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CONCERNING CERTAIN PROBLEMS IN THE SELECTION OF MICROORGANISMS

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A number of industrial branches based on the use of microorganisms have developed in the past 20 years. The growth of the microbiological industry is very rapid and it can be assumed that, in certain cases, vegetable or animal raw materials will be replaced by microbe cultures. The capacity of microorganisms to form different biosyntheses, to produce hundreds of "crops" under industrial conditions and to produce chemical reactions inaccessible to modern chemistry all this speaks of vast prospects for their use. This makes it necessary to promote the development of scientific principles for obtaining experimentally beneficial types of microorganisms and to increase research in the field of their selection. The highly active variants of molds, yeast or bacteria provide the possibility of strongly intensifying microbiological processes. Microbiology and genetics face essentially the same problems which science had to solve at the time the selection of agricultural plants and animals began. However, the microorganisms' variability, which makes it possible to obtain beneficial forms experimentally, is distinguished by a number of specific characteristics. In particular, since many microorganisms lack in sexual processes, hybridization does not have the same great significance for them as in the selection of higher plants and animals. On the other hand, radiogenetics has had good results in obtaining high-yield varieties of cultured plants and highly productive species of farm animals, giving at the same time many variants of microorganisms of practical value. The exceptionally rapid reproduction of microbes makes it possible to realize repeated selections of the needed cultures, and to check repeatedly during a comparatively short time the suitability of the method of selection employed. This requires years in higher plants and animals.

Finally, owing to the specific characteristics of metabolism in microorganisms, it is possible, with the help of radiant energy, to produce resistant variants with altered metabolism. These variants can be much more effective in forming physiologically active substances (antibiotics, vitamins, enzymes, etc.) than the original wild forms.

Allowing a certain schematization, it is possible to speak of three types of the new cultures of microorganisms of practical interest: high-yielding variants, variants with an increased or decreased biochemical function, and variants with special properties.

In the first case, the biomass of microorganisms is valuable in itself. For this reason, the selection of breeds with the highest economic coefficient is carried out. This includes fodder and food yeasts, Leuconostoc cultures (from the polysaccharide capsule of which a blood substitute is prepared) and many other forms. Here progress in the selection of microorganisms is insignificant since the majority of radiomutants reproduce comparatively slowly.

As regards the microorganisms of the second type, the strengthening of this function presents an especially great practical interest. In this case progress in the selection of microorganisms is particularly significant. In this group can be included the variants of penicillin or of Actinomyces, forming increased amounts of antibiotics; variants of Aspergillus, forming hydrolytic enzymes or organic acids; variants of bacteria, yeast or molds synthesizing vitamins or provitamins (riboflavin, ergosterol, B_{12} and others).

Cultures with a decreased or lost function of synthesis of certain substances are less desirable. In the antibiotic industry penicillin variants were produced experimentally which did not form yellow pigment diffused in culture medium; this made it possible to produce colorless penicillin. Analogous pigmentless variants can be obtained from Actinomyces which simultaneously form antibiotics and pigments. Microorganisms incapable of synthesizing a certain vitamin or amino acid and consequently, not developing when they are absent can be used as an extremely fine and precise indicator of a given substance. Variants of pathogenic bacteria which lost their virulence but preserved their immunogenesis are of considerable interest. These cultures are being successfully used for producing living vaccines, which protect man from diseases such as tularemia, pest, etc.

Cultures of the third type are frequently needed in practice. These cultures include those developing at high or low temperatures, high or low rN, resistant to the action of different antibiotics, antiseptics, toxins, salts, etc. As a rule, these types are obtained by cultivating microorganisms at a gradually increasing concentration of toxin or at a gradually rising temperature. This "accustoming" always succeeds and is the property of the adaptation of microorganisms. Ordinarily radiant energy is not used for this and the question has not been decided whether a preliminary irradiation affects the time of microbe's adaptation to the new conditions of life.

Modern physics and chemistry have placed at the disposal of the microbiologist different physical and chemical factors capable of producing hereditary changes in microorganisms. However, these factors are not all equally interesting for the selection of effective cultures of microorganisms. Thus with the help of ultraviolet rays it is possible to obtain in a few minutes a number of variants in the culture of microorganisms. In using gamma-rays for the same number of microorganisms, it would require a prolonged action or a very powerful apparatus.

There are two important problems of radiogenesis the solution of which bears a direct relation to the selection of microorganisms; 1) the relation between the radiation dose and the quantity of variants formed and 2) the effect of the dose on the character of the variants formed.

Observations accumulated during recent years show that an increase in the number of mutants forming under the effect of radiation corresponds to an increase in the dose only to a certain degree. Above this limit the number of variants starts to decrease (Figure 1). Thus, optimal doses do exist and they must be established for each type of microorganism. At the same time one should remember that sensitivity to rays differs not only in various types of fungi or bacteria but also in different strains of one and the same type.

