MUTATION IN CERTAIN PHYTOPATHOGENIC BACTERIA INDUCED BY ACENAPHTHENE

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Mutation in higher plants and fungi is a well-recognized phenomenon. Its artificial induction may be accomplished by various means which are important in obtaining new varieties of useful plants and in the prosecution of mycological and genetical problems.

Blakeslee and Avery (1937) produced mutants in plants by means of an alkaloid of the phenanthrene series, colchicine, which occurs in a high concentration in *Colchicum autumnale*. It is a mitotic poison capable of doubling the number of chromosomes and thus inducing polyploidy. However, attempts to induce changes in fungi and bacteria by means of colchicine have met with failure (Blakeslee, 1939). Jennison (1940) studied the effects of 1 per cent colchicine in nutrient media on ten species of pathogenic bacteria, with no indication of either morphological or physiological changes. Similar results were also obtained by Bonetti and Illenyi (1941). Walker and Youmans (1940) found that 1 per cent colchicine inhibited the growth of *Streptococcus hemolyticus*, but 0.5 per cent and 0.25 per cent had no effect. *Staphylococcus aureus* H developed a somewhat different type of growth in 2 per cent colchicine broth. On solid media wrinkled, waxy, and peculiarly greenish-yellow colonies developed, which obtained only in the presence of colchicine. They reverted to normal type immediately after being transferred to plain media. Thus it appears that colchicine does not disturb the structural apparatus of bacterial cells. In this connection it is interesting to note that colchicine does not affect the cells of *Colchicum autumnale*, from which it is derived (Levan, 1940).

In recent years a chemical of the cholanthrene group, known as acenaphthene, $C_{10}H_6(CH_2)_2$, has been found to cause profound polyploidy in plants (Shmuck, 1938). Extensive studies of this compound indicate that in some respects it may be superior to colchicine in throwing light upon the dynamics of cell division in general and meiosis in particular (Kostoff, 1938*a*, 1938*b*; Navashin, 1938). It has been determined that 7 μg of acenaphthene will cause a profound disturbance in one wheat seed with the formation of a polyploid set of chromosomes (Shmuck and Gusseva, 1939*a*).

METHODS AND RESULTS

The writer has found that acenaphthene induced permanent changes in certain phytopathogenic bacteria. Mutation was induced in *Phytomonas michiganensis* and *Erwinia carotovora* by growing them in nutrient broth saturated with acenaphthene, a medium which was unchanged by previous autoclaving at 15 pounds' pressure for 10 minutes. The inoculum was grown in liquid media, and

only very young cultures were used in the experiments. It was found that the bacteria used in these experiments mutated most readily if recently isolated from their respective hosts. In some cells mutations occurred after two weeks' growth in acenaphthene broth at 28 C. The mutants were detected on agar plates by dilution or streaking. Acenaphthene used in solid media failed to induce any mutation in either form.

Phytomonas michiganensis inoculated into acenaphthene broth at first showed some lag in the rate of growth but soon grew as well as in nutrient broth minus acenaphthene. Apparently a sudden and complete mutation occurs in *P. michiganensis*, since neither intermediate nor typical forms could be found in acenaphthene broth after a certain time, and only a white, slimy, smooth type of colony developed on agar plates. These forms were somewhat more virulent than the parent culture but otherwise have remained similar to the parent, except in colony type, for the last five years under laboratory conditions. There was no deviation from the parent culture so far as sugar fermentation and other reactions were concerned. Morphologically both types were alike. Previously, a similar white strain of *P. michiganensis* had been obtained as a variant in old cultures as well as from field material (Fawcett and Bryan, 1934; Ark, 1944).

Occasionally, when inoculated tubes of the acephthene series were left for some time, a very slow-growing, white, flat type of colony became evident. This proved to be another mutant of *Phytomonas michiganensis*, which was very difficult to maintain in the laboratory and showed no pathogenicity to tomato plants. Unsuccessful attempts were made to change the white race of *P. michiganensis* into the yellow (parental) form by the acenaphthene method.

A recent isolate of *Erwinia carotovora*, from soft rot of carrot, responded to the acenaphthene treatment by producing a mutant. However, a mixture of types prevailed, i.e., normal as well as mutated forms were present when the plates were prepared from 2-week or older acenaphthene broth cultures of the organism. The prevailing type was a grayish-white, compact, flat colony which grew slower than the parent strain. It was only slightly pathogenic on carrot slabs. No physiological or morphological differences were noticeable in common laboratory media.

Phytomonas phaseoli, a yellow pathogen, did not produce any mutants in acenaphthene broth even after very prolonged growth therein.

Thus, acenaphthene does not seem to have similar effects on all species of bacteria. This indication is in agreement with the findings of Shmuck and Gusseva (1939*b*), who found that acenaphthene was not biologically active for the luguminous plants, but strongly so for the cereals.

SUMMARY

Acenaphthene-saturated broth is capable of inducing permanent mutations in *Phytomonas michiganensis* and *Erwinia carotovora*.

Some mutants of *Erwinia carotovora* possessed a reduced pathogenicity for carrot roots, and one mutant of *Phytomonas michiganensis* was nonpathogenic.

Acenaphthene does not act similarly on all species of bacteria.

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