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6. The failure of acid to be selectively sweetened is an indication that the mechanism of sweetness induction by artichoke cannot be the same as that of sweetness induction by miracle fruit.
7. The artichoke extract was prepared from commercially available frozen globe artichoke hearts. These were boiled for 8 minutes, were cooled, and were then blended with ethanol (final concentration, 50 percent) for 5 minutes at high speed. The suspension was centrifuged at 10,000*g* for 30 minutes, the ethanol was evaporated, and the remaining aqueous extract was freeze-dried for storage [yield, 36 to 44 g of powder from 1000 g of raw artichoke hearts (about 40 hearts)]. The concentrations of artichoke extract in this experiment are given as the percent powder by weight.
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27 October 1972

Beggiatoa: Occurrence in the Rice Rhizosphere

Abstract. *A catalase-like activity surrounding the root tips of rice plants favors the presence of* Beggiatoa, *an organism capable of oxidizing the hydrogen sulfide which is toxic to rice and is found in paddy soil conditions.* Beggiatoa *has now been isolated from rice field soil. A mutually favorable interaction between rice and this bacterium is suggested.*

Anaerobic organisms such as *Desulfovibrio* spp. abound in submersed soils and produce large quantities of hydrogen sulfide by the reduction of sulfate (*1*). Theoretical predictions of toxic concentrations of H_2S in rice paddies in Louisiana and Texas (at least 0.1 part per million of H_2S in soils containing abundant iron) (*2*) have been confirmed by field measurements of S^{2-} concentrations with a sulfide electrode (*3*). These minimal concentrations have been shown to inhibit significantly the activities of cytochrome oxidase and other oxidase enzymes in rice seedling homogenates (*4*).

The occurrence of such concentrations of H_2S during the heading-flowering stage of rice plant development in areas of single harvest yields of nearly 6000 pounds of rough rice per acre (5500 kg/ha) led us to postulate detoxification of H_2S by a biological system. *Beggiatoa*, a filamentous bacterium capable of oxidizing H_2S to sulfur intracellularly, was isolated from paddy soil in the Crowley, Louisiana, area and its physiological characteristics were verified. This bacterium is autointoxicated by hydrogen peroxide and the compound must be decomposed externally for optimum growth.

We studied rice plants growing in a greenhouse, a growth chamber, and gnotobiotic nutrient solutions. Using the polarographic method of Rodriguez-Kabana and Truelove (*5*) for the detection of catalase (E.C. 1.11.1.16), we found that a catalase-like activity surrounds the tips of growing rice plants. The results obtained by sampling the greenhouse rhizosphere (Table 1) suggest that in all cases catalase activity was associated with the rice roots. The differences between catalase levels in rice-planted soil and those in controls were highly significant ($F = 5935.5$ with 1 and 597 degrees of freedom). A similar experiment with sterilized soil and the controlled environment of a growth chamber gave similar results (Table 2).

In a subsequent experiment, rice seedlings were disinfested with NaClO for the removal of rice rhizosphere organisms, and the individual seedlings were grown for 10 days in cotton-stoppered tubes containing sterile Hoagland's solution (*6*). The seedlings were then removed and plated on nutrient agar in petri dishes. The plates were examined after 1 week for growth of contaminating organisms (contaminated tubes were eliminated from the test) and the tubes of growth solution were tested for catalase activity. The controls consisted of rice seedlings treated in the same manner as the test seedlings, but killed with propene oxide before placement in the nutrient solution. The catalase units per seedling of 16 uncontaminated rice seedlings after 10

days growth averaged 7.0, with a range of 6.0 to 7.5. Four control seedlings yielded zero catalase activity. These data demonstrate conclusively that catalase activity in the medium can be related solely to the presence of living rice roots.

Attempts to isolate *Beggiatoa* by the classical method of Cataldi (*7*) yielded organisms identified as *Vitreoscilla* spp., which lacked the ability to oxidize H_2S. *Beggiatoa* was ultimately isolated by a method which selected for motile organisms preferring low oxygen tension and the presence of added catalase. The medium contained 0.05 percent sodium acetate, 0.01 percent calcium chloride, 0.2 percent yeast extract (Difco), 0.2 percent agar, and 1000 units of filter-sterilized fungal catalase (Nutritional Biochemicals) which was aseptically added after autoclaving and partial cooling. The *p*H was adjusted to 7.2 with sterile acetic acid.

