drated through a graded ethanol series, coated with gold–palladium and observed in a Cambridge Stereoscan S_4-10 electron microscope. Autofluorescence micrographs were taken on a Carl Zeiss epifluorescence microscope with UV-range filters.

Two pairs of stipels occur on the pinnately trifoliate leaves, one at the petiolule–rachis junction of the first two leaflets and the other at the petiolule–rachis junction of the terminal odd leaflet. SEM observation reveals the presence of 25–30 minute pores distributed mostly at the top of the stipel mound (figure 1). The stipel is composed of parenchymatous tissues, the outermost of which is covered by a thick cuticle (figure 3), interrupted by pores (figure 2). The pore leads to a cavity through an orifice at the base of which is a pyramid-shaped nectary. The whole structure resembles an inverted funnel (figure 4). The wall of the cavity shows cuticular insulation (figure 3). The nectary has three distinct regions, viz. two large basal cells, two stalk cells and 8–14 secretory cells. Peak secretion occurs in the early morning hours and late evening hours. Both aggressive and non-aggressive ants are found foraging on the foliar nectary. The aggressive type of ant was identified as *Camponatus* sp. But the two types never occur together.

Aggressive ants nesting on *E. stricta* offer protection to the plant, for it is a well-known fact that the aggressive behaviour of ants is most pronounced near the nest and can be induced at a food source[8]. But these ants nesting on the leaves cause mild damage to the chlorophyll, since the leaf surface is not exposed to light for a long time and at times the abaxial surface of the leaf is directly exposed to sunlight causing an increase in transpiration. So with respect to aggressive ants on *E. stricta*, a strict symbiotic relation does not seem to exist. The non-aggressive ants nesting in the dried wood are not harmful to the plant.

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MICROPROPAGATION IN DIPLOID INDIAN SQUILL (*URGINEA INDICA* KUNTH.)

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INDIAN squill, a perennial bulbous plant of Liliaceae, contains the cardiac glycosides scillarin A and proscillarin b, and sterols[1]. It has been used since ancient times as a cardiac stimulant and a diuretic. The commercial use of Indian squill as a source of glycosides and sterols is economically not feasible owing to the extremely low rate of asexual multiplication. An earlier report on *in vitro* propagation of Indian squill[2] describes a complex and time-consuming method for regeneration of plantlets via callus culture. The purpose of the work reported here was to develop a simple and rapid method of *in vitro* propagation, through adventive plantlet formation, similar to those reported for other bulb-producing species[3,4].

Diploid Indian squill (*Urginea indica* Kunth.) bulbs were collected from Dona-Paula (Goa) seashore. The dried and outer scales of the bulbs were discarded and the bulbs were washed for one hour under running tap water. These bulbs were then surface-sterilized with 0.1% (w/v) mercuric chloride for 5 min, washed thrice with sterile distilled water, dipped in 95% ethanol and flamed over spirit lamp under sterile conditions. Outer scales from the bulbs were removed and the inner core was cut horizontally into explants of about 1 cm diameter and 0.5 cm thickness. The explants obtained from the lower half of the bulb were termed basal and those obtained from the upper half distal. The explants were placed for one week on basal medium and only those which were found to be free from visible contaminants were used in further experiments.

The basal medium used was of Murashige and Skoog[5] containing 3% sucrose and 0.6% agar; pH was adjusted to 5.8. In the experiments various concentrations of kinetin (0.5–4 mg/l) were added to the basal medium. The media were added in 20 ml aliquots into 25 × 150 mm tubes and autoclaved at 121°C, 1·08 kg/cm² for 20 min. Cultures were maintained at 25–28°C in the dark or under white fluorescent light of 2500 lux in an 8 h photoperiod.

Bulblets produced were separated and placed on a hormone-free MSb medium for 6 weeks for *in vitro* hardening and rooting. Rooted and unrooted propagules were transferred to a soil:sand:compost (1:1:1) mixture in pots which were then placed in a humid chamber for one week prior to transfer to the field. The plantlets were watered twice a week.

Disinfestation of the squill bulbscale was difficult. The least amount of contamination occurred when the bulbs were dipped in 95% ethanol for 30 sec and flamed after treating with 0.1% mercuric chloride for 5 min.

