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Paradoxical Reaction of *Streptomyces avermitilis* to Ultraviolet Irradiation as a Criterion of the Appearance of Highly Active Variants in a Culture

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The paradoxical reaction of *Streptomyces avermitilis* BKM Ac 1301 to ultraviolet light (UV) is revealed. The reaction manifests itself as a drop of lethal and mutagenic activities after exposure to increased dose of UV. Simultaneous changes in the survival of spores and in the number of morphological mutations associated with this reaction are proposed as a criterion for selecting effective doses of mutagen.

Key Words: *streptomycetes; mutagenesis; UV rays; criterion*

Avermectin-based preparations effectively control animal, plant, and human parasites. The nematocidal, acaricidal, and insecticidal activities of avermectins are due to blockade of synaptic transmission in invertebrates. The avermectin complex is produced by *Streptomyces avermitilis* [6]. Highly productive commercial strains are obtained by individual selection of strain and mutagen. We studied the specific reaction of *Streptomyces avermitilis* to ultraviolet (UV) exposure. It was expected that the regularities revealed would be helpful for the choice of effective doses and for the development of highly productive strains.

MATERIALS AND METHODS

Streptomyces avermitilis BKM Ac 1301 strain from the collection of *Farmbiomed* Company was used. An aqueous suspension of resting spores was irradiated with UV (dose range 1000-9000 erg/mm^2) by the conventional method [3]. Spores were kept at a low temperature for 7 days, seeded in Petri dishes with 2% glucose-potato agar, and incubated at 28°C in a thermostat. The lethal effect was assessed by counting the spores surviving after the exposure. Morphologically changed variants (MCV), biochemical mutants (auxotrophs), and changes in biosynthetic activity were assessed. Auxotrophs were detected by total sifting [2] on minimal medium with glucose. Biosynthetic activity (the capacity of a culture to produce avermectin) was assessed by accumulation of avermectins in the mycelium [4].

RESULTS

Figures 1 and 2 illustrate the relationships between the studied parameters of *Streptomyces avermitilis* culture (survival of spores, number of MCV, and activity of avermectin production) and UV dose. All these curves can be divided into two portions reflecting different patterns of changes; the critical

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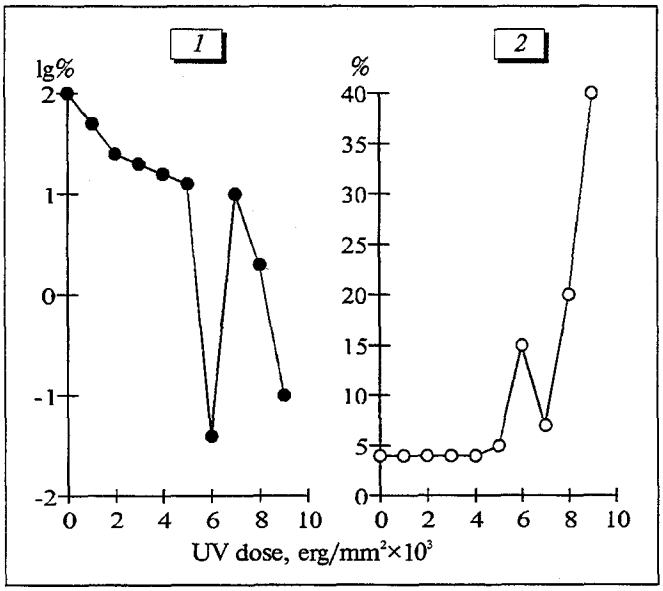

Fig. 1. Relationship between survival of spores (*1*) and number of morphological mutations (*2*), on the one hand, and UV dose, on the other.

point corresponds to 5000 erg/mm². In the 1000-5000 erg/mm² dose range, changes develop gradually. Both lethal and mutagenic effects are weak: at 5000 erg/mm² the survival of spores is 12.5% and the MCV content is 4.2%. Variability shifts towards the low-activity variants, which may be due to a low UV dose [1]. These regularities are consistent with those detected previously for other strains; however, they are observed at high doses which are not commonly used for streptomycetes [1,3].