This medium was distributed in 10-ml quantities in glass tubes (16 by 150 mm) containing 2.5 g of fresh Crowley silt loam rice field soil. The tubes were covered with Parafilm and incubated at 28°C. They were periodically examined over a 10-week period for colonies suspected of being *Beggiatoa*. Suspected colonies were removed by Pasteur pipette to tubes containing 15 ml of sterile water with 0.1 g of $CaCl_2$ per liter, and observed. Trichomes of *Beggiatoa* glide across an agar surface leaving nonmotile organisms behind. This purification process was repeated one or more times, after which the trichomes were transferred to acetate medium. Repeated purification of cultures was necessary to obtain freedom from contaminants.

The axenic cultures were confirmed as *Beggiatoa* on the basis of morphology. Some of the isolates obtained on catalase-enriched acetate media were found to deposit sulfur granules intracellularly when grown in the presence of H_2S, and to produce H_2O_2. Oxidation of sulfide by the organism was determined by pyridine extraction of cells grown in the presence of H_2S (*8*). The production of peroxide in acetate medium without catalase was determined by lead sulfide, ferric ferricyanide, nickel oxide, and phenolphthalein spot-tests devised by Feigl (*9*).

The interaction of *Beggiatoa* with rice roots was studied in four glass tubes (30 by 6 cm) half filled with an aqueous mixture containing 200 g of rice field soil and 3 g of agar per liter. The tubes were autoclaved for 30 minutes at 121°C, and tubes 1, 2, and 3 were planted with aseptically germinated rice seedlings. Tube 4 was planted with rice seedlings killed by propene oxide (control). All tubes received sufficient sterile tap water to produce a layer 1 cm thick on the surface of the agar. After 4 days, tubes 2, 3, and 4 were inoculated with 1 ml of a suspension of *Beggiatoa* trichomes which had been held for 2 days on acetate medium without catalase. Tube 1 was a control and received no inoculum.

Table 1. Catalase activities of rice-planted soils. Abbreviations: GHS, greenhouse soil; BSL, Beauregard silt loam; CSL, Crowley silt loam. The results for pots, 1, 2, and 3 are averages of five replicate determinations. The controls were soil pots without rice plants.

Days	Soil	Catalase units per gram of soil			Control
		Pot 1	Pot 2	Pot 3	
0	GHS	2.1	2.4	2.0	2.2
	BSL	1.0	0.8	0.9	0.8
	CSL	5.1	4.8	5.2	5.2
15	GHS	6.3	6.2	6.5	3.0
	BSL	2.1	2.3	2.0	1.3
	CSL	8.1	8.0	8.5	7.0
30	GHS	8.4	8.8	8.4	3.2
	BSL	6.8	6.9	6.8	2.0
	CSL	12.3	10.8	11.2	6.8
45	GHS	8.2	8.6	8.4	2.8
	BSL	7.0	7.2	7.4	2.0
	CSL	12.4	12.3	12.1	5.2
60	GHS	8.5	8.5	8.8	2.8
	BSL	7.5	7.8	7.6	2.1
	CSL	13.3	14.1	13.8	4.6
75	GHS	8.6	8.2	8.0	2.6
	BSL	7.8	7.5	7.5	1.8
	CSL	14.0	13.8	13.8	4.3
90	GHS	9.0	8.8	8.6	2.5
	BSL	7.4	7.8	7.6	1.8
	CSL	14.0	13.6	13.2	4.0
105	GHS	8.7	8.7	8.7	2.3
	BSL	7.6	7.7	7.8	2.0
	CSL	13.8	13.4	13.0	4.0
120	GHS	8.2	8.6	8.6	1.5
	BSL	5.0	4.8	6.1	1.5
	CSL	12.7	14.5	15.0	4.0
135	GHS	6.2	7.5	8.3	1.6
	BSL	1.0	2.0	1.6	1.5
	CSL	16.1	16.7	14.3	3.8

Table 2. Catalase activity of autoclaved greenhouse soil planted with rice and maintained in a growth chamber. Each value is the average of five replicate determinations. The controls were soil pots without rice plants.

