ESTABLISHMENT OF NEW CELL LINES FROM *AEDES ALBOPICTUS* LARVAL CELLS IN MEDIA CONTAINING CALF AND GOAT SERA

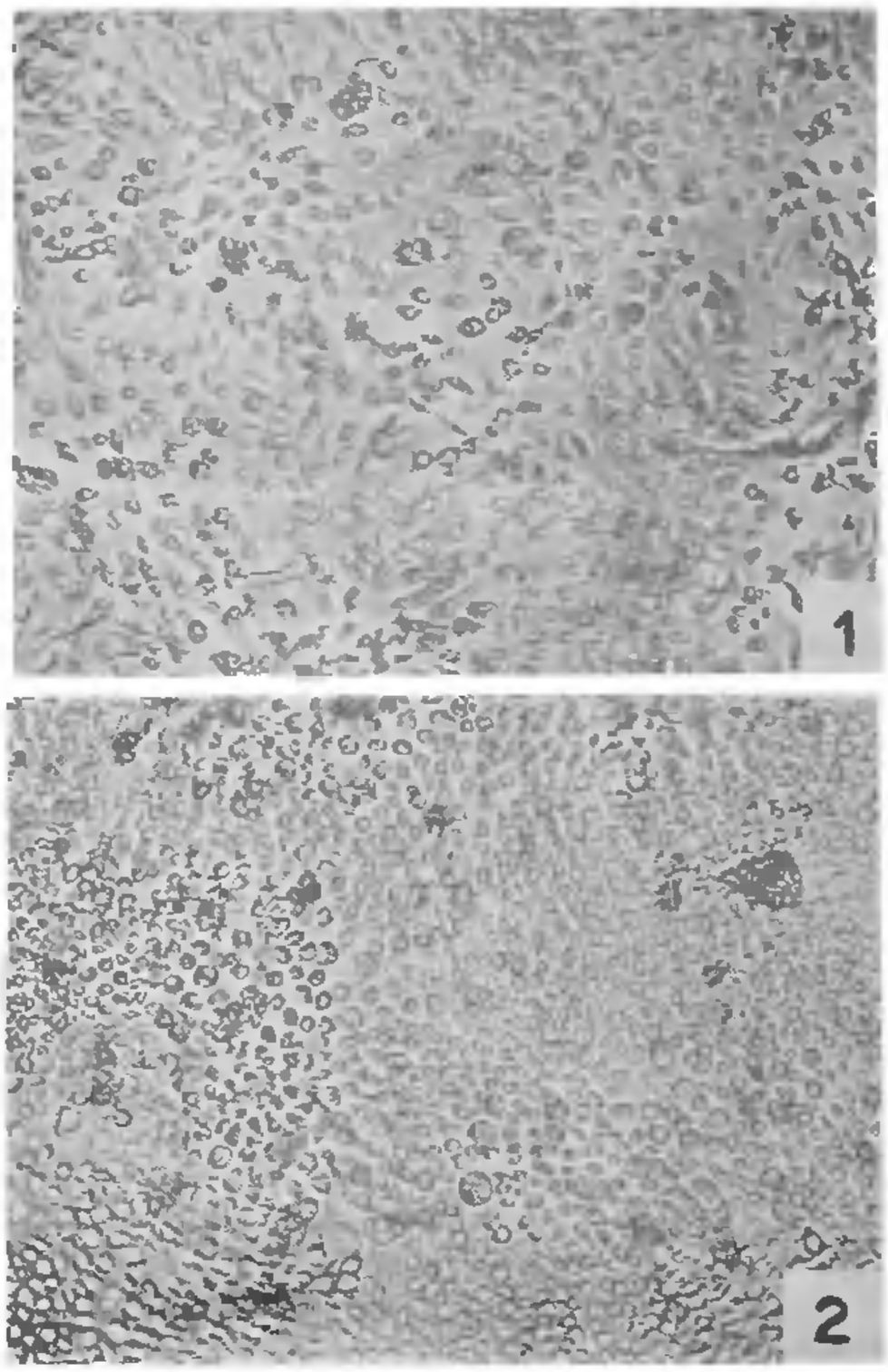
SEVERAL cell lines from larval tissues of *Aedes albopictus* and *Aedes aegypti* have been established by Singh[1] in a culture medium containing 20% fetal bovine serum (FBS). One of the cell lines of *A. albopictus* (ATC-15) has been successfully adapted to grow in the culture medium containing calf or goat serum in place of FBS[2]. Further attempts were made in this laboratory to establish new cell lines from *A. albopictus* and *A. aegypti* larval cells in the culture medium containing calf or goat serum, which are easily and locally available. This communication reports the successful establishment of two new cell lines from larval tissues of *A. albopictus,* one each in the culture medium containing calf or goat serum.

The techniques employed for the preparation of primary cultures, establishment and maintenance of the cell lines, and the culture medium used were essentially the same as described by Singh[1]. In the medium the FBS was replaced with the same volume of calf or goat serum, which were filtered through Seitz and Millipore filters before use. Collection and processing of these sera were reported earlier in detail[2].

The two new cell lines of *A. albopictus,* one in the medium containing calf serum (ATC-89) and the other in the medium containing goat serum (ATC-91), were initiated on 12th and 26th September, 1968 respectively. The cells have been sub-cultured 28 and 37 times respectively before they were stored in liquid nitrogen in the culture medium containing 20% FBS and 10% glycerol. The cells stored in liquid nitrogen were regenerated successfully after 150 days.

