the hyphae. Such a phenomenon could not be detected were it not for the possibilities offered by SEM. It is uncertain as yet to what extent this process determines the actual separation of spores in the hypha.

Even when spores initially appear some twisting is still present (Pl. VI, figs. 1 to 5) and in some cases foldings on the outer surfaces can be recognized in nearly completely formed spores (Pl. VI, figs. 5 to 7).

In the same umbel some hyphae are still at the twisting stage while other are completely sporulated (Pl. VI, figs. 4 and 5).

This shows once again that sporulation is not simultaneous in all hyphae of an umbel.

The phenomenon regards only the hyphae which will give origin to spore chains. There is no twisting of the verticil branches. When the umbel consists of only one hypha, which could give the impression of a simple monoverticil, twisting occurs only on the top part of the same (Pl. IV, fig. 4). By observing such structures when a second spore chain is being formed, it can be seen that the twisting is limited to the part of the hypha where later spores will appear (Pl. IV, fig. 5; Pl. VI, fig. 1).

A comment is necessary on another morphological feature. Up to

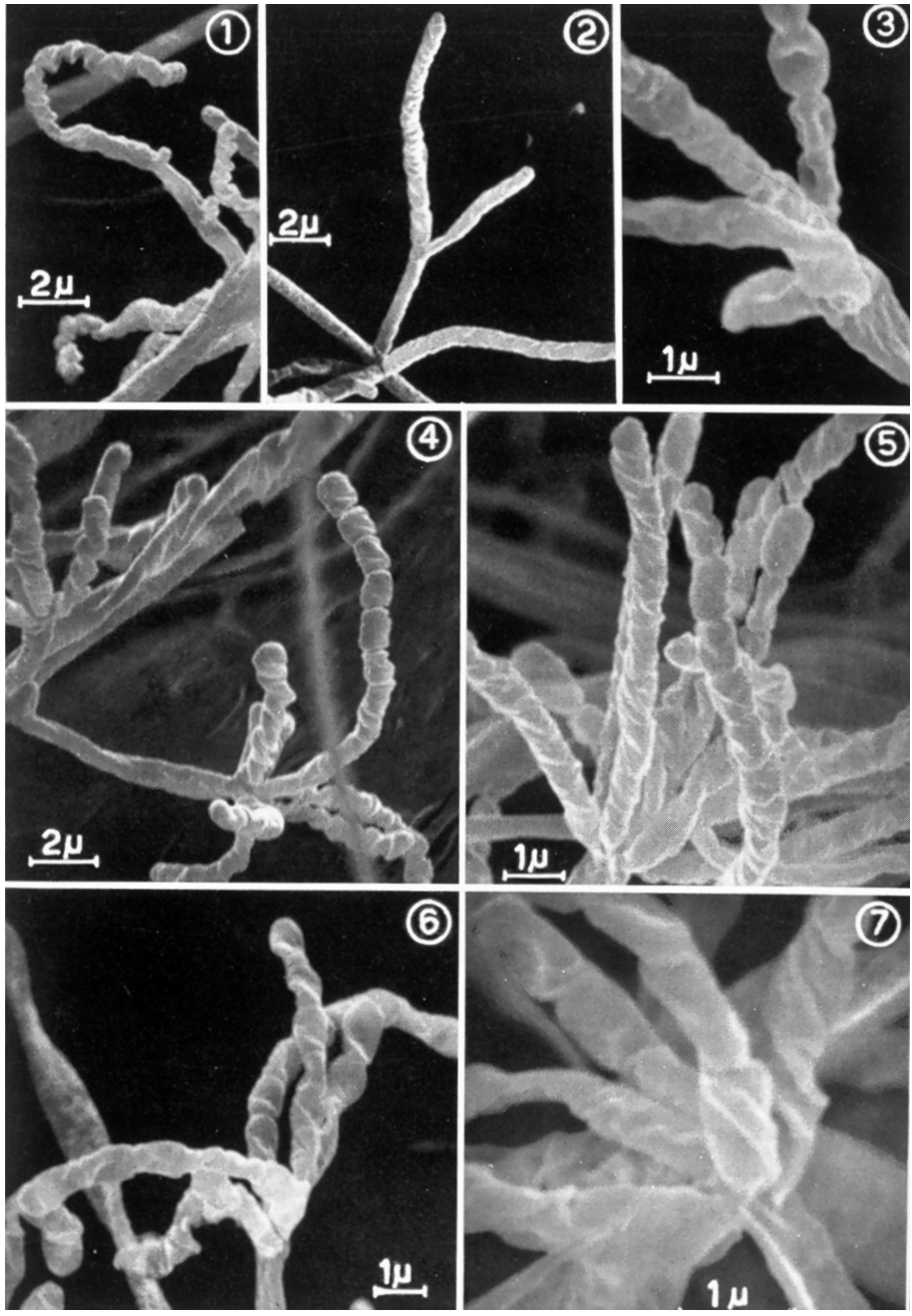

Plate 6.

