DETERMINATION OF BIOGENIC STIMULATORS BY THE YEAST NEPHELOMETRIC TEST

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(Received March 18, 1956. Presented by Academician V. P. Filatov)

As V. P. Filatov [12] and other authors have established, an accumulation of biologically active substances (called biogenic stimulators) occurs in isolated tissues and in whole bodies during the action on the latter of unfavorable factors.

Biological tests are usually used to determine the accumulation of these biogenic stimulators, their chemical nature and action mechanism. The following are some of the tests proposed for this purpose: 1) acceleration of skin defect regeneration in animals [8, 14]; 2) acceleration of epithelial regeneration in isolated frog's eyes [5]; 3) acceleration of plant seed germination and development [2, 10]; 4) heightened enzymatic activity [1, 10]; 5) acceleration of yeast fermentative activity [13].

Some of these tests take a long time. For example, the test of accelerated skin defect regeneration, using a rabbit's ear, takes 30-40 days and needs a large number of experimental animals; the test on plants takes 10-15 days. Therefore, it is important that new tests, which would be simple, quick and exact, be discovered and developed.

Many authors [4 and others] have often mentioned the extraordinary sensitivity of yeast cells to various substances. With this in mind, V. P. Filatov, V. A. Biber and L. A. Adamanis [12] proposed that the fermentative activity of fresh bread yeast be used as a test to disclose biogenic stimulators. It was then discovered that this yeast often varies as to original fermentative activity, which distorts the results in experiments determining biogenic stimulators and does not permit comparison of results from different experiments. The method of L. I. Palladina and A. M. Gudina [6], which is based on stimulating dried bread yeast reproduction, has the same drawback.

To eliminate the biological heterogeneity of yeast cells, N. V. Yanyk and E. P. Karpova [14] proposed that a pure culture of yeast cells be used and their division be computed by counting the yeast cells in a chamber under a microscope. This method, however, has not been widely used since counting the cells in a chamber is a laborious, time-consuming method and is not free from the element of subjectivity.

This method was somewhat simplified by S. R. Muchnik and A. F. Shcherbina's modification of it. Computation of the cells under a microscope was replaced, in this modification by computation of the yeast colonies obtained by culturing the yeast in Petri dishes on a hard agar medium.

A. F. Sysoev [8] successfully used a method measuring yeast reproduction by an electrophotocolorimeter instead of by counting the yeast cells.

G. S. Dyachkov [3] indicated the possibility of determining the concentration of the yeast suspension by a nephelometer.

The purpose of this work was to find the optimal conditions for the use of the nephelometric method and to examine the possibility of converting it into a method allowing the activity of the tissue preparations used in different experiments to be compared, which is extremely important to the standardization of tissue preparations.

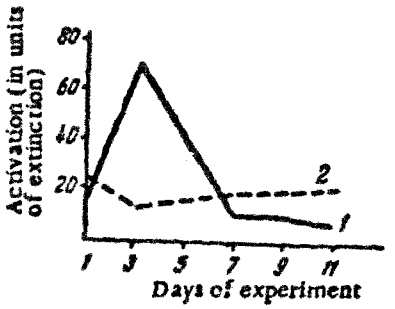

Fig. 1. Activation of yeast reproduction by extracts from preserved tissues compared with that effected by extracts from fresh tissues, depending on the age of the yeast culture (in units of extinction): 1) extract from preserved bull's skin; 2) extract from preserved aloe leaves.

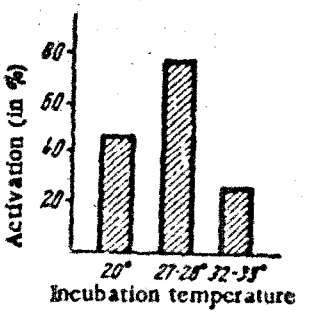

Fig. 2. Effect of yeast reproduction stimulation depending on incubation temperature.

The following questions were considered in our study: 1) the effect of the yeast culture's age; 2) the effect of the temperature in which the yeast was incubated; 3) the effect of the extract concentration on the degree of yeast reproduction; and 4) the frequency of similar results in experiments done at different times.

We believed that the age of the yeast culture was probably extremely important in this test, since it may be assumed that as the age of the yeast cell changes, so does its reaction to the influence of stimulating substances. We compared yeast culture reproduction in yeast of different ages in the same experiment and found that yeast aged 1-3 days reacts most actively to the stimulating substances of the preserved tissue extracts. With the addition of aloe extract to the yeast suspension, the age of the yeast did not seem to be so important, although the intensity of yeast reproduction decreased 2-3 times (Fig. 1) when an older yeast culture was used (7-11 days).

The influence of the incubation temperature on yeast reproduction stimulation was examined at temperatures of 20°, 27-28° and 33-34°. It was then established that the most optimal temperature was 27-28° (Fig. 2).

Data showing the influence of the extract dilution on the intensity of yeast reproduction is given in Table 1.

As the data in Table 1 show, yeast reproduction activation caused by adding an extract from preserved muscular tissues is greatest when the concentration of the extract is 1:5. When the extract is further diluted, the difference between the extracts from fresh tissues and those from preserved tissues is still apparent in dilutions of up to 1:50; extracts with greater dilutions have only a slight influence. As the aloe leaves extract was diluted, the stimulatory effect of the extract from the preserved leaves first increased and reached it strongest expression in a dilution of 1:50. The limit of sensitivity of the test of this extract must evidently be considered a 1:100 dilution.

