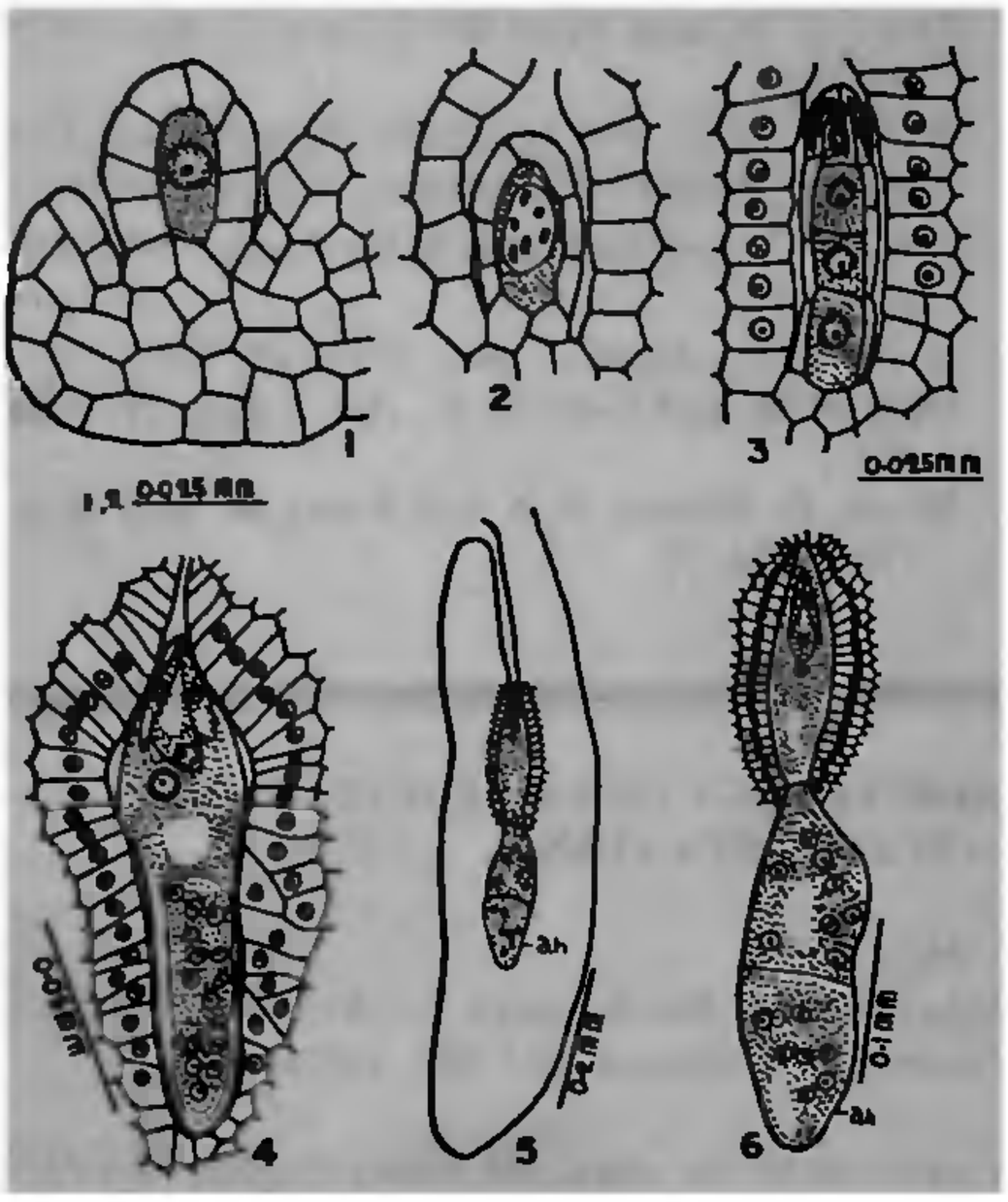

Figures 1–6. 1. Longitudinal Section of young ovule showing archesporial cells. 2. Megaspore mother cell in meiotic division. 3. Megaspore tetrad. 4. Organised embryo sac. 5. Longitudinal Section of anatropous ovule showing mature embryo sac. 6. Embryo sac enlarged from figure 5. (ah, Antipodal haustoria.)

type. The synergids are beak-shaped (Figure 4). Antipodal cells are two in number of which the upper one is binucleate. Later on the number of nuclei in the antipodal cells increase in number in both the cells (Figure 4). The antipodal cells begin to grow in size, become haustorial, crush and absorb the cells of the integument (Figures 5,6) but for a thin membrane. Antipodal haustoria in the tribe Helianthae were earlier recorded in *Bidens pilosa* and *Coreopsis tinctoria*[4].

Fertilisation is porogamous. Endosperm development is of the Nuclear type and Embryo development conforms to the Senecio variation of the Asterad type.

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ASSOCIATIVE NITROGEN FIXING BACTERIA OF BAJRA (*PENNISETUM AMERICANUM*)

R. P. GARG, S. K. NAIR* AND K. R. DADARWAL
Department of Microbiology, Haryana Agricultural University, Hissar 125 004, India.

