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Toxicity of Plankton to Cristispira Inhabiting the Crystalline Style of a Mollusk

Abstract. The crystalline style of *Saxidomus giganteus* supports a large population of *Cristispira* spp. which swim freely in it except near the functional end which impinges on the gastric shield. Here the style is frayed and mixed with ground food material, while amylolytic enzymes and an oxidase are released. Glucosone appears to result from oxidase activity. This proved toxic to cristispira in fairly high dilution. Several tests of the toxicity of extracts of plankton, which forms the food of *Saxidomus*, were made. In every case such extracts proved toxic.

I have previously reported (*1*) that dense populations of an undetermined species of *Cristispira* occur in the crystalline style of *Saxidomus giganteus* Deshayes and that, while the organisms swim freely in that environment in all directions, none, or extremely few, are ever found within a few millimeters of the functional end of the style, which, during feeding, is projected from the diverticulum of the stomach in which it is secreted and rotated against the gastric shield. The cristispira appear to be sensitive to some external adverse influence when they reach this point which causes them to retreat from it. Surmises were discussed as to what this influence might be.

It is well known that the grinding and stirring action of the crystalline style against the gastric shield is accompanied by softening and eventual solution of its material with simultaneous freeing of polysaccharide-splitting enzymes. I have shown that the style material contains a peroxidase which, in association with a substance, or substances, derived from the food material of the mollusk during the grinding process, establishes an oxidizing system (*2*). I suggested that something resulting from oxidation of food material might be responsible for the repulse of cristispira at the head of the style.

A culture of actively swimming cristispira is readily obtained by steeping pieces of styles, which have had their grinding ends, with food material attached, removed, and have been thoroughly washed, in sea water at a temperature not exceeding 5°C for some hours, the style material completely dissolving. Support for the foregoing surmise was obtained by adding to such a culture a few of the severed anterior ends of styles with accumulations of ground food material attached, which resulted in the death of all the cristispira present in a few hours at 5°C. The only means of judging of the death of the organisms in these, and other experiments to be described, has been by the complete cessation of motility, but, since this is usually quickly followed by their disintegration, it seems to be a reliable criterion.

The only substance which has been recognized as probably resulting from the oxidizing activity of the crystalline style system is glucosone, obtained by reacting it with glucose (*2*). Since this substance has been shown to be toxic to many animals (*3*) it seemed of interest to determine whether it were so in relation to cristispira. It was found that addition of 0.5 percent of glucosone to an active culture kept at 5°C killed the organisms completely in 1 hour. It therefore seemed possible that glucosone might be responsible for the repulse of cristispira at the end of the style. However, the production of glucosone under the united action of foodstuff and style has been found to operate only in the presence of glucose as substrate, and there is no evidence of the breakdown of the complex polysaccharides of the food organisms to the hexose stage by style activity. There is some divergence of opinion about this even in application to more simple polysaccharides. Yonge (*4*) found that, among those he studied (including pectin, glycogen, starch, lactose, maltose, raffinose, sucrose, and cellulose), only starch and glycogen were degraded to the hexose stage by the style of *Ostrea edulis*. On the other hand Lavine, in the cases of *Mya arenaria* and *Mactra solidissima* (*5*), and Newell, in those of *Ostrea edulis* and *Mytilus edulis* (*6*), found that the style enzymes degraded cellulose, and the former found glucose in the digest after several days. Newell failed to detect either glucose or glucosone after 25 hours. Possibly digestion was not carried far enough in this case and, even had glucose been derived, no oxidation to glucosone was to have been anticipated in the absence of the component of the food material which is essential to promote the oxidase reaction of the style (*2*).

It would elucidate the situation if it could be determined whether the substance toxic to cristispira occurs in the plankton organisms of which the food material of the mollusk consists before it had been submitted to the action of the style enzymes, but this could be accomplished only if cristispira could be maintained in culture in something other than a solution of style material. This has not been found possible. Nevertheless, a number of samples of plankton have been examined from this standpoint. The results do no more than confirm those previously obtained with food material collected and ground on the styles, but they bring out two points of collateral interest. Plankton was collected in the usual manner over, or in the neighborhood of, saxidomus beds. The material was washed free from coarse components and the remainder filtered through paper, washed, and finely ground. Fractions of the resulting pulp were extracted with as small volumes of sea water as practicable, and again filtered. For tests, samples of the clear, usually colorless, filtrates were added to standard volumes of cristispira cultures; the mixtures were maintained at low temperature (5°C, or lower) for varying periods, and examined at measured intervals for vitality. Checks, with volumes of sea water corresponding to those of plankton extracts in the tests, were maintained under the same conditions and examined at the same intervals.

The results brought out two definite points. (i) All plankton extracts were toxic to cristispira sooner or later, while corresponding checks remained fully active. The periods for complete killing varied considerably in various tests. Quantitative comparisons were not possible, but, with equal volumes of culture and extract, the period was usually between 60 and 90 minutes. (ii) The biological constitution of the plankton was a matter of indifference. This varied very greatly through the period during which the tests were conducted, in some cases phytoplankton predominating, in

others zooplankton, but the toxicity of the extracts to cristispira was not noticeably affected by the proportion of the constituent organisms.