The question of the effect of the dose on the character of variants is less studied. The increase in the total number of variants with a determined dose is not always accompanied by a proportional increase of all their types. Thus, with an increased dose the number of morphological variants deficient in the synthesis of certain substances may increase whereas, at the same time, the frequency of mutants with certain properties (such as in synthesizing organic acids, antibiotics, etc.) may decrease. Therefore it is necessary to determine optimal doses not for forming variants in general but for obtaining cultures with necessary properties. Unfortunately not much experimental data on this extremely important problem exist.

For a long time microbiologists and geneticists paid great attention to a theory according to which the radiation effect hastens the process of "natural" variability (occurring under conditions of laboratory life). In other words, highly effective factors make it possible to obtain the same phenomenon which occurs in population by "itself" and therefore, radiation energy does not produce new forms. Now it is possible to refute this theory with good reason. Active variants of penicillin mold with 100 times more antibiotic content than in its original native forms were obtained under the effect of ultraviolet and roentgen rays. These active forms never appear spontaneously -- that is, under laboratory conditions of the mold's life nor do they exist in soil or in other natural media. Sublethal forms of minute colonies of microorganisms can be produced under the effect of radiation energy. Thus, it is possible to obtain from Aspergillus and Penicillium variants of very fine, brown and smooth colonies devoid of aerial mycelium. Not knowing the origin of such colonies, the micrologist-systematist would categorically deny that they belong to the genus of Aspergillus or Penicillium, as he has not come across them in nature. This already permits us to assert that an external action (in this case the radiation energy) not only increases the number of mutants but also creates new ones and, in this way, plays an important role in form development. This conclusion is a matter of principle.

For the selection of microorganisms the following question is of importance: is it sufficient to irradiate the culture once or is it necessary to repeat the selection and irradiation? Although this question is still in the stage of experimental researcM, the available factual data makes it possible to make certain preliminary conclusions.

In particular, it can be considered established that a single irradiation makes it possible to obtain beneficial forms of microorganisms although their new properties are not always fixed hereditarily. Multiple irradiation is more reliable when after each exposure determined properties are checked and colonies are selected for a new irradiation. This can be illustrated by the diagram on obtaining the variant of Aspergillus nidulans, which forms active protease (Figure 2). This selection by degrees proved its value for the active cultures of penicillin and for the variants of Aspergillus niger, forming increased amounts of citric acid. After each new irradiation, it was possible to isolate a culture more active than the previous one. Of course it is difficult to expect that the properties of the culture will continue to improve infinitely through the selection by degrees. However, the experiment proves that good variants are usually produced only after a four or sixfold irradiation.

The problem of correlation between morphological and physiological changes of variants appearing after irradiation is considered to be among the most interesting and is much discussed. There is no doubt that at the basis of the variability of microorganisms lies the change in metabolism and that the appearance of morphological changes has a secondary character and is related to the realization of a new function or that it reflects those changes in metabolism caused by irradiation.

Between the functional and morphological changes exists a permanent interrelation. However, these generally correct theoretical propositions cannot be proved in every concrete case at the present level of our knowledge.

Let us assume that as a result of irradiation, the mutant of a fungi has lost the capacity of realizing a certain stage in the biosynthesis of one or another amino acid or vitamin. Modern methods of morphological study do not make it possible to discover in each case structural changes in such biochemical mutant or to find morphological deviations in the structure of mycelium, conidium, or colonies. Therefore, the correlation between the structural and functional characteristics is not always fixed in the variant of microorganisms. Does it mean that it does not generally exist? Of course not. Under the effect of radiation the microorganism is changed entirely, its metabolism and its structure.

This conclusion is confirmed by a number of facts established by microbiology, radiobiology and genetics. In the 15-year period of penicillin production in different countries, active species of fungi have been obtained with the help of highly active factors. This permitted the production of penicillin to increase considerably. However, among these species each culture differs from the original native form of penicillin. The native form has round, large and smooth colonies, which form conidium with ease whereas all the cultured forms of penicillin give smooth plicate colonies with less conidium.

A microscopic examination of mycelium confirms the existence of sharp differences between the original native forms and the cultured. The Institute of Microbiology of the Academy of Sciences of the USSR has obtained the cultures of Penicillium chrysogenum, forming more gluconic acid, and of Aspergillus niger, yielding more citric acid. These cultures had a plicate character and differed sharply from the original forms.

It is also frequently possible to distinguish in bacteria a close relation between morphological signs and physiological properties. Thus, in bacteria belonging to coli-typhoid group plicate forms, rather than the smooth ones, lose immunogenic properties. For this reason not a single medical institute producing biopreparations prepares vaccines from plicate variants.

This relation can be reversed. For instance, good results are obtained only from cultures forming plicate colonies in installations for producing bacterial protease from Bac. mesentericus cultures. Considerably less active protease accumulate with the reproduction of smooth forms.

Thus, in many cases it is possible to establish a connection between the structure and function. This connection is evident when, under the effect of radiant energy, weakened and poorly developed forms appear. The external appearance of colonies, accumulating negligible biomass, and the microscopy of mycelium or cells reveal that a given mutant cannot find a practical application as it is not very viable.