Bulblet initiation on the bulbscale occurred at all concentrations of kinetin tested (table 1), whether the culture was maintained in the dark or in the light. Initiation occurred in 100% of the explants and it was independent of the origin of the explant. However, the number of bulblets produced on the distal bulbscale explants was lower than that on the basal bulbscale explants (table 1). Bulblet formation occurred only on the inner (adaxial) surface of the explants. The number of bulblets produced was affected by the concentration of kinetin. Two weeks after the start of culture protrusions appeared on the inner surface of the scale. These developed into bulblets within six weeks, but the number and size of the bulblets were dependent on the concentration of kinetin. There was no significant difference in the number of bulblets on the different explants between 60 and 100 days. The best bulblets, which were about 0.5–1 cm thick and 1–2 cm in height, were obtained at low concentrations of kinetin (0.5 mg/l or 1 mg/l). At higher concentrations of kinetin (2 mg/l or 4 mg/l), although a greater number of bulblets were produced, their growth was stunted. Well-shaped bulblets could be produced on kinetin-free medium, though with less frequency.

Rooting of the bulblets was efficient when the bulblets were transferred and maintained on hormone-free MS medium. Root primordia appeared after one week and the roots grew to about 5 cm in about 4 weeks (figure 3). When rooted and non-rooted bulblets were transferred to sand:soil:compost (1:1:1) mixture and maintained in a humid chamber for one week and thereafter in the field, leaf emergence occurred within 2–3 weeks. Following leaf emergence, most of the bulblets developed roots.

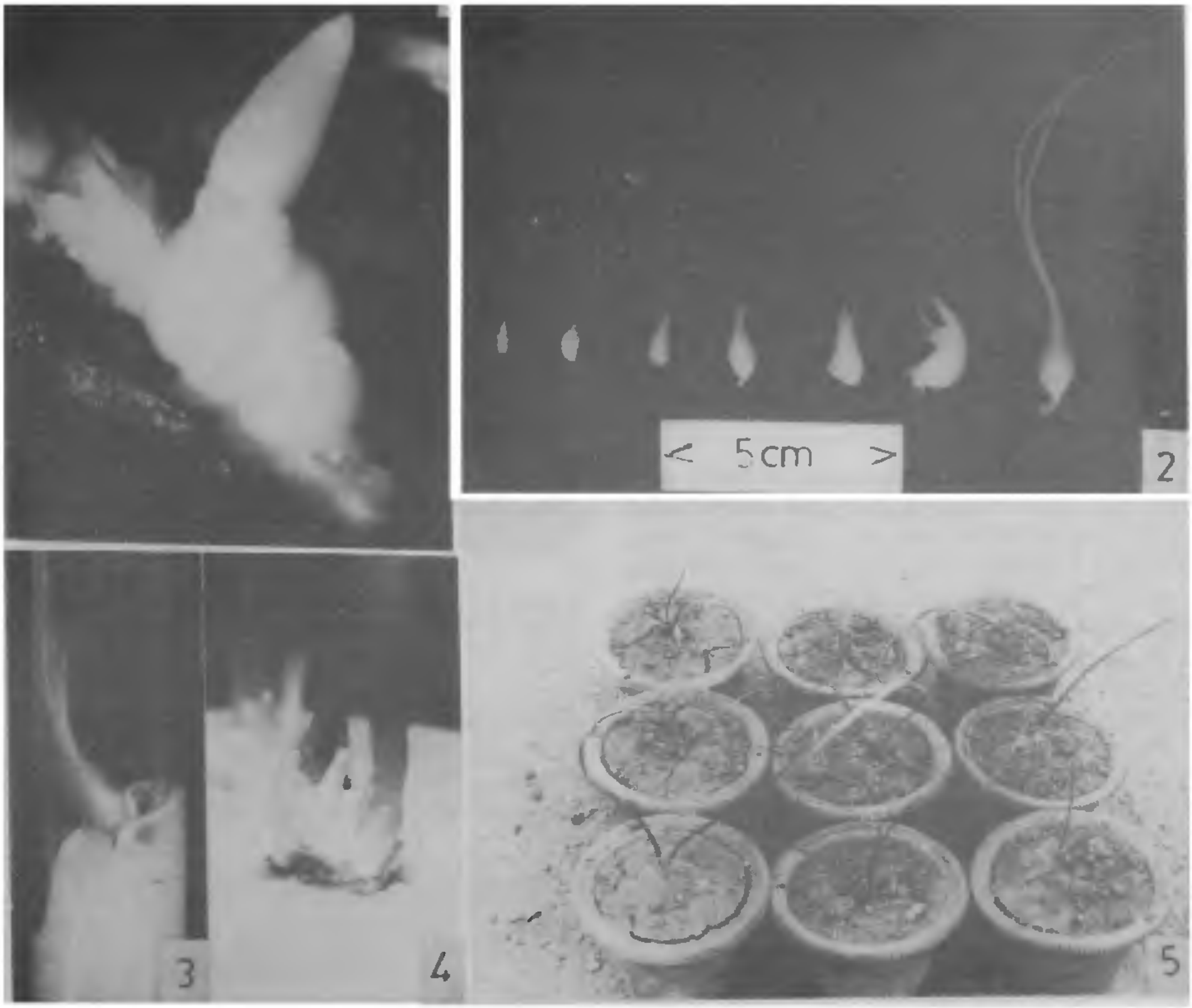
Table 1 *Effect of kinetin concentration on in vitro bulblet formation from bulbscale explants of Indian squill*

