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The facultatively anaerobic bacteria as a source of alcohols in three breeding substrates of cactophilic Drosophila.

Knowledge of the microbial ecology of the necrotic tissues of columnar cacti, the breeding substrates of cactophilic Drosophila of the Sonoran Desert, is very important in elucidating the ecological niche of these Drosophila. The saprophytic yeast flora is probably important as a concentrated protein source and attractant for Drosophila (Starmer et al. 1976). However, very little is known about the bacterial flora; Erwinia carnegieana is believed to be the causal agent of the necrosis (Lieghtle et al. 1942), and at least eight genera of bacteria can be found in Pachycereus pringlei, cardon cactus, and Carnegiea gigantea, saguaro cactus (Graf 1965).

Gas chromatography studies have shown that several alcohols are present in necrotic cacti (Heed 1978). Anaerobic bacteria probably play an important role in alcohol production because the only two saprophytic yeasts which ferment, Torulopsis sonorensis and Candida tenuis, produce only ethanol and are not found in all necrotic samples containing alcohol. Knowledge of alcohol variability as a function of the microflora is necessary for an understanding of the variability at the ADH locus in D. mojavensis (Heed 1978). Here we report the results of a preliminary analysis of the distribution and alcohol production of the facultatively anaerobic bacteria inhabiting the soft rots of three species of giant cacti.

Samples of necrotic tissue of one Carnegiea gigantea (saguaro cactus), two Lamaireocereus thurberi (organ pipe cactus), and two Machaerocereus gummosus (agria cactus) were appropriately diluted and plated on the following three media: (1) 0.3% yeast extract, 0.3% Bacto peptone, 1.5% Bacto agar; (2) tryptic soy agar; (3) nutrient agar (all components and media were from Difco). One set of plates was incubated microaerophilically with CO_2 and another set aerobically at 24°C and 30°C for the initial isolation. Standard microbiological techniques were used for maintenance of the isolates. Identification was made with the aid of the API 20E System (Analtab Products), a miniaturized version of conventional procedures for bacteria identification, and other tests as indicated in Bergey's Manual (Buchanan and Gibson 1974). Although the API 20E System is designed for the identification of clinical isolates in the family Enterobacteriaceae, it can be very useful as a quick screening method in other studies where small, Gram-negative, facultatively anaerobic rods are present. The GasPak anaerobic system (BBL) was used to test for anaerobic growth. Gas chromatography

Table 1. The concentration of each bacteria species in the substrates and the amount of ethanol produced by each species in pure culture.

Bacteria		Colony forming units/cc of sample*					EtOH Production	
Species	No. of isolates	Saguaro (77-30)	Organ pipe (77-31)	Organ pipe (77-32)	Agria (77-33)	Agria (77-34)	No. tested	% EtOH**
Bacillus laterosporus	3	10^5	10^7				2	0-3
Citrobacter sp. A	2			10^7			2	31-34
Citrobacter sp. B	5		10^7			10^8	0	
Enterobacter aerogenes	1		10^6				1	55
Enterobacter agglomerans	1				10^7		0	
Enterobacter cloacae	1					$<10^2$	1	29
Erwinia carnegieana	2		10^7				0	
Escherichia coli	3		10^4	10^7			2	31-40
Klebsiella ozaenae	2	10^3				$<10^2$	1	94
Klebsiella pneumoniae	13		10^8				6	45-91
Leuconostoc sp.	2		10^7			10^7	2	2-3
Staphylococcus sp.	6	10^5		10^7			4	0
Yersinia enterocolitica	1				$<10^2$		0	
Unknown A	2			10^4			0	
Unknown B	1				10^7		0	
Unknown C	1				10^8		0	

*One saguaro sample, 77-30 (Arizona no.), and two organ pipe samples, 77-31 and 77-32, collected at Sil Nagya, Arizona in September 1977; and two agria samples, 77-33 and 77-34, collected at Punta Arenas in Sonora, Mexico in November 1977.