The examples given confirm that the correlation between the function and the form does really exist. This permits one to approach the correct solution of the problem concerning the methods of checking this or other properties of cultures obtained.

Geneticists who in the last 20 years have studied the variability of microbes are inclined to test the function from which the selection is made in all the cultures grown after irradiation. By this "direct" test it is possible to find out the physiological properties of all the mutants. Consequently, the danger of "missing" a valuable form is removed. However, this method is very deficient since it makes necessary the testing of thousands of cultures.

Microbiologists who have studied the type of the culture and its morphological characteristics can avoid this laborious and, to a considerable degree, unnecessary work. In selecting the cultures of Actinomyces rimosus, forming oxytetracyclin, considerably less active cultures are found among the radically changed morphological variants. It is natural that in selection radically changed cultures must be rejected without determining their activity. Furthermore, it is known that Aspergillus, active in protease or in forming citric acid, always changes morphologically and consequently, there is no need to test the activity of all the colonies grown after culturing irradiated conidium. Selection by phenotypic character is widely practiced in the selection of plants and animals, and there is no basis for rejecting it in the selection of microorganisms.

It is quite evident that one cannot be guided by the structure of the cells or mycelium as well as the form of colony in all cases without exception. However, in cases where this relation has been unmistakingly proved one should not perform the extra work of determining the activity of cultures whose colonies did not undergo morphological changes.

Independently of the character of rays, their effect is essentially to produce variants with a decreased viability. The variants lose the capacity to realize one or another biosynthetic process; sometimes sublethal variants may develop, perishing in consequent cultures. On the basis of long experience, microbiologists discovered that radiation energy frequently leads to the degeneration of biological potential and to the formation of "monsters" rejected in nature through selection. Earlier, this appeared to be an entirely correct conclusion as, in fact, the growth and development of microorganisms (the chief indicators of viability), as a rule, slow down in the majority of variants. The successful research on the physiology and biochemistry of mutants made it possible to establish the fact that the concepts of "little viable" or "degenerative" do not have an absolute but a relative significance.

The conditions of life fully satisfying the needs of an original native form prove to be little suitable for the radiomutants produced. Research on the delicate peculiarities of nutrition, respiration and the physiology of growth and development has demonstrated that new variants must also be cultivated under new conditions, particularly in different media.

The following examples can be given by way of illustration. With the help of ultraviolet radiation, a variant with lowered viability was produced from Aspergillus nidulans. As it grew on the surface of a liquid culture medium, it formed a film the weight of which was half as much as the weight of the film of the original culture. However, by cultivating this slowly growing variant in flasks containing rich, well aerated culture medium, it was possible to obtain the same culture as the original.

Therefore, by creating the necessary conditions of life it is possible to prevent the decrease of biological potential, which can be so frequently observed in mutants obtained under the effect of radiation energy.

All this shows that it is very important to study the physiology of variants and that tests of their activity should not be limited to the same conditions under which the original culture was tested. The possibilities for the microbiologist in this respect are much wider than those of the selectionist of higher plants and animals because he can create any conditions in the way of temperature, the composition and reaction of medium as well as the aeration of culture.

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Thus, in obtaining experimentally beneficial forms of microorganisms, microbiology and radiogenetics are confronted by the following important tasks.

First of all, it is necessary to discover the mechanisms lying at the basis of the formation of beneficial variants of microbes under the effect of radiation energy so that the derivation of valuable cultures ceases to be accidental.

The physiology of nutrition, growth and development of variants forming must be studied in further detail. This will make it possible to prevent the decrease of their biological potential.

It is very important to supplement our data on the correlation between biochemical and morphological properties of the variants obtained. The knowledge of morphological signs for the rejection of non-active cultures as well as that of the structural characteristics of beneficial forms can considerably facilitate selection.

Finally, it is necessary to develop methods for growing and preserving experimentally produced variants. These methods should remove the fear of losing the basic and practically important properties of one culture or another.

Naturally all these investigations can succeed only when there is a close contact between microbiologists and physicists and between chemists and technologists. Precisely that very close cooperation would ensure the success already achieved in the selection of microorganisms.

FIGURE APPENDIX

Figure 1. Survival of conidia and formation of morphological variants in Aspergillus nidulans in ultraviolet irradiation of conidium. 1 - survived conidia; 2 - Morphological variants; abscissa-energy in ergs (on conversion to 1 conidium X 10^{-3} at $\lambda = 2537$ Å); ordinate - % of survival of conidia and % of morphological variants.

Asp. nidulans (original)$^{5'}$ ⇢ (n/4)$^{5'}$ [etc.].

Asp. repens (original)$^{10'}$ ⇢ (r/6)$^{5'}$ [etc.].

Figure 2. Diagram of obtaining plicate forms in different cultures of Aspergillus. Dotted arrows designate irradiation tests; the numbers above them designate the length of exposure. Solid arrows designate tests not followed by irradiation with cultures selected after growing.

5253