Occurrence of Actinophage on Lysogenic *Streptomyces violaceoniger*

Actinophages were reported to occur in various species of *Streptomyces* viz., *S. venezulae* S13, *S. griseus* S104, *S. cinnamomeus*, *S. chrysomallus*, *S. olivaceus* and *S. aureofaciens*[1-7]. CLAIR and McCOY[8] isolated 9 streptomycete phages from soil and tested for host range and found that they were specific to the genus. It was demonstrated that *S. violaceoniger* was susceptible to the phage isolated from the homologous host, *S. griseus*[8].

During the study on antagonistic actinomycetes from Punjab soils in 1970–71, large numbers of streptomycetes were isolated. A culture which was identified as *Streptomyces violaceoniger* SV.17 was used extensively for different experiments for approximately one year. The culture was stored in slants without any transfer for 6 months at 4 °C. When the slant was used for transfer, it was found that the subculture did not grow well. After 4 days, the organism exhibited some small circular clear zones. In shaken broth cultures it showed turbidity in contrast to the spherical granular and flocculent growth characteristic of normal streptomycete. So it was thought that *S. violaceoniger* SV.17 might have carried some lytic agents since its isolation but that 'spontaneous' lysis occurred on the first subculture after storage at very low temperature for a considerable period. Experiments were conducted to confirm whether the lysis was due to phages.

Cultures were grown on glucose yeast-extract agar slants (peptone 0.5%; beef extract 0.3%; glucose 0.5%; yeast extract 0.01%; agar 2%; pH adjusted to 7.6 before autoclaving). Glucose yeast-extract broth was used for shaker grown cultures. Two strains of *S. violaceoniger* SV.17 and SV.17–1 were used throughout the experiments. Strain SV.17 looked like an apparently healthy streptomycete which did not exhibit any lysed areas consistently, while the strain SV.17-1 showed plaques in the growth which otherwise looked normal. The agar slant cultures and shaken broth cultures were incubated at 26–28 °C. Shaken broth cultures were grown in 250 ml flasks containing 50 ml of glucose yeast-extract broth on rotary shaker.

S. violaceoniger SV.17 is macroscopically a normal streptomycete. It has a thin branching substrate mycelium and a thick filament of aerial mycelium. The aerial mycelium fragments, although not very rapidly, and produces conidia spherical in shape arranged in spiralled conidiophores. Agar colonies are 2–4 mm in diameter, and are dark gray turning black. A violet-coloured pigment, which turns to black at a later stage of the growth, is produced. The strain, *S. violaceoniger* SV.17–1 is similar to that of the strain SV.17 in most of the characters. When it is grown on agar slants or dishes, the culture produces a true branching vegetative mycelium and typical dense aerial mycelium with chains of conidia on spiralled conidiophores. But numbers of lytic areas with clear zones of 0.5 to 1.5 mm in diameter were observed in the growth. When the plate was examined against light, small circular clear areas were visible. When the plaques were observed microscopically, the lytic areas were found to have hyphae of young substrate mycelium devoid of protoplasm which appeared as clear gaps. Pigment was observed only in the areas other than the plaques. WAKSMAN[7] and SHIRLING[9] observed similar phenomena which they referred to as 'ghost' hyphal walls connecting normal sections of the mycelium. These areas appeared to be the sites of phage release.

Both strains of *S. violaceoniger* were grown in shaken broth cultures. The flasks, in which SV.17 were inoculated, exhibited small spherical flocculent granules of macroscopic diamensions submerged in clear broth and the medium was free from turbidity, which is characteristic of the growth of streptomycete in broth[7,9]. But the flasks in which the strain SV.17–1 was grown, showed extreme opaque turbidity. The contents of the flasks in both cases were centrifuged at 3000 rpm for 15 min and the supernatants were passed through the bacteriological filter under suction. When 1 ml of the filtrate obtained from SV.17 was added to 4-day-old shaken broth cultures of the same strain, it did not have any effect. When 1 ml of the filtrate obtained from the flasks containing turbid growth (SV.17–1) was added to 4 days old shaken broth cultures of strain SV.17 the flocculent granular growth started disintegrating within 24 h from the time of addition of the filtrate and turbidity produced in place of clear broth. SHIRLING[9] reported that the turbidity was due to the direct consequence of mycelial breakdown following irregular and scattered lysis of segments within the hyphae and also due to short surviving segments. Lytic debris also contributed to the clouding. Experiments were repeated 3 times and consistent results were obtained.

The fact that *S. violaceoniger* SV.17, which was used for nearly 1 year and kept unattended for 6 months at 4 °C, produced spontaneously a variant which exhibited lysis, suggests that the organism carried the phage as a prophage from the time of its isolation from the soil. The organism, which may be considered as a lysogenic host, did not exhibit lysis as long as host and phage coexisted in a state of equilibrium and lysis occurred when there was a shift in the host-phage equilibrium. The occurrence of an actinophage on *S. violaceoniger* in nature has not been reported so far[10].

Zusammenfassung. Nachweis eines Actinophagen in der Kultur eines für den betreffenden Phagen wahrscheinlich lysogenen Stammes von *Streptomyces violaceoniger*. Das Vorkommen von Aktionsphagen von *S. violaceoniger* in der Natur ist bisher nicht beschrieben worden.

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