Kinetin (mg/l)	Mean number of shoots per bulbscale explant	
	Distal	Basal
0	2.65 ± 1.15	3.30 ± 0 78
0.5	3.90 ± 0.99	4.95 ± 0.92
1.0	4.90 ± 0.94	7.00 ± 0.89
2.0	7.60 ± 1.68	9.51 ± 1.56
4.0	7.75 ± 1.37	9.40 ± 1.60

Values are mean number of bulblets (± S.D.) developed on each 1 × 0.5 cm bulbscale explant after 40 days in culture. Each treatment was repeated twice with ten replicates.

In vitro bulblet formation has been observed in growth-regulator-free medium[6,7], or when low concentrations of cytokinins are used[4,8]. In Indian squill bulblet formation was recorded in growth-regulator-free medium. Addition of kinetin, however, enhanced the frequency of bulblets produced per explant, as reported for red squill[4]. Alternative procedures[2] described for *in vitro* regeneration of diploid *U. indica* Kunth. utilize 2,4-D and NAA to produce a callus intermediate from which bulblets can be derived and subcultured.

In liliaceous plants, development of adventitious plantlets has been reported from almost every plant part[9]. Our results clearly prove that adventitious bulblets can be induced on primary explants, contrary to the earlier report. *In vitro* bulblet formation on explants of Indian squill was found to be independent of photoperiod, as reported earlier in *Lilium*[10]. Regeneration of complete plantlet from the bulblet was most successful when rooting was induced on the bulblets immediately after their formation on the explants. Prolonged culture of bulblets on medium containing kinetin (1 mg/l) produced deformed shoots and rhizogenesis was found to be difficult on such shoots. Subculture of the bulblets on hormone-free basal medium produced roots and healthy shoots simultaneously, thus reducing the time required for rooting and hardening. The method described here for *in vitro* propagation of Indian squill is much superior to the earlier reports in its rapidity and in the simplicity of the medium.



Figures 1–5. **1.** Adventive plantlet formation on explant, after 3 weeks of incubation on MSb + 1 mg/l kinetin (× 2.5); **2.** Various stages of bulblet development; **3.** Development of shoot on bulblet after 3 weeks of culture on hormone-free MS medium (× 0.6); **4.** Deformed shoots developed due to prolonged incubation on MSb + 1 mg/l kinetin (× 2); and **5.** Plantlets, after 2 months of transfer to sand : soil : compost (1 : 1 : 1) mixture (× 0.6).

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ISOLATION OF NEW CYANOPHAGE An-1

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SINCE the discovery of a cyanophage in 1963[1], more than 100 types of cyanophages have been recorded in the past 25 years. These cyanophages have been broadly categorized into two main types—one infecting filamentous cyanobacteria and the other to unicellular or colonial forms[2]. During our studies on cyanophage infecting unicellular alga *Anacystis nidulans*, we have found a new type which is being reported in this communication.