Days	Catalase units per gram of soil				Control
	Pot 1	Pot 2	Pot 3	Av.	
0	2.4	2.2	2.3	2.0	2.3
7	3.5	3.7	3.7	3.6	2.5
14	4.9	4.6	4.8	4.8	2.8
21	5.5	5.5	5.4	5.5	3.5
28	6.0	6.1	6.0	6.0	3.8
35	6.2	6.3	6.2	6.2	3.5

The contents of the tubes were examined repeatedly during an 18-day period after planting. At the end of this period reisolation of the organism was attempted by the technique described above. The organism was reisolated from tubes 2 and 3 only. *Beggiatoa* was not observed in tube 1, which was uninoculated, nor in tube 4, which was planted with seedlings killed by propene oxide. During the course of the experiment, water samples aseptically drawn from tubes 2 and 3 were found by microscopic examination to contain actively motile trichomes of *Beggiatoa*. No motile trichomes were observed in tube 4 at the end of the 12th day. Thus, it was concluded that the survival of *Beggiatoa* was favored by the presence of growing rice plants.

The known occurrence of rice-toxic concentrations of H_2S in rice fields, the isolation of *Beggiatoa* from rice fields, and the demonstration of catalase in the rice rhizosphere suggest that *Beggiatoa* may be involved in a relationship existing between the sulfide-producing anaerobes and the sulfide-sensitive rice root in submersed rice field soils. Toxic peroxides produced by *Beggiatoa* may be decomposed by rice-root catalase, and sulfides toxic to the rice root may be oxidized by *Beggiatoa*. Thus, the H_2S-oxidizing bacteria (*Beggiatoa*) could limit antagonistic interactions between the two extreme organisms (*Desulfovibrio* spp. and *Oryza sativa* L.) by utilizing characteristic products of both. The further elucidation of these phenomena is necessary for a better understanding of similar submersed environments characterized by low levels of soluble sulfides, such as anaerobic seas, swamps, marshes, and estuaries.

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28 August 1972

Centric Fusion and Trisomy for the LDH-B Locus in Brook Trout, Salvelinus fontinalis

Abstract. *Cytogenetic analyses showed that a trisomic male brook trout of genotype* BB′B″ *for one of the lactate dehydrogenase subunit loci had a karyotype with two extra arms appearing as a metacentric chromosome. The metacentric chromosome probably arose through centric fusion of two acrocentric or telocentric chromosomes—one of which carried the locus for subunit B—followed by nondisjunction.*

In the course of screening a population of brook trout for use in intragenic recombination studies (*1*) at the locus specifying the B subunit of the ubiquitous system of lactate dehydrogenase (LDH) in trout (*2*), a male showed a zymogram pattern that indicated trisomy for three *LDH-B* alleles—genotype *BB′B″*. Breeding and cytogenetic analyses of this male confirmed the trisomy and indicated that it probably arose through a spontaneous centric fusion of two acrocentric or telocentric chromosomes, followed by nondisjunction.

Starch gel electrophoresis of eye tissue (*2*) permits unambiguous detection of all genotypes for the *LDH-B* locus. This is so because gene dosage effects, as well as allelic differences, are reflected in differentially stained homo- and heterotetramers of unique electrophoretic mobility formed from the A and B subunits specified by the *LDH-A* and *LDH-B* loci (Fig. 1).

The breeding results for the parental generation in which the trisomic male was found (family M290), for two families produced by this male (0-37 and 0-38), and for families produced by two heterozygous male sibs (0-39 and 0-40) are presented in Table 1. The trisomic male arose in a family in which the proportions of offspring indicate the expected segregation of *LDH-B* alleles, if the trisomic offspring is excluded.

