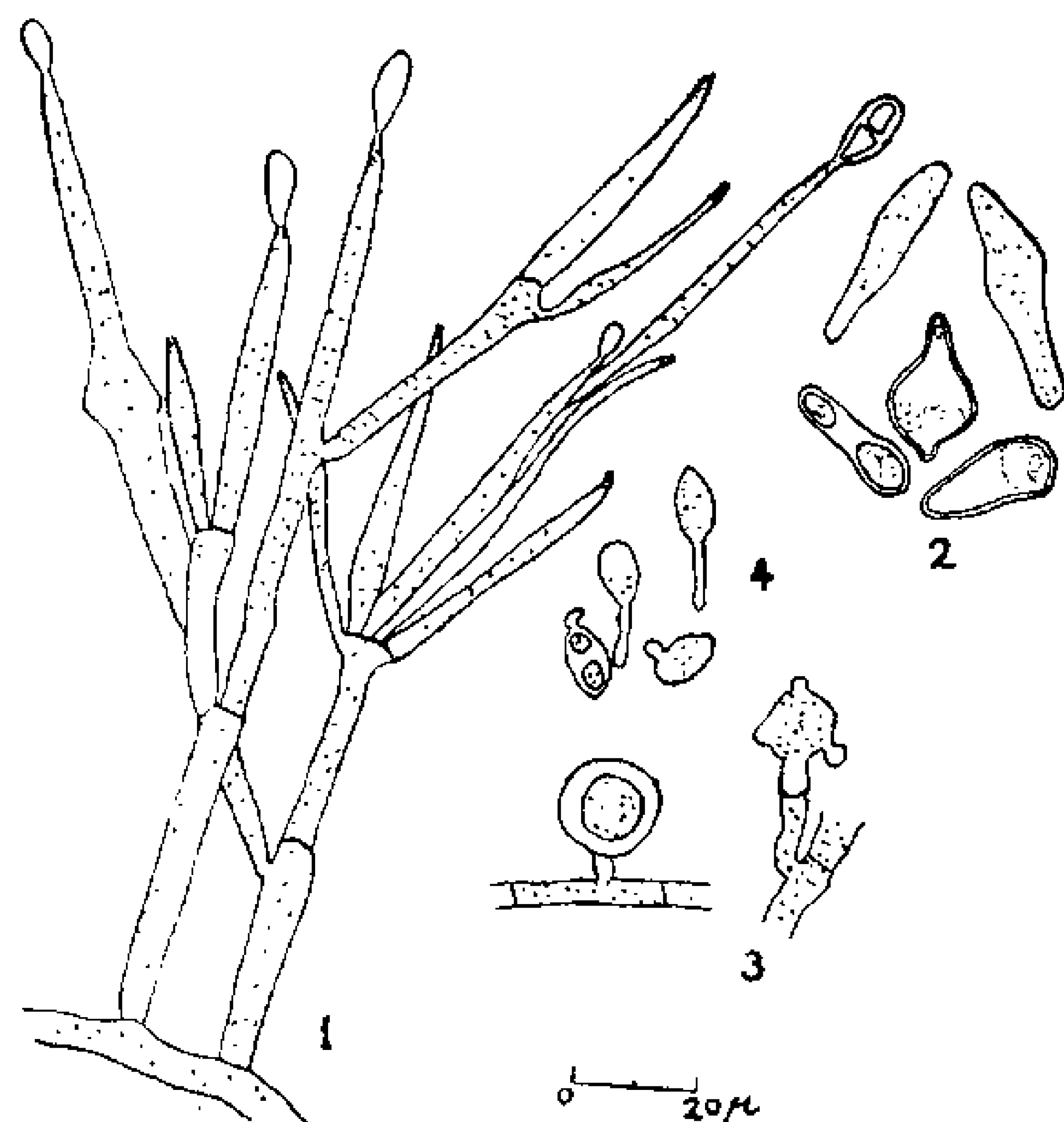
The growth of the fungus was found to be the best on Richard's medium in which after a week the diameter of the colony was 42 mm.



FIGS. 1–4. *Cephalosporium kashiensis* sp. nov. Fig. 1. Branched phialides and conidia. Fig. 2. Conidia. Fig. 3. Acrogenous chlamydospores with hyaline stalks. Fig. 4. Germinating conidia.

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Department of Botany, Banaras Hindu University, Varanasi-5, *October* 25, 1967.

R. Y. Roy.
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ON THE IDENTITY OF *TEPHROSIA JAMNAGARENSIS* SANTAPAU AND *T. AXILLARIS* A. R. SMITH FROM GUJARAT

A NEW species of *Tephrosia* Pers., namely *T. jamnagarensis* was described by Santapau in 1958 based on fruiting twigs from Jamnagar in Saurashtra and the *type* (*Santapau* 7522) has been deposited in the Blatter Herbarium, Bombay. He could not examine any flowering specimens of the new species but even in the absence of any flowers, it was obviously quite distinct from all other known species of *Tephrosia*. The new species is closely allied to *T. strigosa* Sant. and Mahesh. differing in the structure of leaves, size and form of legumes and the brevity of the peduncles. Subsequently in the *Flora of Saurashtra* (1962), he has indicated that Shri Ahluwalia has also recollected the same species with young flower buds on 24th August 1954 from Victoria Bridge in Jamnagar. Recently Ahluwalia and Smith (*Kew. Bull.*, 1967) have described another new species of *Tephrosia*, viz., *T. axillaris* A. R. Smith from Jamnagar itself based on both flowering and fruiting twigs. An analysis of the description of *T. axillaris* indicates that it fully conforms to the type of *T. jamnagarensis* and the description aptly fits the fruiting twigs and leaves of *T. jamnagarensis*. Moreover, *T. axillaris* is also partly based on the collection from Ahluwalia from Victoria Bridge, the identical locality indicated by Santapau in *Flora of Saurashtra* but the date of collection is given as 25th August 1954. There is no doubt that both *T. axillaris* and *T. jamnagarensis* are identical in all respects, though for the first time the floral details have been described under *T. axillaris*. *T. jamnagarensis* is not so rare and a careful survey is bound to reveal additional localities in Gujarat State.