**Expressed as % ethanol (v/v) x 10^3, minimum value-maximum value.

(1/8 inch x 5 foot stainless steel column packed with Poropac Q at 140°C and 170°C oven temperatures) was used to determine if methanol, ethanol, acetone, 2-propanol, and 1-propanol were produced both in the rot pockets and by the isolates (grown in 0.5% glucose fermentation broth for 3-4 days).

Forty-six isolates representing 9 genera, 13 species, and 3 unknowns were obtained from the 5 samples of necrotic cactus (Table 1). The majority are in the family Enterobacteriaceae and are true phytobacteria. Yersinia enterocolitica, Escherichia coli, and Klebsiella ozaenae are not true phytobacteria. Klebsiella pneumoniae, previously associated only with inflammations of the respiratory tract, has recently been found in the heartwood and sapwood of living redwood trees (Bagley et al. 1978). Enterobacter aerogenes, E. cloacae, and Erwinia carnegieana are the only species from our study that were isolated from saguaro and cardon by Graf (1965).

Of the volatiles tested, only ethanol was produced in detectable quantities by the bacterial isolates. This corroborates White and Starr (1971) who found that 65 of 71 strains of Enterobacteriaceae produced ethanol. The cacti contained not only ethanol but also the other four volatiles as well.

Three conclusions can be made. First, the diversity of genera is high when compared with the yeasts which are represented by only four genera (Starmer et al. 1976) in the cactus substrates. Given the small sample size of five reported here, this is a minimum estimate of the diversity. Secondly, there is less similarity within than among substrates, i.e., the two agria samples have no species in common but share three species with saguaro and organ pipe, and the two organ pipe samples have only one species in common and share five species with saguaro and agria. Thirdly, the facultative anaerobes contribute only to the ethanol content. Alcohols are also produced by bacteria (i.e., obligate anaerobes) not isolated in this study (unpublished data). This investigation is a first approximation of the facultative anaerobes and suggests a much higher diversity of these bacteria than yeasts.

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Zacharopoulou, A., G. Yannopoulos and N. Stamatis. University of Patras, Patras, Greece. Cytological localization of the "cn" (cinnabar) locus in D. melanogaster.

For localizing cytologically the "cn" locus we have used the deficiencies induced by a wild lethal chromosome II (Symbol 23.5). The previously mentioned chromosome was isolated from a female captured in 1978 from the same natural population of Southern Greece (Peloponesse-Patras) where the male recombination factor 31.1 MRF was found by Yannopoulos and Pelecanos (1977). Wherever heterozygous F_1 23.5/dp b cn bw sons of the cross ♀♀ dp b cn bw; ve x 23.5/CyL^4 ♂♂ were individually mated with dp b cn bw; ve virgin females, high frequencies of cn individuals were observed, sometimes reaching up to 2.50%. Moreover, the 23.5 chromosome was found to induce sterility, male recombination, etc. (Stamatis, in preparation). Ten cn individuals derived from different F_1 23.5/dp b cn bw males were separately mated with dp b cn bw; ve virgin females. Salivary chromosomes from third instar larvae were then examined for deficiencies. Eight out of the ten males tested have shown detectable deficiencies in the 2R chromosome, namely: (1) Df(2R)43C;44C, (2) Df(2R)42E;43F, (3) Df(2R)43D;44A, (4) Df(2R)43B;44D, (5) Df(2R)43C; 44C, (6) Df(2R)42E;44A, (7) Df(2R)43D;43E, (8) Df(2R)43E. The breakpoints were recognized on the basis of photograpic maps of Lefevre (1976).

An approximate estimation of the distribution of the breakpoints on the chromosome is presented in Fig. 1. The figure shows that all eight deficiencies include the region $43E_3$-E_{14}. This finding strongly suggests that the "cn" locus is located in this region.

References: Lefevre, G., Jr., 1976, in The Genetics and Biology of Drosophila 1a (A. Ashburner and E. Novitski, eds.); Yannopoulos, G. and M. Pelecanos 1977, Genetical Research (Cambridge), 29:231-238.