COLCHICINE AS A MUTAGENIC AGENT FOR *STREPTOMYCES GRISEUS*

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Several investigators have caused mutations in morphology and in the ability of *Streptomyces griseus* to produce streptomycin with X-rays or ultra-violet light, Waksman and Harris (Proc. Soc. Exptl. Biol. Med. 71: 232, 1949), Appleby (J. Gen. Microbiol. 2: 80, 1948), but use of colchicine as a mutagenic agent has not been reported. Colchicine is a well known agent for producing chromosomal variations in higher plants and might increase either the metabolic functions of the Actinomycetes or produce other aberrations. The culture of *Streptomyces griseus* used for these studies had been maintained in our laboratory several years and showed no propensity for spontaneous variation. Sterile stock solutions of colchicine in distilled water were kept at 4°C in the dark to prevent oxidation to the inactive aldehyde and were used within three weeks. All studies were carried out using concentrations of colchicine ranging from 0.001 to 5.0 percent.

Colchicine was toxic to *S. griseus* as shown by the inhibition or abnormal germination of spores in broth containing the reagent. There is a rough, inverse relationship between the concentration of colchicine and the length of the germ tube or the amount of secondary branching. Washed spores were suspended in broth solutions of colchicine and allowed to germinate at 28°C for 48 hours. Below 0.05 percent colchicine, germination was normal, greater than 90 percent, whereas in concentrations of colchicine from 0.1 to 1.0 percent there was a gradual decrease both in percent germination and branching of the germ tube. The percent germination decreased from 80 percent in 0.1 percent colchicine to 40 percent in 1.0 percent colchicine and no rudimentary mycelial mats formed at concentrations greater than 0.1 percent. The germ tubes were progressively shortened in 1.0 to 4.0 percent colchicine with no branching. Above 4 percent colchicine germination was only 10 percent and germ tubes were mere stubs.

Two methods of determining changes in the colony morphology were used: (1) exposing washed spores to the various concentrations of the reagent, allowing spores to germinate, and then plating them; (2) streaking normal spores on agar plates containing graded concentrations of colchicine. Three types of variation occurred with greater frequency than in the controls. The most frequent variants were normal, well-sporulating colonies with a sector of dead-white, asporogenous hyphae and some entirely asporogenous colonies, such as have been described by other investigators. The second variant was a slow growing asporogenous

colony which first appeared brown and butyrous with sparse aerial hypae. After 20 days the aerial hypae increased in number and length until at the end of 40 days the colony resembled the asporogenous variants. To determine whether this variant arose only after prolonged adsorption of colchicine on the germinating spore, the spores were washed in distilled water prior to plating them. This treatment did not decrease the number of such variants. The third type was a giant form, 19-25 mm. in diameter with otherwise normal appearance which arose only on agar containing 0.002 percent colchicine. None of the abnormal forms were stable and on subsequent transfer all reverted to the normal form.

Attempts to induce physiological variants of *Streptomyces griseus* which would produce abnormal yields of streptomycin were uniformly unsuccessful. Subcultures of normal and variant colonies developing from spores exposed to 0.001 to 5.0 percent colchicine were transferred to a corn steep soybean and Emerson media in shake flasks. The absence of variants with different capacities for producing streptomycin indicates that colchicine is a less favorable agent for this purpose than X-rays or ultra-violet light.