

The Study of a Cause of the Variation of the Antibiotic Properties of a Bacterium

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Antagonisms indicate the antibiotic possibilities of a microorganism. If one organism hinders or prevents the growth of a second it is said to be antagonistic to that organism. Antagonism may be caused by one organism over crowding the second organism, or by one organism utilizing the nutrients and thus causing the second organism to stop growing, or by one organism producing a substance which is toxic to a second organism.

A bacterium was isolated from a germinating Kentucky bluegrass seed after it was observed to inhibit *Rhizopus nigricans*. It was decided that this organism should be studied for its possible antibiotic properties. *Rhizopus nigricans* is a difficult fungus to inhibit because of its stoloniferous nature. Originally the bacterial inhibitor was so powerful it not only inhibited *Rhizopus* at a considerable distance, but it affected this mold in such a manner that it tended to pile up on the opposite side of the plate from the inhibitor.

This bacterium produced a thermostable substance which inhibited *Rhizopus nigricans*, *Helminthosporium carbonum*, and *Trichophyton inter-digitale*. After several months and repeated subcultures, the culture lost all ability to inhibit *Rhizopus nigricans*.

A microscopic examination of the bacterial culture revealed the organisms were rods of approximately the same size and shape and from microscopic examination there was no evidence that we were working with a mixed culture.

The possibility of a mixture of strains was investigated. The bacterium was plated using standard dilution techniques and a study of individual colonies were made. Seven types of colonies were recognized and studied.

Variant number 1 grew with a spreading growth on a slant with a glossy center and dull edges. It was a circular, flat, opaque colony with a moruloid surface and erose margin.

Variant Number 2 grew with a spreading growth on a slant, with a glossy center and dull edges. It was a circular to slightly irregular, flat colony, glossy and translucent in the center, dull around the edges with a moruloid surface and erose margin.

Variant Number 3 grew with a spreading growth on a slant, glossing in the center and dull and opaque around the edges. It was a circular, convex colony with a tendency to produce concentric buckles. It was opaque with moruloid surface and undulate margin.

Variant Number 4 grew with a spreading growth on a slant, was

glossy in the center with dull edges. It was a flat, circular, opaque colony with a moruloid surface and erose margin.

Variant Number 5 grew with a spreading growth on the slant and was glossy and translucent. The colony was circular, raised, and translucent with moruloid surface and undulate margin.

Variant Number 6 grew with an echinulate growth on the slant. The colony was irregular, raised in the center, translucent with a finely granular surface and entire margin.

Variant Number 7 was filiform on the slant. The colony was circular, raised in the center, translucent with a finely granular surface and entire margin.

It will be seen from this analysis that these strains do not show a great deal of visible variability. This would strengthen the idea of species purity. However there were slight growth variations in these various strains and they were tested to determine their power to inhibit.

No difference was noted in differential media. All strains were gram-positive, motile rods. In nutrient broth, a pellicle was formed but no sediment was produced. Gelatin was liquified glucose and sucrose were fermented with the production of acid but no gas. Lactose was not fermented. Nitrates were reduced to nitrites. No indole was produced. No hydrogen sulfide was produced. There seems to be a tendency for some of these variants to revert to a common form.

The original culture and all variants were tested against *Helminthosporium*, *Rhizopus*, and *Trichophyton*.

There was no difference in the inhibition of *Helminthosporium* by any of the variants or the original culture. However, when *Rhizopus* was used as a test organism variants Number 3, Number 6, and Number 7 inhibited *Rhizopus*. The other strains showed no antagonism to *Rhizopus*.

When *Trichophyton interdigitale* was used as a test organism variants Number 3, Number 6 and Number 7 inhibited *Trichophyton*. Variants Number 6 and Number 7 were better inhibitors producing antagonism from a greater distance. The other strains produced no antagonistic substance for *Trichophyton*.

Trichophyton interdigitale is the organism causing "athletes foot". It would be of value to find an organism that could produce an antagonistic substance to inhibit *Trichophyton*. If the substance could be isolated it could be used as treatment for the disease. Many antibiotic substances have looked promising on a test plate but have proven toxic when used internally as treatment for disease. The danger of toxicity in the antibiotic treatment of *Trichophyton* is not great because it is an external disease and local treatment could be made.

It is apparent that the culture is made up of a number of different strains. The strains differ in their antagonistic characteristics. If some of the ineffective strains dominate the culture, the whole culture is impotent. If, on the other hand, the antagonistic strains dominate the culture, antagonisms toward fungi are exhibited in varying degrees. It seems reasonable that this is an adequate explanation for the variations of antagonisms shown by the original culture.