Occurrence of a Lysogenic *Streptomyces* sp. on the Nodule Surface of Black Gram (*Vigna mungo* (L.) Hepper)

M. RANGARAJAN,* A. DAVID RAVINDRAN, AND K. HARIHARAN

Centre of Advanced Studies in Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

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A lysogenic *Streptomyces* sp., strain NS.A4, which was isolated from the nodule surface of black gram (*Vigna mungo* (L.) Hepper), was found to inhibit rhizobia of fast- and slow-growing strains of cowpeas and soybeans. It exhibited plaques when there was a change in cultural conditions. Repeated culturing of the organism in nutrient agar and broth confirmed the infection of *Streptomyces* sp. strain NS.A4 by an actinophage. Addition of the culture filtrate of *Streptomyces* sp. strain NS.A4 to shaken broth cultures of three other *Streptomyces* spp. resulted in phage infection.

Actinomycetes, which are resident microflora of the rhizospheres of a number of legumes, are known to inhibit rhizobia (1, 3, 4–6) and cause unsuccessful nodulation in the field (2, 11). Several kinds of microflora, including fungi, occur on the surface of nodules, which may affect the development of nodules and the fixation of nitrogen by bacteroids at the postinfection stage (8). The actinomycetes, which are antagonistic to soil microorganisms, are inhibited by their viruses in nature (7), but information on the occurrence of actinophage on antagonistic actinomycetes residing on the nodule surfaces of legumes is not available.

Recently, during an investigation on the interbiotic relationship between resident microflora of the rhizosphere and nodules of certain tropical legumes and their rhizobia, a number of actinomycetes were isolated in Ken Knight agar (10) and tested against rhizobia for their antagonistic effect in nutrient agar by cross-streak assay (13). Preliminary results of the investigation revealed that several of the actinomycetes isolated from the nodule surface of black gram (*Vigna mungo* (L.) Hepper) inhibited both fast- and slow-growing *Rhizobium* strains of the cowpea group and *Rhizobium japonicum*. Zones of inhibition ranged from 3.0 to 17.5 mm in diameter, depending upon the strain. One among those isolates, strain NS.A4, exhibited a few small, circular, clear lytic zones on the streak which were not seen at the time of isolation. These lytic zones resembled plaques of actinophages. As it was thought that these lytic zones might have been caused by actinophages, experiments were conducted to confirm this hypothesis and also to determine whether the phage from one actinomyce (strain NS.A4) might infect other antagonistic actinomycetes.

Four isolates of actinomycetes, viz., strains NS.A2, NS.A5, RS.A5, and NS.A4, which were later identified as *Streptomyces* spp., were grown in nutrient agar and broth (peptone, 0.5%; beef extract, 0.3%; agar, 2%; pH adjusted to 7.0 before autoclaving). The agar plate cultures and shaken broth cultures were incubated at 26 to 28°C. Shaken broth cultures were grown in 250-ml flasks containing 50 ml of nutrient broth on a rotary shaker for 4 days. Strains NS.A2, NS.A5, and RS.A5 appeared to be healthy streptomycetes producing true branching vegetative and typical dense aerial mycelia with chains of conidia on spiral conidiophores. For strain NS.A4, several lytic areas with clear zones of 0.5 to 3.0 mm in diameter were observed. When the plate was examined against light, small, circular plaques 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. Shirling (9) and Waksman (12) observed similar phenomena which they referred to as "ghost" hyphal walls. These areas appeared to be the sites of phage release.

Shaker-grown broth cultures of the three isolates of *Streptomyces* sp. strains NS.A2, NS.A5, and RS.A5 exhibited small, spherical, flocculent clumps of macroscopic dimensions submerged in clear broth, and the medium was free from turbidity, which is characteristic of the growth of *Streptomyces* species in broth (9, 12). However, the flasks in which strain NS.A4 was grown showed extremely opaque turbidity. The contents of all four flasks were centrifuged at 3,000 rpm for 15 min, and the supernatants were passed through Millipore filters under suction. When a sample (1 ml) of the filtrate obtained from strain NS.A2, NS.A5, or RS.A5 was added to 4-day-old shaken broth cultures of the same strain or two other strains and incubated for 2 days, there was no effect. When 1 ml of the filtrate obtained from the turbid growth of strain NS.A4 was added to 4-day-old shaken broth cultures of strain NS.A2, NS.A5, or RS.A5, flocculent, granular growth started disintegrating within 48 h from the time of addition of the filtrate, and turbidity occurred in place of clear broth. Shirling (9) reported that the turbidity is caused by mycelial breakdown after irregular and scattered lysis of segments within the hyphae and also by short surviving segments. Lytic debris also contributed to the clouding. Repetition of the experiments by adding 0.5 ml of the culture filtrate of strain NS.A4 to 5 ml each of shaken broth cultures of NS.A2, NS.A5, and RS.A5 in test tubes resulted in turbidity, confirming the earlier observations of phage infection. Dilution titer plaque assays were carried out in nutrient agar media. The results revealed that the culture filtrate of strain NS.A4 was able to cause plaques in three other strains of *Streptomyces* up to a 1,000-fold titer (10-3). Experiments were conducted thrice, and consistent results were obtained.

Occurrence of an actinophage in an antagonistic *Streptomyces* sp., strain NS.A4, residing on the nodule surface of a legume is significant because of the inhibitory nature of the organism to rhizobia. Although the organism was isolated

* Corresponding author.
from the nodule surface of the legume, it is possible that it may reduce soil rhizobial population in nature, inhibit symbiosis, and cause poor nodulation.

We considered *Streptomyces* sp. strain NS.A4 to be a lysogenic host as it did not exhibit plaques at the time of its isolation from Ken Knight agar but exhibited several plaques when grown in nutrient agar. It is not known whether ingredients of Ken Knight agar suppressed the development of plaques. In nature, *Streptomyces* sp. strain NS.A4 might have carried the virus as a prophage and thus did not exhibit lysis when it was isolated in Ken Knight agar. As long as host and phage coexisted in a state of equilibrium, lysis did not occur. When there was a disruption in the host-phage equilibrium caused by a shift in cultural conditions, phage dominated, resulting in the formation of plaques. The occurrence of a lysogenic *Streptomyces* sp. on the nodule surface of black gram has not been reported previously.

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**LITERATURE CITED**