Based on the data given above, we can assume that the most optimal conditions for the use of the yeast test are the following: age of yeast culture – 1-3 days (better to keep to some one of these days), incubation temperature – 27-28°, dilution of extract from animal tissues – from 1:5 to 1:50, from aloe leaves – 1:50 to 1:100 per wet weight of tissue.

EXPERIMENTAL METHODS

The experiments were done on a pure culture of Saccharomyces cerevisiae yeast, strain No. 47, using Rider's medium.

The method we developed is as follows: sterile test tubes (with cotton plugs) were filled, under sterile conditions, with 1 ml of the experimental extract in a corresponding dilution (usually 2 dilutions were tested – one from the optimal zone and another more concentrated). Then 4 ml of Rider's solution was poured into the test tubes (contents of Rider's solution, per 1 liter: 3 g ammonium sulfate, 1 g monohydrogen potassium

phosphate, 0.1 g dihydrogen potassium phosphate, 0.4 g calcium nitrate, 0.5 g sodium chloride, 0.7 g magnesium sulfate, 20 g saccharin), and 2 ml of a yeast suspension from a pure culture of Saccharomyces cerevisiae yeast with an extinction of 0.05 (optical density), according to the electrophotocolorimeter was added. We prepared the yeast suspension in the following manner: first a suspension was prepared from a 3-day old yeast culture on brewing wort. The pure yeast culture was maintained continuously by culturing two loops of liquid yeast culture, or one loop of the culture from the hard agar medium, in 5 ml of brewing wort. To obtain the yeast suspension, the pure yeast culture, in the 5 ml of wort, was poured into 100 ml of boiled tap water and shaken for 20 minutes by an apparatus for that purpose. Six ml were taken from this suspension and transferred to the next 100 ml of water. After this had been shaken for 10 minutes, the extinction of the resulting yeast suspension was determined; it had to measure 0.05 on the scale, and the layer of fluid in the vessel had to be 1 cm thick. If these measurements were not obtained, more water or more of the concentrated yeast suspension was added, depending on which was necessary.

TABLE 1

Activation of Yeast Reproduction Effected by Extracts from Preserved Tissues (in per cent of Reproduction Intensity Effected by Extracts from Fresh Tissues) with Different Dilutions of the Extracts.

Extracts	Dilution of extracts per wet weight of tissue							
	1 : 5	1 : 10	1 : 20	1 : 50	1 : 100	1 : 200	1 : 500	1 : 1000
From bull's muscles .	150	140	134	130	104	108	109	108
From aloe leaves . .	123	132	159	160	146	143	—	106

EXPERIMENTAL RESULTS

We observed that the yeast remained cloudy for a day after acid fixation, and that the cloudiness sometimes recurred.

Biological activity was expressed figures of extinction (optical density) after taking the reading from the control test tubes and then translated per 1 mg of the dense residue of the extract.

This methods permits biological activity to be determined in many types of extracts simultaneously. The advantages this method possesses over other methods of determining biogenic stimulators is the speed and simplicity of its execution, the availability and economy of the materials needed and, finally and most important, the objectivity of its results and the possibility of comparing data obtained from different experiments.

The following example is a good example of this: in two experiments done at different times, the same extracts were tested, but with a different list of ciphers.

Water was poured into the control test tubes (there were 8-10 of these) instead of the extract. When the yeast suspension was poured into the test tubes, the flask containing the suspension was shaken constantly to prevent the yeast cells from settling. The test tubes were carefully shaken and placed in an incubator at a temperature of 27-28° for approximately 17-20 hours. After the 17 hour period in the incubator, the first two control test tubes were taken out and 1 ml of 50% by volume sulfuric acid was added to stop yeast growth; then the test tubes were shaken and the extinction was determined on an electrophotocolorimeter — it had to be 0.100 units on the scale. If the extinction had not yet reached this figure, the test tubes were left for another hour in the incubator, after which the extinction was again determined on the next two control test tubes, etc., until an extinction of 0.100 units was reached.

Measuring the concentration of the original yeast suspension and that of the control test tubes after incubation established a background for this test which was constant, thereby permitting the data obtained in differ-

ent experiments to be compared.*

After the indicated density of the yeast cell suspension was obtained in the control test tubes, all the test tubes were removed from the incubator, 1 ml of 50% sulfuric acid was then added, the test tubes were carefully shaken, poured into the electrophotocolorimeter container, and the extinction was measured. Water was poured into the control vessel.

TABLE 2

Results Obtained from Testing the Extracts

Extracts	Experiment I	Experiment II
From fresh aloe leaves	118	119
From preserved aloe leaves	123	129
From fresh placenta	123	119
From preserved placenta	135	130

The data in Table 2 shows the closeness of the results from both experiments and, therefore, the possibility of using the proposed test in order to standardize tissue preparations.

It could be proposed that the suggested yeast test method could also be widely used to determine other biologically active substances, vitamins, hormones, auxins, etc., which, added to a yeast suspension, would stimulate or inhibit yeast reproduction.

SUMMARY

Optimal condition for the A. F. Sysoev's yeast nephelometric test have been established. They are: age of yeasts 1-3 days, incubation temperature 27-28°; suspension of animal tissue 1 : 50 to 1 : 100 (live weight of tissue). The method thus modified may be used for standardization of tissue preparations.

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*Since it is very difficult to ascertain if the readings of the control test tubes are exactly 0,100 on the scale, one may equalize the data by rounding it off to 0,100. The permissible deviations are between 0,090 and 0,120.

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