*Present Address: Department of Plant Pathology, College of Agriculture, Vellyani, Trivandrum, India.

BAJRA (*Pennisetum americanum*) is one of the major millet crops grown during rainy season in Northern India. This crop is grown with limited amount of fertilizer application. Because of its rapid and vigorous vegetative growth, it consumes more solar energy for CO_2 fixation which could supply more carbon compounds to associative nitrogen fixing bacteria in root zone. In a survey of such bacteria under unirrigated conditions, we observed *Pseudomonas azotogenesis* as major nitrogen fixing bacteria[1]. In this article we report the bacteria which are associated under irrigated conditions.

Root samples of bajra (*Pennisetum americanum*) var HB-3, grown in canal irrigated farm area of Haryana Agricultural University, Hissar, were collected randomly from 5 locations at tillering, preflowering and seed formation stages. The adhered soil particles on the root surface were gently removed and washed with sterilized water. The roots were cut into 1–2 cm long pieces and once again washed with sterilized water. Washed roots (5 g) were then suspended in 45 ml of sterilized phosphate buffer (0.05 M, pH 7.5) for 1 h to get diffused the rhizoplane bacteria into the buffer. The buffer was dilution plated on Dobereiner's modified medium[2] in which the malate was replaced with mannitol (10g) and succinate (5g). The roots were then macerated in sterilized phosphate buffer and dilution plated for count of bacteria present inside the roots. The plates were incubated at 28 ± 1° C for 4 days and all the colonies appeared on the plates were assayed for nitrogenase activity on the same medium slopes. The expression of nitrogenase activity was taken as the parameter for considering a colony to be active N_2 fixer. The nitrogenase activity was determined in 15 ml tubes containing 5 ml medium. After transfer of colonies to medium slopes the tubes were incubated at 28° C for 48 h. The cotton plugs of tubes were then replaced with suba seals and 1 ml of air was replaced with 1 ml of C_2H_2, (air: C_2H_2 9:1). The tubes were further incubated for 24 h and C_2H_2 formed was determined with Nucon 5500 gas chromatograph using dual FID and porapak R columns. The major groups of bacteria showing nitrogenase activity were

TABLE 1

Morphological and biochemical characteristics of the two groups of bacteria

Characteristics		Representative Isolate Number BH 25	BH 124
Morphological:			
Shape, motility		Rod, motile	Rod, non-motile
Gram reaction		G-ve	G-ve
Capsulation	(1) Nutrient agar	−	−
	(2) Nitrogen free medium	+	+
Biochemical:			
1. Acid production from arabinose, xylose fructose, mannose, galactose, sucrose, maltose, lactose, mannitol, sorbitol, N-acetyl glucosamine.		+	+
2. Gas from lactose		+	−
3. Catalase		+	+
4. β-galactosidase		+	+
5. Growth on KCN		±	±
6. Starch and gelatin hydrolysis		−	−
7. Indole		−	−
8. M.R.		−	+
9. V.P.		+	−
10. Utilization of Acetate, malate, pyruvate succinate, fumarate, citrate, malonate		+	+
11. Lysine decarboxylation		−	+
12. H_2S from TSI		±	±
13. Ornithine decarboxylation		+	−
14. Urea hydrolysis		−	+
15. Phenylalanine deamination		−	−

identified upto generic level by standard procedures[3].

Two types of colonies were predominantly observed in root washing and in the macerated root samples which showed nitrogenase activity *viz* (a) mucoid gummy, raised transparent colonies with air bubble trapped within the colonies, identified as *Enterobacter* type, and (b) small mucoid colonies with whitish tinge and without trapped gas in the colonies, identified as *Klebsiella* sp. (Table 1). The frequency of both types varied from 1.0×10^6 to 1.9×10^6 in rhizo plane and 8×10^3 to 1.6×10^4 in macerated roots (table 2).

TABLE 2

Viable count of nitrogen fixing bacteria associated with bajra

Growth Stage	Root washing ($\times10^4$/g)	Macerated roots ($\times10^4$/g)
Tillering stage (30 d)	10.00	0.81
Pre-flowering stage (60 d)	18.6	1.50
Seed ripening stage (90 d)	19.7	1.61

The counts are for nitrogenase positive colonies of both *Enterobacter* and *Klebsiella* type appeared on modified Dobereiner's medium.

The specific nitrogenase activity of two representative isolates of the *Enterobacter* sp. and *Klebsiella* sp. showing the highest activity per tube, was determined and compared with standard strain of *Azotobacter chroococcum* (A 41). Nitrogenase activity was determined as stated above on solid slants and after assay, the bacterial growth was harvested in normal saline. The cell protein in the suspension was estimated by Lowry's modified method[4] and the nitrogenase activity was then calculated as nM of C_2H_2 reduced h^{-1} mg^{-1} cell protein.

The two isolates along with *Azotobacter chroococcum* (A 41) were also grown under stationary conditions for 10 d in modified Dubereiner's medium containing mannitol as the sole carbon source. Nitrogen content in the broth the cultures was then estimated by conventional Micro-Kjeldahl's method.

