Cultivation of Intracellular Microorganisms of *Ptyelus sexvittatus* Walker (Cercopidae, Homoptera) with Special Reference to Antibacterial Drugs. Absar M. KHAN (Department of Zoology, Aligarh Muslim University, Aligarh (India)) Received Nov. 25, 1975. *Botyu-Kagaku*, 41, 67, 1976.

13. *Ptyelus sexvittatus* Walker (Cercopidae, Homoptera) の細胞内微生物の培養とその抗細菌剤の効果

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アワフキムシの類 *Ptyelus sexvittatus* はインドにおいて棉やヒマの重要害虫で，毎年大きな害を与えている．この昆虫のマイセトーム mycetome に微生物が共棲しているが，この微生物について全く研究されたことがなかった．そこでこの微生物をマイセトームから単離し，形態的，生化学的，培養基での増殖について調べた結果，*Klebsiella* sp. と同定した．各種の抗細菌剤の *Klebsiella* sp. に対する抗菌力を調べた結果，クロロマイセチン，ストレプトマイシンなどは効果があったが，フラダンチン furadantin は効果がなかった．

Introduction

Although the difficulties in culturing the intracellular microorganisms have been surmounted through the studies made by many investigators[1-13]. All of them claimed that the microorganisms isolated from mycetomes could be easily grown on artificial media. In many cases these microorganisms have been classified as symbionts or symbiotes and thus beneficial to insects. *Ptyelus sexvittatus* Walker a common pest of cotton and castor causing tremendous loses every year in India. Nothing is known about the mycetomal microorganisms or symbionts of this insect and their role. In case the role of such microorganisms in the metabolism is worked out this may help in evolving suitable biological control. Frank[14], Koch[15], Behrenz and Technau[16] and Gabrani[13] studied the effect of various drugs on the inhibition of mycetomal microorganisms. Therefore, these findings open a new vista in the insects control research by elimination the symbionts.

In the present investigation an attempt has therefore, made to isolate intracellular microorganisms from the mycetomes of *P. sexvittatus* and estimate the total counts of these microorganisms ; effect of antibacterial drugs on the mycetomal microorganisms *in vitro*.

Materials and Methods

After etherization of the insect and removing the legs and wings, the remaining portion of the body was sterilized in 1 : 1000 solution of mercuric chloride in 80 per cent ethanol. They were rinsed in sterile normal saline. The dissections were performed in sterilized condition under a stereoscopic microscope and the mycetomes after removal from the abdomen were transferred to cultured tubes containing sterlize normal saline. It was then macerated by means of sharp needle and transferred to nutrient, broth tubes and incubated at 37°C for 24 hours. The details of other techniques were same as given in the Manual of Microbiological Methods (1957). The form, arrangement, size of bacterial cells and it reaction to gram stain, mortality of vegetative cells, growth on different media and other tests on biochemical aspects were made. The estimation of organism growth were made in neubauer hemacytometer counting chamber. The total number in all the four squares were counted and an average of the three counts was taken for establishing the final number. The classification, morphology and biochemical tests employed were as given in Berge's Manual of Determinative Bacteriology[17]. The antibiotic and sulpha drugs sensitivity test on the mycetomal bacteria were done by disk and turbidimetric methods[18]. In disc method the filter paper disc impregnated with different drugs *viz* (achromycin, chloromycetin, streptomycin, terramycin, erythromycin, furadantin, penicillin and sulphathiazole) were kept on blood agar plates which were previously

seeded with pure culture. These plates were incubated at 37°C for 24 hours. The zone of inhibition was measured from edge to edge in millimeter (mm) scale.

In the turbidimetric method the (penicillin, chloromycetin, streptomycin, and erythromycin) with four different concentrations were used. In each culture tube 8 m*l* of Nutrient broth, 1.0 m*l* of 24 hours old standarized culture and 1.0 m*l* of antibiotics were taken. In control, tubes were simultaneously prepared in the above manner except that the antibiotic solution was replaced by an equal amount of normal saline. The tubes were placed at 37°C for 24 hours. The reading were made at a wave length of 400 milliminon in Spectronic 20 Bauch and Lomb Spectrophotometer.

