

ANTIBIOTIC PRODUCING MICRO-ORGANISMS FROM WEST PAKISTAN SOILS

Part 1.—*Aspergillus Quadrilineatus*

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A mould isolated from local soil has been identified as *A. quadrilineatus*. The organism has been shown to elaborate three different active materials at different stages of its growth, viz., penicillin, ridulin and nor-ridulin, and quadrilineatin. The elaboration of these active materials has been established by various tests.

During the past two decades random screening of micro-organisms for antibiotic properties has proved greatly fruitful.¹ The screening of micro-organisms for antibacterial properties is going on all over the world. It is estimated² that the number of fungi alone is about 100,000 and consequently the scope for search in antibiotic producing fungi is very large indeed.

Experimental

Fourteen pure cultures of moulds from local soils near Shahdara, District Sheikhpura, Lahore Division, were isolated on Czapek-Dox medium by usual technique.³ Out of the moulds isolated the present investigation deals with one of these. It has been characterised and its antibacterial properties have been studied. The initial determination of the antagonist was carried out by strip test using *S. aureus* as test organism.⁴

The four-day old culture of the organism on Czapek-Dox medium was subjected to taxonomic studies.⁵ Lactophenol has been used as mounting medium in all microscopic work.

COLONY CHARACTERISTICS

Greyish in colour with purple tinge, floccose mycelium, spreading slowly and slightly wrinkled, reverse purple reddish, perithecia developing abundantly.

Heads: short, columnar, slightly greenish, length 62-67 μ , width 3-4 μ .

Conidiophore: Smooth wall, dull brown, length 62-75 μ , width 3-4 μ .

Primary sterigmata: length 5-6 μ , width 2-3 μ .

Secondary sterigmata: length 5-6 μ , width 2-2.5 μ .

Conidia: Globose, pale yellow, diameter 3-4 μ .

Huelle cells: Brownish spherical, embedded

in mycelium.

Perithecia: Diameter 129-147 μ .

Ascospore: Purple red, wall smooth.

This data indicates the inclusion of the mould in *Aspergillus nidulans* group. The organism is akin to *Aspergillus quadrilineatus*.

ANTIBACTERIAL ACTIVITY

The test for antibacterial activity was carried out by the method of Vincent and Vincent.⁶ The procedure consisted of spreading uniformly thick layer of nutrient agar in a petri dish and then inoculating the solidified layer with test organism. The inoculum consisted of two-hour culture of the test organism. Sterilized filter paper disc, wetted with the solution of the test material, was laid on the surface of seeded plate. The results were recorded as positive or negative depending upon the formation of zone of inhibition after 24-hour incubation of the plate at 30°C.

The organism was grown on two different media: (1) Czapek-Dox and (2) Raulin-Thom.

The culture broth of 2, 4, 8, 16, 24 and 32 days growth was tested for antibacterial activity using three different test organisms: (1) *S. aureus*, (2) *S. albus* and (3) *E. coli*.

The results of these tests (given in Table 1) indicate the antagonistic character of the mould. A second series of tests was carried out by using penicillin resistant strain of *S. aureus* and *Mycobacterium tuberculosis* on Lowenstein Jensen medium in order to establish the identity of the active material.

The culture broth was but little active up to 4th day. The active material in the sixteen-day old culture broth was unstable at pH 2 and at pH 10 after 4 hours and was inactivated by copper sulphate solution and hydroxylamine solution.⁷

TABLE I.

Age in days	Czapek Dox medium			Raulin Thom medium		
	<i>S. aureus</i>	<i>S. albus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>S. albus</i>	<i>E. coli</i>
2	—	—	—	—	—	—
4	—	—	—	—	—	—
8	—	+	—	+	+	—
16	+	+	—	+	+	—
24	+	+	—	+	+	—
32	+	+	—	+	+	—

TABLE 2.

Age in days	8	16	24	32
<i>M. tuberculosis</i>	—	—	+	+
Penicillin resistant <i>S. aureus</i> .	—	—	+	+

— indicates test material is inactive.

+ indicates test material is active.

The inactivity of the material to *E. coli* and penicillin resistant strain of *S. aureus* established the presence of penicillin.

The 24-day old culture contained an active material other than penicillin. The antibacterial spectrum of this material covers penicillin resistant *S. aureus* and *M. tuberculosis*. The presence of nidulin and nor-nidulin is probably indicated.⁸

The culture broth of the organism after 32 days yielded flocculant dark orange precipitate with Brady's reagent due to quadrilineatin, another active pigment.⁹

Discussion

The mould isolated from local soil indicates

to be of *A. nidulans* group and a species of *A. quadrilineatus*. The mould exhibited the elaboration of three different active materials at different stages of its growth. The activity of the culture upto 16th day is predominantly due to penicillin. The study of antibacterial spectrum, instability at low and high pH and inactivation by copper sulphate and hydroxylamine establish its identity.

At a later stage the change in antibacterial spectrum which covers both penicillin resistant *S. aureus* and *M. tuberculosis* and the solubility of the active material in organic solvents like ether and chloroform indicates substances like nidulin and nor-nidulin.

The orange red precipitate with Brady's reagent confirms, the elaboration of an active pigment quadrilineatin by the organism in 32-day old cultures.

The combination of these three active materials elaborated by a single micro-organism at different stages of its growth has not hitherto been reported.

References

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