sidered these contents in *P. rectinatà* as those of tannin, but the present authors observed (on microscopical examination) that the contents of *P. sylvestris* were motile. With a view to ascertain if these yellowish brown contents were living organisms, some of the aerial roots collected from different plants of *P. sylvestris* were inoculated on meat-infusion agar, Czapek agar, Congo-red media of Kellerman[4] and of Leonard[6] after that they had been treated by a mercuric chloride solution as recommended by Harrison and Barlow.[3] The plates on incubation revealed the growth of some colonies: yellow colonies were observed on meat-infusion agar; on Kellerman's medium the colonies (whitish) were practically not absorbing the dye; on Leonard's they were clearly coloured but were not typically looking like those of *A. radiobacter*; the Czapek agar gave rise to slimy white colonies like those of *Klebsiella pneumoniæ*.

The apparently different organisms isolated on these media were eventually proved to be one and the same species; the pigment appearing on meat-infusion agar was lost on Czapek for mucilage and *vice versa*. The micro-organism on routine cultural examination consistently revealed the following characteristics:—

Morphology: Rods, 0·54 to 0·75 by 1·13 to 1·41 microns, actively motile, peritrichically flagellated, non-capsulated, non-spore bearing and found in groups. Gram negative. *Infusion agar slant*: Abundant, slightly slimy, translucently greenish-yellow. *Agar colonies*: Minute, moist, raised, circular, greenish-yellow. *Czapek agar*: Abundant, slimy, white; no sign of yellow pigment. *Infusion broth*: Heavy, general turbidity, thin pellicle, mucoid deposit. *Peptone water*: Moderate, otherwise same as in the broth. *Potato*: Abundant, lemon-yellow to light red pigment. *Gelatine colonies*: Slimy, not well-pigmented; gelatine not liquefied. *Nitrates*: Reduced to nitrites. *Indol*: Not formed. *Hydrogen sulphide*: Not produced. *Sugar media*: No observable reaction; abundant growth in presence of mannite, glucose, lactose, dextrine and maltose, but not saccharose. *Starch*: Feebly hydrolysed. *Milk*: Strongly alkaline. *Pigment*: Slightly soluble in water, soluble in 95 per cent. alcohol, insoluble in chloroform, ether and carbon disulphide. *Carbohydrates agar*: Whitish colonies, very slimy instead of pigmented. Presumably mucilage prevents chromogenesis by cutting off the oxygen supply. *Carbohydrate-free agar*: Solidified peptone water proved to be a poor medium; meat-infusion agar (with sugars eliminated by *E. coli growth*) was the best for chromogenesis. *Adaptation Power*: Eight months' adaptation on infusion agar made it lose to a great extent its slime production on Czapek; the property was regained in serum-milk. *Optimum temperature*: 28° C. *Nitrogen fixation*: Slight power.

All the observations lead to the conclusion that this is a new species; but because of its occurrence in the endophytic state and of its resemblance to *A. radiobacter* (despite very many well marked differences) the authors are inclined to label this new species as a variant of *Alcaligenes radiobacter*.

SUMMARY

Presumably a new micro-organism, but labelled for convenience as a variant of *Alcaligenes radiobacter*, isolated from the aerial roots of *Phœnix sylvestris* is described at length.

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A PRELIMINARY NOTE ON THE ANTIBACTERIAL SUBSTANCE FROM *ASPERGILLUS FLAVUS*

WHITE (1940) first observed that *Aspergillus flavus* when grown on certain liquid medium yielded a filtrate that showed anti-bacterial activity against gram-positive and gram-negative bacteria. Glister (1941) obtained anti-bacterial concentrate from *Aspergillus flavus* but did not isolate it in a crystalline form. Bush and Goth cultivated *Aspergillus flavus* on the surface of the liquid medium and obtained an anti-bacterial substance. This substance which is soluble in ether and water was called Flavicin. It was extracted by the authors with isopropyl ether in an atmosphere of carbon dioxide. The crude product, toxic to mice, was found to be innocuous after purification. Waksman and Bougie (1943) used six strains of *Aspergillus flavus* and five of *Aspergillus flavus orizæ*, the latter yielding little or no anti-biotic substance. According to them two factors were responsible for the anti-biotic activity, namely, aspergillic acid, which is active both against gram-positive and gram-negative bacteria and flavicin mostly active against gram-positive bacteria and, therefore, very similar to penicillin. The strain of *Aspergillus flavus* under submerged condition of growth, produced enough flavicin to be compared favourably with penicillin produced by the best strain of *Penicillium notatum* grown under similar conditions. McKee and MacPhillany (1943) found from *A. flavus* antibacterial substance unlike aspergillic acid and closely resembling penicillin in its biological and chemical nature. Menzel *et al.* (1943) gave