The data obtained in the 5000-9000 erg/mm² range are particularly interesting: changes in the three parameters are abrupt, considerable, and (most important) synchronous. A dose of 6000 erg/mm² decreases the survival by three orders of magnitude (from 12.5 to 0.35%), the number of MCV increases by 4 times (from 4.2 to 16.6%), and the variability range increases almost 2-fold due to the appearance of plus-variants with an activity of 170.6% of the maximum control level. In other words, both lethal and mutagenic effects of this UV dose increase substantially. However, at 7000 erg/mm², mutagenic and lethal effects drop to levels almost equal to those for 5000 erg/mm²; the survival of spores increases by 2 orders of magnitude (from 0.35% to 5.9%), the number of MCV decreases more than 3-fold (from 16.6% to 5.2%), the variation range becomes narrower than at 5000 erg/mm², and the maximum activity is only 53.3% of the maximum control level. The next two doses induce a noticeable decrease of the survival of spores and increase in the MCV count, the variation range is virtually the same, and the plus-variants are absent.

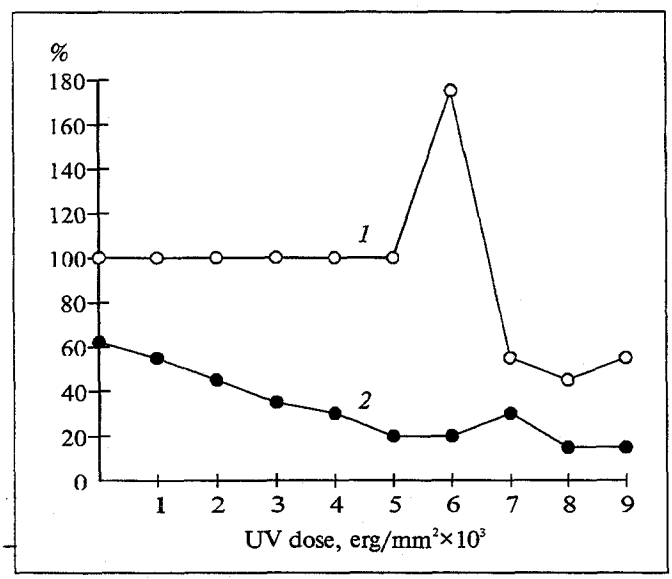

Fig. 2. Relationship between biosynthetic activity and UV dose. The maximum activity of variants of control inoculation is taken as 100%; maximum (*1*) and minimal (*2*) activity.

Obviously, with the exception of the parameters obtained for a dose of 6000 erg/mm², the reaction of *Streptomyces avermitilis* to UV irradiation does not differ from the ordinary response. In fact, increased UV dose reduces survival of spores and increases the number of MCV. Although the MCV count declines after attaining the maximum, it may rise constantly as the dose increases; generally, the variation range decreases after attaining the maximum. Two specific features should be stressed: first, the resistance to UV, since the maximum lethal effect is 99.92% for a dose of 9000 erg/mm². Second, the absence of auxotrophs among morphologically changed variants, which may be due to genetic peculiarities of *Streptomyces avermitilis*: only one tryptophan-defective mutant was detected after exposure to UV, however; after a certain time period it reverted to the prototroph state [5].

The effects caused by a dose of 6000 erg/mm² cannot be regarded as an artifact. They were recorded for the three parameters, and an inverse dependence between the survival of spores and MCV count was observed: the higher the survival of spores, the lower the MCV count. This commonly recognized relationship proves the reliability of these effects, since changes in one parameter are paralleled by changes in the other.

Based on the coincidence of changes in the three parameters, we propose to employ this effect as a criterion of the appearance of plus-variants in the culture while selecting the effective dose of a mutagen. The criterion is an abrupt and pronounced increase in lethality and mutagenicity of irradiation which disappears with a further increase in the dose.

On the curves illustrating the survival of spores and MCV count this effect will plot as two peaks directed towards each other.

The effect observed in the present study is paradoxical, particularly in its second part. The lethality and mutagenicity of UV are expected to increase as the dose increases, although not so substantially. However, disappearance of this effect with further increase of the dose is absolutely unexpected, and the increase in spore survival is very strange. We failed to find any information regarding this effect in the available literature. Interestingly, after the paradoxical effect the spore survival curve changes from exponential to sigmoid. The MCV count curve shows only a rapid increase in this parameter.

An investigation of the nature of this paradoxical reaction is not our goal, since the problem requires a complex approach and special methods. We plan to reveal the conditions, specific features, and ranges within which this effect is exerted for other physiological states of the culture, other strains, cultures, mutagens, *etc.*

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