The question whether the oxidizing activity induced by the combined action of the style peroxidase and a food constituent is responsible for the toxicity of the mixed extracts to cristispira still remains open. It may depend on a constituent of plankton organisms entirely unrelated to their participation in the oxidase activity, which is quite general in the styles of lamellibranchs (*7*), though relatively few of them harbor cristispira.

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Evidence for Mixed Cytoplasm in Heterocaryons of Colletotrichum lagenarium

Abstract. Spores of race 2 but not of race 1 of *Colletotrichum lagenarium* are ingested by myxamoebas of strain NE-30 of the slime mold *Acrasis rosea*. Sporulating colonies of three heterocaryons of *C. lagenarium* involving auxotrophic strains with different color markers of races 1 and 2 were inoculated with myxamoebas. Although spores of race 1 produced on homocaryons were not ingested by the myxamoebas, they were ingested when produced on heterocaryons involving race 2. Spores from sectors of the heterocaryons yielded colonies of race 1; spores from these colonies were not ingested by the myxamoebas.

Although the intermingling of nuclei of different genotypes has been emphasized in considering heterocaryosis, it is reasonable to assume that the component strains not only contribute nuclei but also cytoplasm in the fusion of their hyphae to produce a heterocaryon (*1*). Results from a study of the ingestion of spores of heterocaryons of *Colletotrichum lagenarium* (Pass.) Ell. & Halst., the fungal incitant of anthracnose of cucurbits, by myxamoebas of strain NE-30 of the slime mold *Acrasis rosea* Olive & Stoianovitch indicated that these heterocaryons may have a mixture of cytoplasm characteristic of each component strain.

Table 1. The component strains of heterocaryons of *Colletotrichum lagenarium*. Abbreviations: cho, choline; nic, nicotinic acid; try, tryptophan; gly, glycine; pdx, pyridoxine; ade, adenine.

Heterocaryon	Resistant strain			Susceptible strain		
	Race	Color	Nutritional requirements	Race	Color	Nutritional requirements
A	1	Black	cho, nic, or try	2	Orange	gly, pdx
B	1	Yellow	cho, nic, or try	2	Orange	gly, pdx
C	1	Yellow	cho, nic, or try	2	Black	ade

Spores of race 2 but not of race 1 of *C. lagenarium* are ingested by the myxamoebas of strain NE-30 of *A. rosea* (*2*). For convenience, the former race has been termed "susceptible," and the latter, "resistant." When myxamoebas are added to the surface of a sporulating colony of race 2 and incubated at 22°C for 10 days, the area of ingested spores resembles a crater; the spores of race 1 are not ingested and the area of inoculation is not altered in appearance. The mycelium of a susceptible colony is not ingested.

Three heterocaryons (Table 1) involving auxotrophic strains of races 1 and 2 which differed in the color of their spore masses and mycelium were prepared (*3*). With prolonged incubation on bean agar, the heterocaryons displayed obvious sectors with the color of one or the other component strain. Mycelial fragments of young heterocaryotic colonies were transferred to bean agar and incubated until the sectors exhibited spore masses. Myxamoebas of strain NE-30 were added to the surface of large sectors and incubated.

Spore masses on all sectors of each heterocaryon were ingested by the myxamoebas, regardless of the color of the sector. In heterocaryon A, for example, spore masses on the black and on the orange sectors were ingested. Spore masses on the control colonies of the black component strain were not ingested. These observations indicated that the spores of the resistant race produced by a heterocaryon involving susceptible and resistant races could be ingested by the myxamoebas.

Mycelial fragments were taken from sectors displaying the color associated with the resistant component strain, transferred to bean agar, and incubated until spore masses appeared. At this time, the colonies were not sectored and resembled those of the resistant component. Myxamoebas were added to the surface of the colonies, which were then incubated. Some colonies were susceptible and others were resistant. With continued incubation, all susceptible and no resistant colonies developed sectors. In other words, the former colonies were heterocaryotic, and the latter, presumably, homocaryotic. Since sectoring was not detected in three to five serial transfers from the resistant colonies, it may be assumed that these colonies were indeed homocaryotic.

Spore masses were taken from sectors with the color associated with the resistant component strain in each of the heterocaryons and suspended in water; appropriate dilutions of each suspension were plated on bean agar to obtain individual colonies. Many spore masses yielded only colonies with the color of the resistant strain; 5 percent of the colonies from some spore masses displayed the color of the susceptible component. No colonies were sectored. Depending on their color, the colonies were either susceptible or resistant. For example, spores from the black sectors of heterocaryon A gave black resistant or orange susceptible colonies. These observations indicated that resistant colonies had been produced from susceptible spores produced by the heterocaryons.

Colonies of susceptible and resistant strains involved in each heterocaryon were grown on bean agar so that they were either in contact or almost in contact at the time of sporulation. Myxamoebas were added to the center of each colony. The spore masses of susceptible but not resistant strains were ingested. This observation indicated that diffusable materials probably had not been responsible for the ingestion of spore masses on sectors with the color associated with the resistant strain.

In summary, spores produced from mycelium that was heterocaryotic for susceptibility and resistance are sus-