CHAPTER 4

SOME STUDIES ON CHAINIA OCHRACEA

Various cultural, morphological and biochemical properties of the sclerotial actinomycete, Chainia ochracea, were described by Kuznetsov. With regard to the spore-bearing apparatus of the culture, it was briefly reported that 2-3 week-old colonies of the organism produced 'islets' of white aerial mycelium with spiral sporophores on Czapek's medium with glucose, and on soyabean agar. During the present studies it was found that the organism possessed some additional cultural and morphological characteristics which have remained undescribed so far. A detailed account of these characteristics, especially the spore-bearing organs, is given in the present chapter.

1. External characteristics of C. ochracea on various media

Czapek's sucrose agar

Growth profuse, raised, folded, wet, deep yellow (9K7) sclerotia not evident; a pink diffusible pigment formed;
aerial mycelium absent or in traces.

**Inorganic salts starch agar**: 
Growth good, slightly raised, yellow (11J6); reverse similar; sclerotia not evident; diffusible pigment absent; aerial mycelium in traces.

**Glycerol asparagine agar**: 
Growth good, light yellowish-brown with colourless, thin spreading margin; centre whitish; sclerotia present; aerial mycelium present, in traces (Fig.1); diffusible pigment absent; cultures show colourless slimy exudate during the early stages of growth.

**Yeast extract malt extract agar**: 
Growth good, raised, folded, light brown (14L7); reverse similar; sclerotia not well-formed; microscopic examination of small colonies, however, revealed extensive sclerotial growth in the form of a rosette, surrounded by a thin ring of mycelial growth of normal hyphae, diffusible pigment not evident; aerial mycelium poorly developed, distributed sparsely in older colonies, sometimes forming a thin ring of whitish growth around the central rosette.

2. **Micromorphology of C. ochracea**

2.1. **Aerial sporophores**

Microscopic examination of the 2-3 weeks old agar plate
cultures of *C. ochracea* revealed some aerial mycelium which
was not very prominent externally. The aerial mycelium was
mostly localised over the central portion of the colony and
bore simple or branched sporophores with loose terminal
spirals. The aerial mycelium was scarcely observed towards
the periphery of the growth and hardly any was found towards
the spreading margin, especially on GAA. Instead,
ocasionally a few simple or branched aerial sporophores with
loose irregular terminal spirals were observed to leave the
substrate growth and rise upwards into air away from it,
without forming a typical aerial mycelium. Aerial sporophores
towards the peripheries of the colonies on YEA are seen in
Fig.2.

2.2. *Substrate sporophores*

 Besides the aerial sporophores borne directly on it, as
described above, the thin spreading peripheral growth
produced extensive substrate sporophores with a growth habit
similar to that of the substrate mycelium. Substrate mycelium
which trailed along the agar surface and that which grew
underneath it bore these sporophores in large numbers (Figs. 3
and 4). The sporophores presented a similar texture and
growth habit as the substrate mycelium and merged
indistinguishably with the latter except for their terminal
compact spirals. The thickness of the substrate sporophores
and the substrate mycelium was also similar and only a careful microscopic examination of colonies revealed these structures. Sometimes substrate mycelium was also observed to show spore-like structures (Fig. 4). Sometimes, it was helpful to remove a small piece of the agar culture from the margin of a colony, slice from it a thin layer of the growth using a sharp safety razor, and examine it under the microscope for the substrate sporophores.

The substrate sporophores were easily distinguishable from the aerial sporophores on several accounts. The aerial sporophores emerged from the substrate growth, rose into air, and presented a dry texture. They were appreciably larger in thickness than the substrate sporophores and could be easily observed under low magnification. Substrate sporophores were not normally distinguishable under this condition (Fig. 2). Besides, the aerial sporophores bore loose irregular spirals and were generally branched.

Substrate sporophores could not be observed in the light-microscope in areas showing thick mycelial growth (mat) but there appeared to be an indirect evidence to suspect that they could be present as spores and detached spirals were also obtained from the substrate growth which did not show any microscopic evidence of aerial sporophores.
Fig. 1. **Chainia ochracea**; four single spore isolates on GAA after 15 days of incubation at 25°C showing sparse white aerial mycelium; about X 1.