detailed comments on anti-biotic substance elaborated by a strain of *Aspergillus flavus* and an unclassified mould. The authors fully explained the chemical nature of pure aspergillic acid in crystalline form which was tested against and staphylococci and other organisms in high dilutions. *Jones et al.* (1943) also found similar substance using the same strain of the fungus, but one of the variants showing Chlamydospores gave more potent antibacterial substance than others.

MATERIALS AND METHODS

A strain of *Aspergillus flavus* which was found to contaminate a flask of tomato juice and giving a zone of inhibition (25 mm.) against *Staphylococcus aureus* by cup-and-plate method was selected for the work. This fungus when grown on Difco Peptone-agar at pH 7·6 gave an inhibition zone of about 15 mm. (when the growth is 2 mm. in two days). The fungus when grown on Sauboraud's medium (glucose-agar) showed yellow spores at first, which gradually became green, changed to deep green and later dirty brown in colour. The strain was maintained in Sauboraud's medium, but before inoculation it was cultivated in test-tubes containing bran sterilized by autoclaving. Yellow green spores developed in these tubes in three days when they were easily dispersible, and inoculated on the surface of the liquid medium. Cultivation of spores direct from Sauboraud's medium gave similar results.

Strains of *A. flavus* isolated from different sources, namely, bread, wheat, flour, fruits, etc., were found to give antibacterial substances in approximately same quantities when grown under similar conditions. All the cultures were maintained at the room temperature varying from 25° C. to 35° C. at Calcutta and no degenerative changes were noticed. A strain of *A. flavus orizæ* which was used for the routine production of Taka-diastase was tried for its antibacterial activity with respect to the development of antibacterial substance in different media described elsewhere. This did not produce any antibacterial substance whatsoever. Some of the strains when used to produce diastase were found to be as rich in diastase as any of the preserved strains of *Aspergillus flavus oryzæ*.

Various laboratory media were tried to find one that would give a maximum titre of antibiotic substance against *Staphylococcus aureus*. As a rule liquid media were used, but semi-solid medium containing ·5 per cent. agar gave

TABLE I

Serial No.	Medium	pH	Growth and sporulation	Maximum dilution of the filtrate in which antibacterial activity (a.a.) is marked against *Staphylo aureus*.
1	Doglas broth	7·6	Thin mat. Few spores 7th day.	1/40 dil.
2	Semi-solid agar	7·6	Thick white mat. Spores Nil.	1/80 dil.
3	Bacto-peptone 2%	6·7	Soft moist mat. Spores Nil.	1/10 dil.
3*a*	Bacto-peptone 2%	7·4	Soft moist mat. Spores Nil.	1/40 dil.
4	Bacto-peptone 2% & 2% cane sugar ..	6·7	Thin mat with green & then brown spores. Spores 3rd day.	No a.a. in 1/10 dil.
4*a*	Bacto-peptone 2% & 2% cane sugar ..	7·4	Thin mat with green & then brown spores. Spores 3rd day.	1/10 dil.
5	Bacto-peptone 2% & sod. acetate 1%	6·7	Thin moist mat. Spores Nil.	No a.a. in 1/10 dil.
5*a*	Bacto-peptone 2% & sod. acetate 1%	7·4	Thin moist mat. Spores Nil.	No a.a. in 1/10 dil.
6	Bacto-peptone 2% & 2% cane sugar & sod. acetate 1%	6·7	Thin moist mat. Spores 3rd day.	No a.a. in 1/10 dil.
6*a*	Bacto-peptone 2% & 2% cane sugar & sod. acetate 1%	7·4	Thin moist mat. Spores 3rd day.	1/10 dil.
7	Casein peptone 2%	6·7	Moist mat. Spores Nil.	1/10 dil.
7*a*	Casein peptone 2%	7·4	Moist mat. Spores Nil.	1/40 dil.
8	Casein peptone 2% & cane sugar 2%	6·7	Thick mat with moist areas on surface. Spores Nil.	1/10 dil.
8*a*	Casein peptone 2% & cane sugar 2%	7·4	Thick mat with moist areas on surface. Spores Nil.	1/50 dil.
9	Casein peptone 2% & sodium acetate 1%	6·7	Thick mat. Spores Nil.	1/50 dil.
9*a*	Casein peptone 2% & sodium acetate 1%	7·4	Thick mat. Spores Nil.	1/10 dil.
10	Casein peptone 2% & cane sugar 2% & sod. acetate 1%	6·7	Thick crumpled mat with dew drops like moist areas on the surface. Spores Nil.	1/10 dil.
10*a*	Casein peptone 2% & cane sugar 2% & sod. acetate 1%	7·4	Thick crumpled mat with dew drops like moist areas on the surface. Spores Nil.	1/80 dil.