Results

The growth of organism on different media presented the following characters. The colonies in *Nutrient agar*: were raised, slimy and some what yellowish with entire margin ; on *Blood agar* : white, convex and smooth surface, on *Gelatin stab*: dirty white, no liqualification with some gas bubbles, on *Nutrient broth*: it showed the turbidity. In general appearance the organism was short, oval shaped (5.6 micron in length by 0.4 micron in width) and arranged in pairs. It was gram negative, non-sporforming, non flagellated, non-motile and capsulated. The organism gave a negative methyl red (MR) and positive Voges-proskauer (VP) test. It reduced nitrate and was not able to produce indol and hydrogen sulphide (H_2S). The litmus milk test gave positive test without coagulation. Citrate was utilized and there was no haemolysis on blood agar plate. The organism was able to ferment lactose and glucose with the production of acid and gas whereas, sucrose, maltose and mannitol were fermented with the production of acid only (Table 1). Therefore, on the basis of the above morphological and biochemical characters the organism isolated from the mycetomes of *P. sexvittatus* was identified as *Klebsiella* sp.

The estimation of total counts of growth of *Klebsiella* sp. was made at a wave length of 400 millimicron, the number of cells were 20×10^{-6} per m*l*.

The table 2 shows the details of inhibition zone obtained against various drugs by the disc method whereas the Table 3 indicates the results of the antibiotic sensitivity pattern as obtained by optical density by turbidimetric method.

Table 1. Details of biochemical reaction on the mycetomal organisms isolated from *P. sexvittatus*

Source of mycetomal organism	Test or substrate	Reaction
	Methyl red (MR)	(−)
	Acetyl methyl carbinol (VP)	(+)
	Nitrate	(+)
	Indol	(−)
	Hydrogen sulphide (H_2S)	(−)
Ptyelus sexvittatus	Urea	(−)
	Citrate	(+)
	Litmus	(+)
	Lactose	AG
	Glucose	AG
	Sucrose	A
	Maltose	A
	Mannitol	A
	Hemolysis on blood agar	(−)

A = acid; AG = acid and gas; (−) = negative and (+) = positive.

Table 2. Zone of inhibition produced by different drugs on *Klebsiella* sp. of *P. sexvittatus*

S. No.	Treatment	Disc potency μgm	Inhibition zone (mm)	Remarks
1	Achromycin	10	22	Inhibition
2	Chloromycetin	10	30	Inhibition
3	Streptomycin	10	30	Inhibition
4	Terramycin	10	24	Inhibition
5	Furadantin	10	16	No inhibition
6	Sulphathiazole	10	20	Inhibition
7	Erythromycin	10	28	Inhibition
8	Penicillin	10 IU*	20	Inhibition

* International Units.
Each figure is a mean of three readings.

Table 3. Optical density of 24 hours old culture of *Kebsiella* sp. of *P. sexvittatus*

Treatment	Optical density in four different antibiotics concentration				
	0.5 mg/ml	1.0 mg/ml	1.5 mg/ml	2.0 mg/ml	Control
Penicillin	0.310	0.292	0.272	0.252	
Chloromycetin	0.252	0.222	0.110	0.720	
Streptomycin	0.260	0.244	0.226	0.110	
Erythromycin	0.377	0.337	0.323	0.310	
Normal Saline					0.523

Each figure is a mean of three readings.

Discussion

Although the microbiology of various orders of insects have been exhaustively studied by Glaser[1], Brues and Dunn[19], Steinhaus[20], Brooks and Richards[21], Musgrave and Miller[4], Brook[9], LeBlance and Musgrave[22] and Buchner[10] however, there is a paucity of information regarding the microorganism associated with the *P. sexvittatus*. In the present investigation an attempt has been made to diagnose as accurately as possible within the limits of these studies. The *Klebsiella* sp. was successfully raised on nutrient agar, potato agar, blood agar, nutrient broth, failed to liquefy gelatin and was gram-negative, non-sporforming, non-flagellated, non-motile and capsulated. These findings are therefore in accord with those of Steinhaus[21,25] and Bucher and Stephens[23] where they also found association of gram negative bacteria in coccid and grasshopper respectively. Crawford *et al.*[7] was able to isolate gram negative bacteria from the mycetocyte of weevil. The sensitivity pattern of antibiotic and sulpha drugs in disc method showed that chloromycetin and streptomycin produced marked inhibition zone (30 mm) followed by erythromycin, penicillin, terramycin, achromycin, sulphathiazole. The furadantin failed to inhibit growth (Table 2). In the test where inhibition was measured by using optical density as criteria the inhibitory effect of chloromycetin was most effective followed by streptomycin, erythromycin, and penicillin (Table 3). They are thus in accordance with the results obtained by Frank[14], Brooks and Richards[21], Koch[15] and Gabrani[13].

Summary

The mycetomal microorganisms isolated from mycetomes of *Ptyelus sexvittatus* is gram negative, non-flagellated, non-sporforming, non-motile and capsulated. It is cable of growing luxriantly on different media. The organism has been identified

as *Klebsiella* sp. on the basis of morphological and biochemical tests. In sensitivity test chloromycetin and streptomycin produced inhibitory zones. The furadantin failed to inhibit the growth.

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