Since *T. jamnagarensis* has been validly published earlier in 1958, *T. axillaris* should be treated as a synonym under the former species. The citation should now read as follows:

Tephrosia jamnagarensis Santapau in *Proc. Nat. Inst. Sci. India*, 24B : 133, t. 1, 1958, et *Fl. Saur.*, 1 : 134, 1962.
= *T. axillaris* A. R. Smith in *Kew Bull.*, 21(2): 311, t. 1, 1967.

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Botanical Survey of India, 7-Koregaon Road, Poona-1, *June* 13. 1968.

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METHODS OF CULTIVATION, STANDARDIZATION AND QUANTITATIVE ESTIMATION OF *ENTAMOEBA HISTOLYTICA*

FOR my studies on *Entamœba histolytica* at Carnegie Institute of Technology, Pittsburgh, Pa. U.S.A., I have found the following methods for the cultivation and quantitative estimation of amœba as very convenient and accurate. The strain of *E. histolytica* used in these studies was first isolated by Tobie (1949) at sigmoidoscopy from a patient with amœbic

dysentery. The organism was later transferred to a dog and subsequently isolated in pure culture. The strain was later maintained by Dr. C. W. Rees of the National Institute of Health, Bathesda, Maryland, and is now known as NIH strain 200. The choice of this strain was made on the basis of its rapid growth, which reaches a maximum count after 48 hr. of incubation, and because of the large size of the trophozoites.

Cultivation of E. histolytica.—The Modified Shaffer and Frye medium (MS-F) (Reeves, Meleney and Frye, 1957) was used for culturing amoebæ in the laboratory. The MS-F medium consisted of 1·5 g. of thiomalic acid first dissolved in 1000 ml. of distilled water; Trypticase (BBL) 2%; Dextrose (Difco) 1%; sodium chloride, 0·25%; and $K_2HPO_4 . 3H_2O$, 2%, adjusted to pH 7·0 with 1 M sodium hydroxide. The medium was dispensed in 12 ml. portions in screw cap tubes (16 mm.) and autoclaved at 121° C. at 15 pounds for 15 min. All inoculations were made into the above medium and incubated at 37° C. over a period of 48 hr.

A second cell line was also maintained on Endamœba Medium (Difco) (Cleveland and Collier, 1930) slants. An inoculum consisting of 0·5 ml. of a 48 hr. culture of amœba and 0·5 ml of penicillin inhibited *Bacteroides symbiosus* cells was used on the slants. The slant culture also received 0·5 ml. each of Horse-Serum-Saline (1 : 6) (Difco). These slants were incubated at 37° C.

Standardization of Organism for Inoculum.—A 48 hr. culture of *B. symbiosus* grown in thioglycollate medium was centrifuged and the cells were suspended in 0·85% physiological saline. The turbidity was adjusted to 35% T with a Bausch and Lomb Spectronic 20 Spectrophotometer at a wavelength of 650 mμ. The bacterial count was established in Levy's Hemacytometer counting chamber. A saline suspension of *B. symbiosus* at 35% transmittance has a total count of $6·5 \times 10^6$ cells per ml. In all experiments 0·5 ml. of the above standardized penicillin inhibited cells was used as an inoculum.

A 48 hr. old culture of *E. histolytica* strain NIH 200 grown in 12 ml, portions of MS-F medium gives approximately 4×10^5 trophozoites per ml. as counted in Levy's chamber. An inoculum of 0·5 ml of this culture was used in all experiments.

Methods of Enumerating the Amœbæ.—All counts were made using a Levy's hemacytometer. The standard procedure used in counting the amœbæ was to make a 1 : 10 dilution of 1 ml. of amœba culture in MIF (Merthiolate-Iodine-Formalin) saline solution. The MIF (Sapero and Lawless, 1953) gives a bright colour stain to the amœbæ. The MIF stain-fixative consists of 10% solution of Tincture of Merthiolate (No. 99 Ely Lilly and Co. 1 : 1000) in distilled water; 10% formaldehyde (USP); and 2% glycerin. To each 1 ml. of MF is added 0·1 ml. of freshly prepared 5% Lugol's solution.

MIF stain-fixative is a recommended procedure used by the U.S. Naval Medical Center for the identification of amœbæ in the stool specimens. In this research (Ahmad, 1964), however, the stain was exclusively used for counting amœbæ in the Levy's chamber. Each 1 ml. of amœba culture to be counted was added to 3 ml. of MIF and 6 ml of physiological saline. This gives 1 : 10 dilution of the culture in which all amœbæ are fixed and stained. Counting in this manner minimizes the chances of overlooking the amœbæ in the chamber, and moreover, because of 1 : 10 dilution of the culture, there are fewer bacteria under the field. All counts were made in duplicate, counting all squares in the central chamber. An average of two counts was used for establishing the final number.

Dept. of Biology, Delhi College, Delhi, *May* 23, 1968. SOHAIL AHMAD.

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