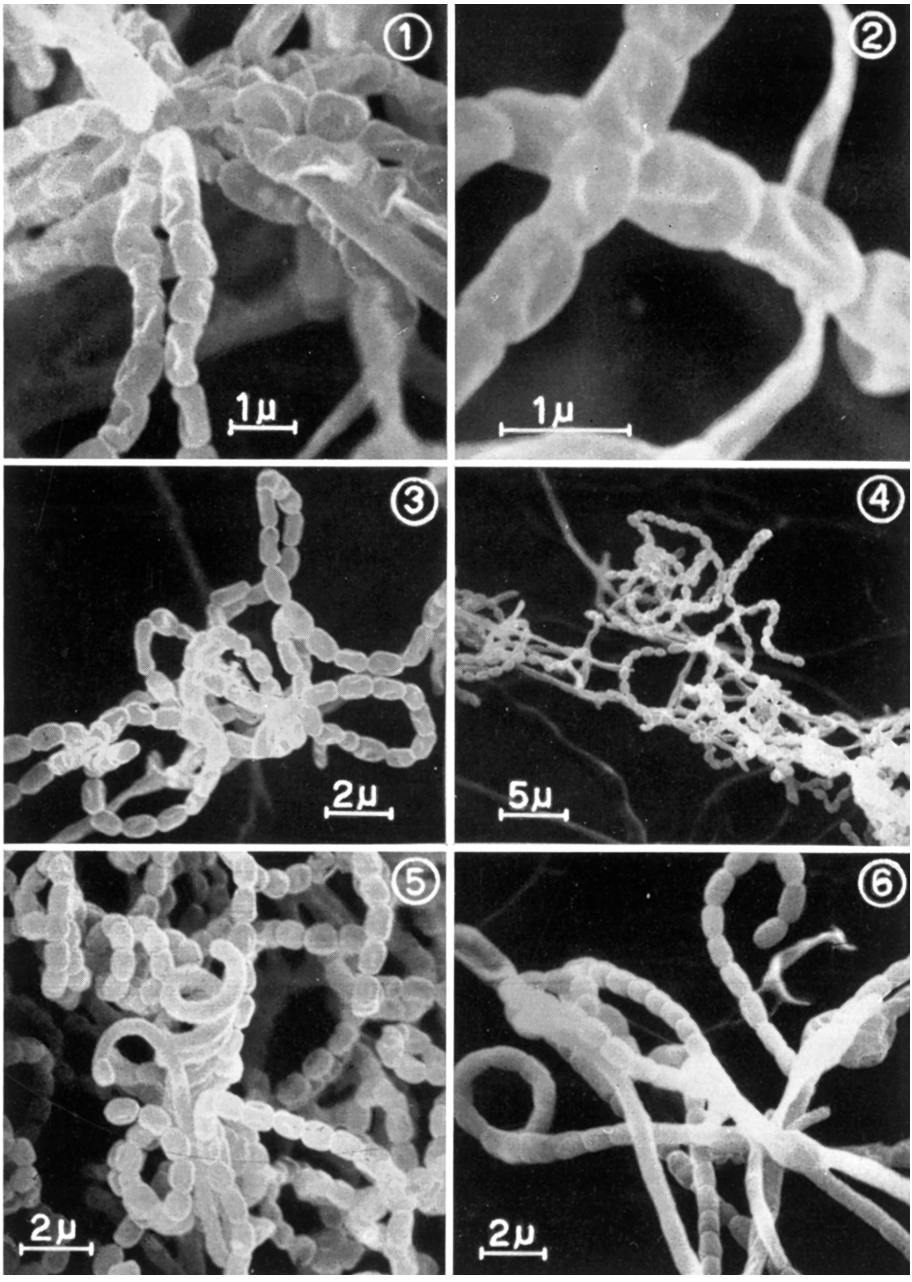

Plate 7.

now no *Streptoverticillium* species has been isolated showing spines or hairs on the spore surface. All spores are practically smooth (PI. VII, figs. 1 and 2). Small irregularities could be recognized by transmission electron microscopy (Locci et al. 1969), but in the light of the present results the residual foldings on the spore surfaces, as described above, could be responsible for such an appearance.

The absence of structures which are common among other actinomycete spores may have some significance. This added to the fact that no true spirals are formed in this genus, notwithstanding the twisting of sporulating hyphae, may lead to some speculations in regard to the position of these organisms in respect to other actinomycetes. However further experimental work is necessary before even an hypothesis could be put forward.

c) Conformation of pseudoverticillate actinomycetes. Cultures excluded from the genus *Streptoverticillium* on the basis of morphological structure (Locci et al. 1969) were re-examined by scanning electron microscopy. The features of such species as previously determined were confirmed.

In figs. 3 to 6 (Pl. VII) the sporulating structures of some pseudoverticillate cultures are illustrated. It can be clearly seen that such conformations cannot be confused with those of true verticils.