very satisfactory results. Various modifications and combinations of Czapeck-dox synthetic media with and without cane-sugar and also in combination with steep water, yeast extract and peptone were tried without any encouraging result. At this stage it was found that nutrient agar which was prepared from trypsin-digested meat gave a good growth of the fungus at pH 7·6 without spore formation and showed inhibition against *Staphylococcus aureus*. So trypsin-digested broth was selected for further study of the fungus. The initial pH of the medium at which antibacterial activity was marked was between 7·4 to 7·6. Trials were conducted with casein-peptone (trypsin-digested), bacto-peptone with and without sucrose at pH 6·7 and 7·4. Sodium acetate was supplemented in similar series. The results of all these experiments are shown in Table I. From third day after the inoculation the antibacterial activity was observed and the maximum development of antibacterial substance was noticed between ninth and thirteenth day depending on the growth of the fungus mat.

The following characteristics may be pointed out regarding the development of the antibacterial substance :—

(1) Development of the antibacterial substance depends on the particular strain of the fungus.
(2) Formation of good mat is essential but without sporulation.
(3) Sporulation is harmful for the development of antibacterial substance.
(4) pH 7·4 gives a better result for development of antibacterial substance than a pH 6·7 or 6.
(5) In all cases antibacterial substance was found to develop between nine and thirteen days after inoculation of fungus depending on the quality of mat-formation.
(6) The final pH of the medium was found to be between 8·4 and 9 when the maximum antibacterial activity developed.

CONCENTRATION

An attempt was made to concentrate the filtrate containing the antibacterial substance and the following process was adopted.

The fungus-free filtrate which was slightly alkaline in reaction was treated with acetic acid till the pH was 3·5. This was then adsorbed by activated charcoal (about 2 per cent. W/V). (A second adsorption is necessary if the filtrate is not clear.) Charcoal was filtered after 3 hours when clear filtrate was recovered. The charcoal was dried on the filter-paper. The dry charcoal was then eluted with ether or chloroform and refluxed for 4-6 hours.

The elution was found to be incomplete with ether and, therefore, in later experiments it was eluted with chloroform. After the elution charcoal was filtered off and the clear dark orange coloured chloroform extract was evaporated to dryness.

The residue was treated with 2 per cent. sodium bicarbonate and tested for antibacterial substance. It was found that the total antibacterial unitage in the whole fluid could be approximately recovered. Further work on the isolation of the active principle is under progress.

The advantages of antibiotic substance from *Aspergillus flavus* in the tropics over penicillin obtained from *P. notatum* are manifold, important of which are :—

(1) The fungus is easily isolated from every-day contamination in the tropics.
(2) It requires no special method for the preservation of spores but in the case of *Penicillium notatum* a special method has to be followed to maintain spores to have maximum yield of penicillin.
(3) The cultures may be cultivated at the room temperature of the tropics varying from 25°-35° C. in a place like Calcutta and no special arrangement is necessary in an air-conditioned chamber for the regulation of temperature during incubation for the optimum yield of penicillin.

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STUDIES IN THE SULPHUR FORMATION AT KONA, MASULIPATAM—PART II

THE isolation and general characteristics of *Vibro desulphuricans*, Konæ has previously[1] been demonstrated to be responsible for sulphate reduction in the sulphur areas at Kona. It was of interest to elucidate in greater detail the physical and physiological requirements of the organism for its growth and functioning.

1. *Temperature*.—The organism was inoculated in the stock medium and incubated under anaerobic conditions at various temperatures for 72 hours. The results are shown in Table I.

TABLE I

Temperature of incubation °C.	25	30	37	45
Blackening after ....days	5	3	No Blackening	No Blackening

2. *Thermostability*.—The culture, inoculated in the stock medium was subjected to different temperatures for measured periods; It was then immediately cooled under running tap water (temp. 25° C.) and thereafter incubated at 30° C. for 72 hours, and examined after this period. Table II gives the results.