During the present studies, four test organisms, viz. *Anacystis nidulans*, *Gloeocapsa* sp., *Gloeothece* sp. and *Synechococcus cedrorum* were selected for initial screening against 19 strains of cyanobacteria being maintained in the National Facility for BGA Collections. The screening was done by soaking 5 mm diameter sterilized filter paper discs in the culture

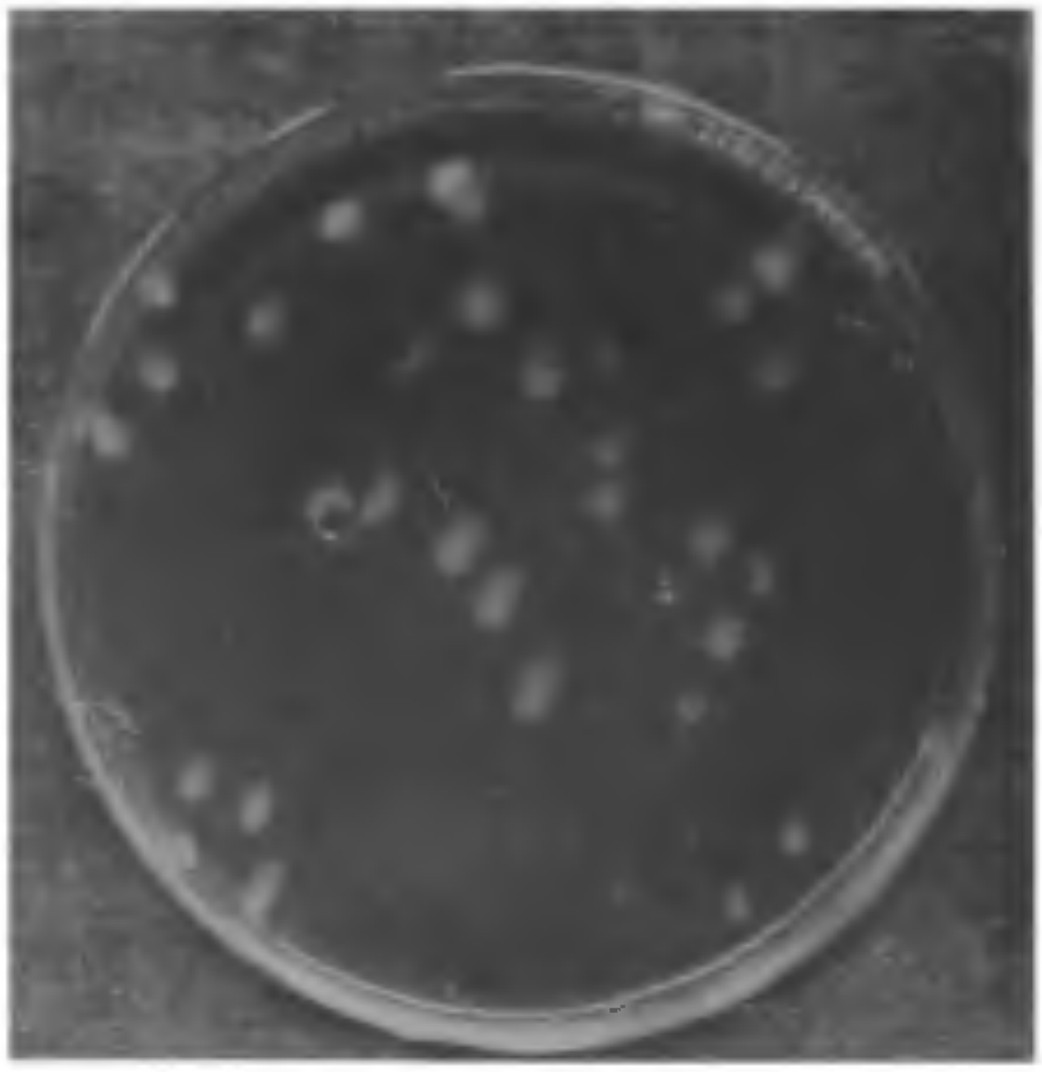

Figure 1. Plaques of An-1 on the lawn of *Anacystis nidulans*.