The specific nitrogenase activity of the *Enterobacter* BH 25 and *Klebsiella* BH 124 when compared with

TABLE 3

Relative nitrogenase activity and nitrogen fixation by associative nitrogen fixing bacteria of bajra and A. chroococcum

	Klebsiella sp.	*Enterobacter* sp.	*A. chroococcum*
Nitrogenase activity	630.24	905.64	1021.84
Nitrogen fixed	11.53	8.49	14.7

Nitrogenase activity is expressed as nM C_2H_2 reduced h^{-1} mg^{-1} cell protein. The value for nitrogen fixed is mg nitrogen fixed g^{-1} of mannitol in broth after incubation for 10 d.

Azotobacter chroococcum were about 88.7% and 61.5%, respectively (table 3). The amount of nitrogen fixed per gram of carbon source was 11.5 mg by *Klebsiella* and 8.5 mg by *Enterobacter* as compared to 14.7 mg by *A. chroococcum*. Interestingly, the *Enterobacter* BH 25 showed higher nitrogenase activity than *Klebsiella* BH 124. However, the nitrogen fixed per gram of carbon source was more by the later bacterium. This was subsequently found to be due to the fact that the *Enterobacter* BH 25 possesses conventional hydrogenase and loses much energy in wasteful hydrogen production (data unpublished).

The counts of nitrogenase positive colonies, made in this study, are mainly of those bacteria which were growing aerobically on agar surface. The oxygen sensitive bacteria, however, were not studied in the present studies because of limitations in isolation and characterization of such bacteria. Also precise condition under which these bacteria express nitrogenase activity are not well known.

Our earlier studies with this crop showed that under dryland conditions, *Pseudomonas azotogenesis* was the main aerobic associative N_2 fixing bacteria[1], under irrigated conditions, however, there was a change in the associative forms and the major taxonomic types, found to be associated with bajra, were *klebsiella* sp. and *Enterobacter* sp.

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GUT BACTERIAL FLORA OF COWPEA WEEVILS

Y. F. NEELGUND AND S. MEENA KUMARI
Department of Studies in Microbiology, Gulbarga University, Gulbarga 585 106, India.

THE cowpea weevils*, *Callosobruchs analis* (Fabricius) and *C. maculatus* (Fabricius) (Coleoptera: Bruchidae) are serious pests of peas, beans and pulses[1,2]. Though bacteria associated with different groups of insects have been surveyed, the knowledge of bacteria associated with the storage insects[3,4] is scanty. In the present work it is aimed to report the bacteria associated with the digestive tract of two storage insect pests, *C. analis* and *C. maculatus*. This appears to be the first report of bacteria from cowpea weevils.

*Identified by Commonwealth Institute of Entomology, London.

The samples of pea and blackgram along with the infested cowpea weevils, *C. analis* and *C. maculatus* were collected from various godowns and maintained in the laboratory in clean sterilised transparent boxes. Living adult insects were randomly chosen and subjected to surface disinfection[5]. Effectiveness of the technique was decided by dipping some of the randomly chosen pretreated insects in nutrient broth which turned turbid within 24 hr with contaminated insects; and these were discarded.

After surface disinfection, hundred adults of each of the two insect pests were dissected aseptically and the guts obtained were grouped in 20 sets separately, for further study. Each set (having 5 guts) was homogenised in a known quantity of peptone broth and was incubated at 30 ± 1°C for 48-72 h, which served as the original stock suspension. Later, out of this suspension, 1 ml broth was serially diluted six times. One ml of such dilutent was pourplated, in duplicate, on each of the five media; nutrient agar, macConkey agar, sodium azide agar, blood agar, glucose agar, for the quantitative estimation of microflora, and was incubated at 30°C for 48-72 hr. Thus an average of 40 plate counts from each media was used for the calculation of the gut population. The bacterial populations were identified[6,7] and analysed statistically.

Results indicated that a total of eighteen bacterial types were found associated with the gut of the two cowpea weevils. These represented *Bacillaceae, Micrococcacea, Pseudomonadaceae, Enterobacteriaceae*, and *Flavobacteriaceae*. They were *B. cereus, B. circulans, B. subtilis, B. megaterium, B. coagulans, B. polymyxa, B. licheniformis, Micrococcus roseus, M. varians, M. luteus, Pseudomonas aeruginosa, Aerobacter aerogenes, A. cloacae, Citrobacter intermedius, C. freundii, Klebsiella pneumoniae, Proteus mirabilis* and *Flavobacterium lutescens*. Of the bacteria isolated, *A. aerogenes, A. cloacae, C. intermedius, C. freundii, Klebsiella pneumoniae* and *Ps. aeruginosa* are reported to be human pathogens[8,9]. *B. subtilis* is a food spoiling and food poisoning organism[7]. *A. aerogenes, A. cloacae, P. mirabilis, Ps. aeruginosa, B. subtilis, B. cereus* and *B. megaterium* are reported to be potential insect pathogens[10]. *M. roseus, M. varians, M. luteus, B. licheniformis, B. coagulans, B. circulans, B. polymyxa* and *Flavobacterium lutescens*