On the basis of such observations another species (*Streptomyces caespitosus*) can now be excluded from the genus *Streptoverticillium*. In fact the culture (Pl. VII, fig. 5) shows closely packed spore chains but irregularly arranged along a branched hypha.

Discussion. The utility of SEM in the study of actinomycetes has already been pointed out by Williams & Davies (1967). This technique is particularly suitable for *Streptoverticillium* species, because of their tridimensional structure. Due to their size a clear interpretation of the arrangement of sporulating structures by light microscopy is not easy. The closely packed arrangement presents a further difficulty.

In addition to elucidating with greater ease features that had previously been defined by light microscopy, SEM also revealed the existence of phenomena otherwise undetectable.

Transmission electron microscopy is not of much use in this regard. Contact sampling of aerial mycelium rarely shows more than a few hyphae or fragments of spore chains.

In this respect one of the advantages of SEM is that the entire original structure may be examined. This is true to such an extent that the use of light microscopy in actinomycete work becomes obsolete (Williams & Davies 1967). SEM employed at different enlargements gives both a general view of the mycelium structure and also investigates the same sample at magnifications which up to now had to be carried out separately by transmission electron microscopy. Due to the large size of samples which can be examined, variously differentiated areas can be observed. In this way the development of the mycelium can be followed accurately as shown in the enclosed documentation.

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Explanation of photographs

Plate I – Sporulating structures in *Streptoverticillium* species. General and particular appearance of verticils (figs. 1 to 3), umbels (fig. 4), short branched verticils (fig. 5) and true biverticils (fig. 6) are illustrated.
1) *Streptoverticillium griseoverticillatum,* IPV 1976, on Czapek agar (CA), age: 4 days.
2) *Stv. mobaraense,* IPV 2058, on starch agar (SA), age: 5 dd.
3 and 6) *Stv. kentuckense,* IPV 1780, on potato agar (PA), age: 4 dd.
4) *Stv. baldaccii,* IPV 1339, on PA, age: 15 dd.
5) *Stv. netropsis,* IpV 1720, on CA, age: 4 dd.

Plate II – Spore germination (figs. 1 to 4), primary and secondary mycelium (fig. 5) and strands of secondary mycelium hyphae (figs. 6 and 7).
1 to 4) *Stv. baldaccii,* IPV 1339, on PA, age: 30 hours.
5) *Stv. kentuckense,* IPV 1780, on PA, age: 4 dd.
6 and 7) *Stv. baldaccii,* IPV 1339, on PA, age: 2 dd.

Plate III – Synnemata-like structures (figs. 1 and 2) and beginning of verticil formation (figs. 3 to 9).
1 and 2) *Stv. baldaccii,* IPV 1339, on PA, age: 30 hours.
3, 6 and 9) *Stv. salmonis,* IPV 2019, on SA, age: 3 dd.
4, 5 and 7) *Stv. baldaccii,* IPV 1339, on PA, age: 7 dd.
8) *Stv. biverticillatum,* IPV 1594, on SA, age: 3 dd.

Plate IV – Further verticil development (figs. 1 to 4), twisting of sporophore hyphae (figs. 4 to 6) and beginning of umbel formation (figs. 5 and 6).
1 and 4) *Stv. cinnamoneum,* IPV 2013, on SA, age: 3 dd.
2 and 3) *Stv. baldaccii,* IPV 1339, on PA, age: 5 and 7 dd. respectively.
5) *Stv. kentuckense,* IPV 1780, on PA, age: 4 dd.
6) *Stv. parvisporogenes,* IPV 1972, on CA, age: 15 dd.

Plate V – Twisting of sporophore hyphae prior to sporulation (figs. 1 to 6) and actual spore formation (figs. 3 and 6).
1, 2, 4 and 5) *Stv. salmonis*, IPV 2019, on SA, age: 3 dd.
3 and 6) *Stv. kentuckense,* IPV 1780, on PA, age: 4 dd.

Plate VI – Twisting of sporophore hyphae and spore formation in *Streptoverticillium* species. Residual foldings on the spore surface can be observed (figs. 4 to 7).
1 and 6) *Stv. mobaraense,* IPV 2058, on SA, age: 5 dd.
2) *Stv. flavopersicum,* IPV 2010, on SA, age: 12 dd.
3) *Stv. netropsis,* IPV 1720, on CA, age: 20 dd.
4) *Stv. salmonis,* IPV 2019, on SA, age: 3 dd.
5 and 7) *Stv. baldaccii,* IPV 1339, on PA, age: 15 dd.

Plate VII – Spore chains in *Streptoverticillium* species (figs. 1 and 2) and sporulating structures of pseudoverticillate actinomycetes (figs. 3 to 6).
1 and 2) *Stv. baldaccii,* IPV 1339, on PA, age: 15 dd.
3 and 4) *Streptomyces mediocidicus,* IPV 2031, on PA, age: 20 dd.
5) *Streptomyces caespitosus,* IPV 2085, on V-8 agar, age: 3 dd.
6) *Actinomyces circulatus,* IPV 1540, on CA, age: 5 dd.