The trisomic male was testcrossed to two females of *BB* genotype to give the families 0-37 and 0-38. In each family the ratio of six offspring genotypes fit that expected if there had been random assortment of three chromosomes into equal numbers of functional n and $n + 1$ gametes. Moreover, the data from the two families are homogeneous enough to be combined ($\chi^2 = 0.82$, $P > .95$); as expected, the number of progeny with the six genotypes are nearly equal, as are the number of trisomic and disomic offspring (55 and 53, respectively). Typical zymogram patterns used to classify the six genotypes are shown in Fig. 1. The high concentration of the B_4 homotetramer in genotypes *BBB″* (slot 1) and *BBB′* (slots 3 and 5) contrasts to the lower concentration of this tetramer in genotypes *BB″* (slot 6) and *BB′* (slot 4). More bands are present in the zymogram from a *BB′B″* fish (slot 2), and the second band from the origin in this zymogram is a doublet representing A_3B' and A_3B'' heterotetramers.

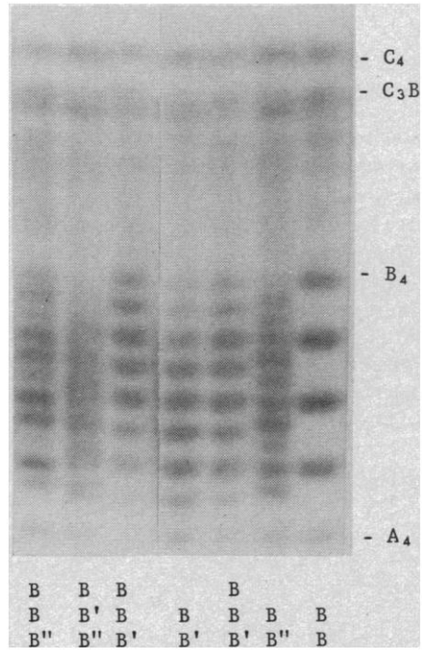

Fig. 1. Starch gel zymograms of eye tissue LDH from progeny of a *BB* disomic female crossed with a *BB′B″* trisomic male brook trout. Genotypes are designated at the bottom of each slot; homotetramers of A and B subunits of the ubiquitous system as well as tetramers of the C subunit of the eye system are shown along the side (origin and cathode at bottom; anode at top).

Two *B′B″* male sibs from family M290 were testcrossed to *BB* females to produce the families 0-39 and 0-40 (Table 1); the ratios of offspring in both families are close to the 1 : 1 expected for offspring of disomic heterozygotes. Also, one female and three male sibs of the *B′B″* genotype from family M290 were used in other crosses (*1*); all four produced results expected of normal disomic segregation. Thus, no unusual genetic results were recorded from six normal siblings of the trisomic male studied.

Cytological studies were made on the trisomic male after the breeding experiments and on some of his progeny from family 0-37. Squash preparations were made from gill and kidney tissues that were finely minced, treated with hypotonic saline, and fixed in Carnoy's fixative; the technique has been described (*3*). Chromosomes were stained with 1 percent aqueous crystal violet and photographed with a Leitz Ortholux photomicroscope. The normal brook trout modal chromosome number is $2n = 84$, with 100 total arms distributed as 16 metacentrics and 68 acrocentrics (*3*, *4*). In contrast, all 25 cells examined from the trisomic male had two extra arms. The modal count (15 cells) was 85 chromosomes with 17 metacentrics and 68 acrocentrics. One cell had 87 chromosomes with 15 metacentrics and 72 acrocentrics; six cells had 86 chromosomes with 16 metacentrics and 70 acrocentrics; and three had 84 chromosomes with 18 metacentrics and 66 acrocentrics. While some of this variation might be due to counts made on disrupted cells from squash preparations, such intraindividual variation due to Robertsonian variation or centric fusion and fission is common in Salmonidae (*3*, *5*). A karyotype of a cell showing the modal chromosome number with the extra metacentric appears in Fig. 2.

Among young fry from family 0-37, limited chromosome counts on known LDH genotypes were informative but not definitive. One metaphase in a *BB′B″* individual showed 102 arms distributed as 85 chromosomes with 17 metacentrics, a distribution identical to