The cell population of both the new cell lines consisted of multiple cell types as observed in the original *A. albopictus* cell line[1,3]. In both the cell lines round epithelial-like and spindle-shaped fibroblast-like cells formed the major part of the cell population during the first ten passages. A change in the cell population of both the cell lines was observed during the course of further passages. After about 20 passages the calf serum cell line had more of spindle-shaped fibroblast-like cells (60 to 70%) and less of round epithelial-like cells (Fig. 1); whereas, the goat serum cell line consisted mostly of round epithelial-like cells (Fig. 2). The cell population in both the cell lines consisted of 70 to 80% diploid cells ($2n = 6$), and the rest were polyploid.

Attempts to establish new cell lines from *A. aegypti* larval cells in the medium containing calf or goat serum were not successful.



FIGS. 1–2. Fig. 1. *Aedes albopictus* cell line in medium with calf serum (Living culture), × 80. Fig. 2. *Aedes albopictus* cell line in medium with goat serum (Living culture), × 80.

I wish to thank Dr. K. R. P. Singh for his advice and keen interest during the course of the present study.

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Poona (India), *August* 11, 1972.

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RELATIONSHIP BETWEEN VIRULENCE AND AUXIN PRODUCTION BY *RHIZOBIUM* ISOLATES FROM GROUNDNUT

AMONG the various native rhizobia avirulent strains often predominate[1]. Hence scrupulous selection of rhizobial strains is necessary to ensure effective nodulation. However, assessment of

virulence of rhizobia is quite laborious and time-consuming[2,3]. Hence attempts are being made by some workers to correlate the virulence of the rhizobial isolates with their various biochemical characters. Gupta and Sen[4] reported that there was a positive correlation between the rate of glucose consumption by the rhizobial isolates and their virulence. Shemakhanova and Oleinikov[5] were able to distinguish virulent and avirulent lucerne bacteria by the specific activity of their dehydrogenases in pure culture. The present study is to assess the relationship between auxin producing capacity of the rhizobial isolates and their virulence.

Rhizobium sp. was isolated from groundnut (*Arachis hypogaea* L.) from different parts of Tamil Nadu. The virulence of these isolates was tested by growing groundunt in a nitrogen-free mineral agar medium[6] and inoculating with the various isolates. After 30 days, the number of nodules formed, weights of individual nodules and root and shoot lengths of the plants were assessed.

Indole acetic acid (IAA) production by these isolates was assessed by growing them in yeast extract mannitol broth containing 0·1% DL-tryptophan. The inoculated flasks were incubated under darkness for 10 days at 28 ± 2° C and then culture filtrates were obtained. The filtrates were adjusted to pH 3·0 with 1 N HCl and extracted with equal volume of peroxide-free ether at 20° C for three times. The ether was evaporated *in vacuo* and the residue was dissolved in 2 ml methanol. The methanol extract was spotted on Whatman No. 1 paper and developed descendingly with *n*-butanol : acetic acid : water (4 : 1 : 1) for 10–12 hr in darkness. The paper was sprayed with Salkowski reagent. The detected IAA was quantitatively estimated by using Salper reagent[7]. Three replications were maintained in all the experiments and the experiments were repeated once.

The results presented in Table I revealed that only three out of eight isolates were highly virulent. The virulent isolates produced more number of nodules and the nodules were also bigger. The inoculation with effective strains increased shoot and root elongations also. The isolates which produced more number of nodules and bigger nodules produced more IAA also *in vitro*.

Nodule and gall formation in plant tissues has been attributed due to the accumulation of auxins[8]. Auxins have been found to be responsible for the shoot and root elongation[9]. Hence it is likely that bigger nodule formation and increases in shoot and root elongations may be due to production of auxins by the virulent rhizobia. Novikova and Irtuganova[10] reported that *Rhizobium lupini* did not produce IAA *in vitro*. However, IAA production by *R. meliloti*[11] and by *R. trifolii*[12] has been reported. Georgi and Beguin[13] could not obtain any correlation between the quantity of IAA produced by the isolates of *R. meliloti*, *R. trifolii*, *R. leguminosarum* and *R. phaseoli in vitro* and their virulence. Dullert[14] suggested that *R. lupini* induced the synthesis of IAA in root nodules by the host (lupine) itself. IAA production by *Rhizobium* sp. from groundnut has not been reported previously. Further studies on this line to examine other species and isolates of *Rhizobium* for their ability to produce IAA could lead to this character being used for identifying virulent strains of the organism.

TABLE I

Effect of different isolates of Rhizobium *sp. on the nodulation and vigour of groundnut plants*

Isolate No.	Place of collection	Number of nodules per plant	Mean wt. of nodules in mg	Shoot length in cm	Root length in cm	IAA production in ppm
1	Aliyarnagar ..	6	18·4	14·0	12·5	40·0
2	Kinathukadavu ..	11	21·0	14·5	11·0	15·0
3	Pollachi ..	13	19·5	15·3	12·2	45·0
4	Tindivanam ..	60	29·5	18·6	13·5	75·0
5	Tirupathur ..	10	18·0	14·7	12·0	52·3
6	Tiruvannamalai ..	84	33·5	19·0	14·0	102·0
7	Vellore ..	20	23·8	16·0	13·0	57·5
8	Villupuram ..	49	32·0	18·0	13·7	82·3

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