Fig. 2. **C. ochracea**; aerial branched spiral sporophores; emerging from the substrate growth (in background) on YEA plate after 21 days of incubation at 25°C; note the absence of a well-defined aerial mycelium; X 325.
Fig. 3. *C. Ochracea*; substrate spiral sporophores towards the edge of a colony on GAA plate after 21 days of incubation at 25°C; a solitary sclerotial granule is seen in the centre, X 800.

Fig. 4. *C. ochracea*; as in Fig. 3, showing a number of isolated substrate sporophores; substrate mycelium also shows spore-like bodies (arrows); X 750.
3. **Morphological characteristics of single spore isolates of Chainia ochracea**

Spores from a GAA slant culture were plated on GAA and YEA plates. The cultures were incubated at 25°C, and a number of colonies were examined microscopically after 10-18 days of growth, or later. The substrate sporophores were observed as usual towards the peripheral regions of many colonies. These structures were very prominent in those colonies which showed a thinner spreading margin. Invariably in these colonies the margin did not show appreciable sclerotial activity although sclerotia were observed in the central region. Substrate sporophores were not, however, observed in areas where sclerotial hyphae predominated.

4. **Microscopic observations of the cover-slip cultures**

Coverslip cultures of *C. ochracea* also confirmed the presence of substrate sporophores. A number of these structures formed on YEA are seen in Fig.5. Some of them were found to arise independently on the substrate mycelium, whereas others were found as simple terminal endings of the main hyphae which had spiralled into coils. The former type of sporophores were simple or branched and are shown in Fig.5. In this picture lateral branch initials can be seen on many of these sporophores near the coils. A solitary unbranched sporophore with a terminal compact spiral is shown in Fig.6. The sporophore appeared to stain slightly
Fig. 5. *C. ochracea*; substrate sporophores with tightly coiled terminal spirals; cover slip culture, YEA, 96 hrs, crystal violet, X 825.

Fig. 6. *C. ochracea*; a solitary substrate sporophore with a terminal coil arising directly from the substrate mycelium; cover slip culture, GAA, crystal violet; X 1900.
more intensely than the substrate hyphae. A magnified view of a number of substrate hyphae with terminal compact spirals are shown in Fig. 7. These hyphae did not show any differences in their stainability from the bulk of growth.

Sometimes, the substrate sporophores did not exhibit the usual type of structure as described above. Some of these sporophores were straight, some curved, while others formed loose to compact coils. They appeared to fragment into spores from early stages of their initiation and the length of these sporophores was highly variable. Whether this signified a basipetal mode of development of the spores in these structures is not certain. Many of the fragmenting sporophores are presented in Fig. 8 (light microscopy) and Fig. 9 and 10 (scanning electron microscopy).

Occasionally, substrate hyphae were also seen to fragment into spores. A mass of such fragmenting hyphae with lateral sporophores is seen in Fig. 11.

Production of the unusual type substrate sporophores and the fragmentation of the substrate hyphae as reported above appeared to be somewhat variable as revealed in the cover slip cultures of a number of single spore isolates. Sclerotia were, however, found in all these isolates as in the parent culture.
Fig. 7. C. ochracea; substrate growth with some hyphae terminating into tightly coiled spiral sporophores; cover slip culture, GAA, 72 hrs, crystal violet, X 2160.

Fig. 8. C. ochracea; substrate growth showing straight or spiral sporophores with chains of spores; cover-slip culture, GAA, 96 hrs, crystal violet, X 860.
Fig. 9. *C. ochracea*; scanning electron micrograph of substrate growth showing sporophores with chains of spores; note the formation of smooth, oval to elongated spores with a central depression; cover slip culture, GAA; X 3000.

Fig. 10. *C. ochracea*; scanning electron micrograph of a solitary sporophore with elongated spores. Details as in Fig. 9, X 10000.
Fig. 11. C. ochracea; substrate mycelium and substrate sporophores showing fragmentation into spores; cover slip culture, YEA, Crystal Violet; X 2100.
The results of the above studies have thus revealed that, besides presenting a characteristic sclerotal growth and the aerial sporophores\textsuperscript{26}, cultures of \textit{C. ochracea} produced substrate sporophores on the substrate mycelium which grew on the surface of agar or underneath it. Substrate sporophores, with chains of spores, on the substrate mycelium have not so far been described in any of the streptomycetes or chainiae, and this feature thus appeared to be a unique morphological characteristic of the organism, which may probably have an important bearing on its taxonomy.