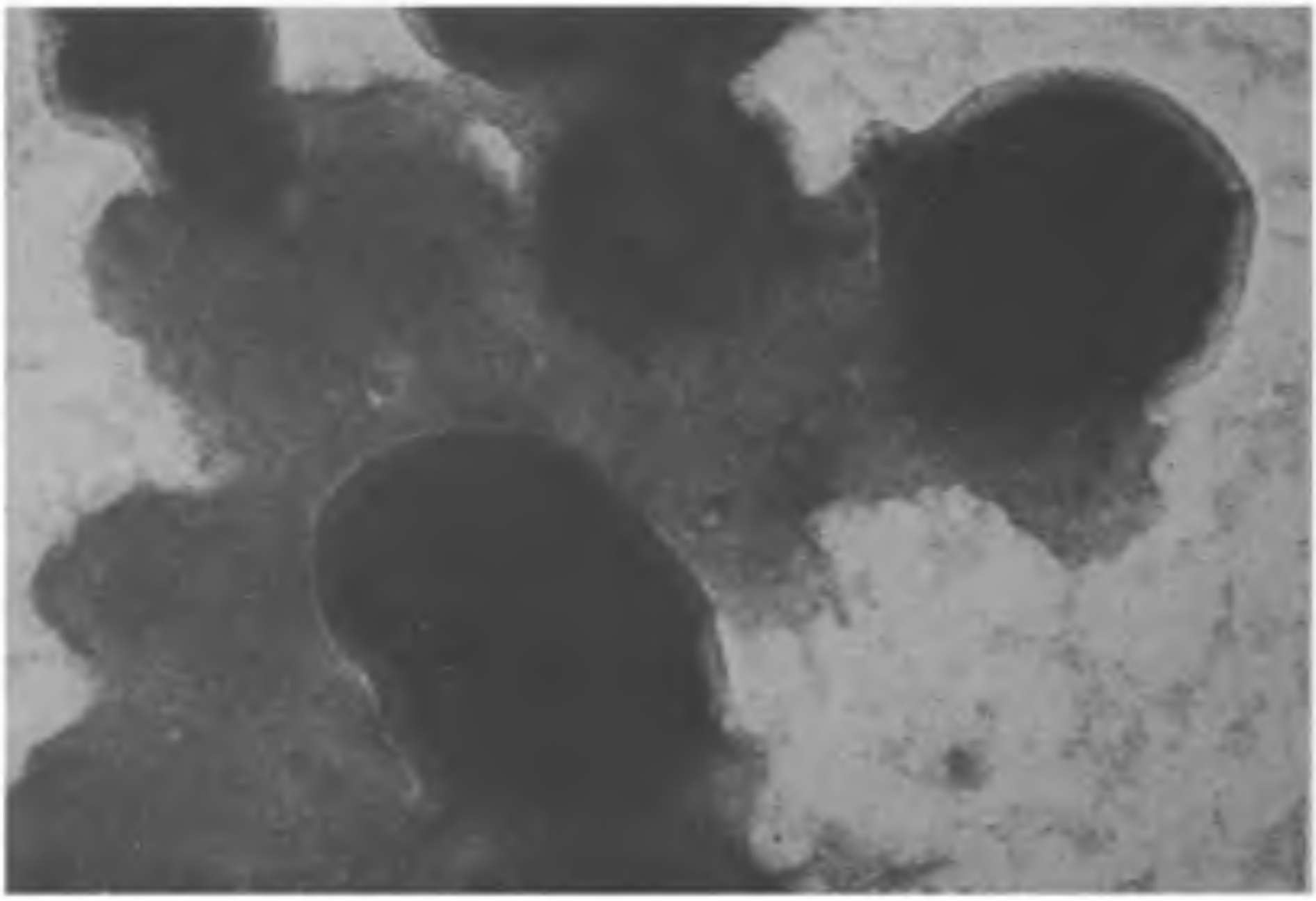

Figure 2. Head-tail type cyanophage An-1 specific to *Anacystis nidulans*.