INVESTIGATION OF THE PRODUCTION OF GROWTH-PROMOTING AND GROWTH-INHIBITING FACTORS BY ULTRA-VIOLET IRRADIATED MICROÖRGANISMS

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INTRODUCTION

The alleged proliferation-promoting effect of sub-lethal doses of ultra-violet radiation for microörganisms has been the subject of considerable controversy. The earlier literature is reviewed elsewhere (Duggar, 1936; Sperti, Loofbourow and Dwyer, 1937a). Recently, various investigations have lent support to the positive point of view. Thus, our coworkers and ourselves published evidence in 1937 that yeast and animal tissue cells injured by ultra-violet radiation release proliferation-promoting factors into the suspending medium (Fardon, Norris, Loofbourow and Ruddy, 1937; Sperti, Loofbourow and Dwyer, 1937a, b). Concurrent investigations of Florence Meier indicated that sub-lethal doses of ultra-violet stimulate the proliferation of unicellular green algae. In 1938, Hollaender and Duggar reported carefully controlled experiments in which the division rate of Escherichia coli was similarly stimulated by sub-lethal ultra-violet irradiation.

More recent investigations in our laboratories, of the factors obtained from ultra-violet injured cells, have indicated that the proliferation promoters are hormone-like substances ("intercellular hormones") produced by living cells and released into the intercellular fluid as a physiologic response to injury (Loofbourow, Dwyer and Morgan, 1938). Spectrographic studies of the active concentrates (Loofbourow, Schmieder, Stimson and Dwyer, 1937) have shown them to be nucleic-acid-like, and subsequent investigations by chemical and spectrographic methods (Cook, Loofbourow and Stimson, 1938; Loofbourow, Cook and Stimson,
1938) have substantiated these indications and afforded evidence that the active materials contain adenine, guanine, pentose and phosphorus, but are free from protein, sulphur, pyridine, or pyrimidines.

The question of whether microorganisms other than yeast respond to ultra-violet injury by the production of proliferation-promoting factors similar to those obtained from yeast seemed worth investigating and we consequently undertook such studies, beginning in 1937. In a previous publication (Loofbourow and Morgan, 1938) we reported preliminary results on the effects of preparations from ultra-violet irradiated yeast on bacteria, in which it was found that concentrated cell-free fluids from irradiated yeast suspensions stimulate bacterial growth. We wish now to present studies of the effects of similar preparations from Staphylococcus aureus, Aerobacter aerogenes, and Escherichia coli as well as further investigations of the effects of preparations from Saccharomyces cerevisiae on the above microorganisms and on Diplococcus pneumoniae.

METHODS

The preparation of the cell-free filtrates from the irradiated and non-irradiated suspensions followed closely techniques previously described (Loofbourow and Morgan, 1938). The microorganisms were suspended in 0.85 per cent sterile saline. A portion of the suspensions was irradiated in quartz test tubes, with constant stirring, using unfiltered ultra-violet radiation from a Burdick A.C. quartz mercury arc, operated at 65 volts across the arc. The irradiation distance was 25 cm. for yeast and either 25, 40 or 50 cm. for bacteria. The remainder of the suspension was kept at the same temperature as the irradiated portion (30° to 35°C.) throughout the irradiation period. After irradiation, both suspensions were filtered through Berkefeld N filters. Samples from the filtrates were tested for growth stimulation, both before and after taking them to dryness in an oven at temperatures of 56° or 90°C. The dried samples were made up to a concentration of 30 mgm. per ml. and autoclaved at 20 pounds for 15 minutes.
Since previous results with irradiated yeast indicated that the proliferation-promoting materials are produced in greatest quantity if the irradiation conditions are such that prolonged exposure is required before extensive killing of the organisms takes place, experiments were first conducted in each instance to determine the concentration of microorganism suspension which would withstand at least a 20-minute irradiation period before 90 to 95 per cent of the organisms were killed. These concentrations were then adopted as the minimum ones to use in preparing irradiation products. In some instances, heavier suspensions were employed, in order that the killing time might be prolonged. As a means of duplicating concentrations, optical density measurements with a photoelectric photodensitometer were used (Beck, 1937; Loofbourow and Dwyer, 1938).

The suspensions for irradiation were prepared as follows: In the case of yeast, Fleischmann’s bakers’ yeast from commercial cakes was used. Known weights were suspended in saline to give the required concentrations. *Staphylococcus aureus* was grown on nutrient agar (Difco) in culture tubes or Roux bottles. Twenty-four-hour cultures were washed off with saline. The concentrations of the suspensions (in saline) to be irradiated were adjusted to predetermined values with the aid of the photodensitometer. *Aerobacter aerogenes* and *Escherichia coli* were grown on nutrient agar (Difco) in Roux bottles. The organisms were washed off and brought to the desired concentrations as in the case of *S. aureus*. In all but the early experiments with *S. aureus*, the organisms were washed repeatedly with saline before suspending them for irradiation.

The growth potencies of the concentrates were tested by adding them to cultures of the organisms in liquid nutrient medium, and comparing the growth after 24 hours with that in controls.

In the case of yeast, Reader’s (Reader, 1927) medium was used for the growth tests. The test organism was a strain of *S. cerevisiae* which had been cultured in our laboratories for about two years on solid Saboraud’s medium (Difco). Serial dilutions in Reader’s medium of the preparations to be tested
were introduced in quantities of 1 ml. into rocker tubes (Norris and Kreke, 1937). The tubes were inoculated with \textit{S. cerevisiae} suspended in Reader's medium in such concentration (determined photoelectrically) as to make the final value in the rocker tubes 0.064 mgm. per ml. (wet weight) after addition of sufficient medium to bring the total volume to 25 ml. The tubes were rocked approximately 60 times a minute for 24 hours in a water bath at 30°C. The final concentrations of the suspensions were then measured photoelectrically.

In the growth tests on bacteria, cultures were grown in nutrient broth (Difco), or Hartley broth, in culture tubes, at 37.5°C. The materials to be tested were added to the tubes in serial dilutions. The tubes were inoculated with suspensions of organisms washed with saline from 24-hour slants, the inoculating suspensions being adjusted to the same concentration (using the photodensitometer) in succeeding experiments in order to insure uniformity of seeding. The concentrations of the inocula used for each organism were chosen by experiment to give easily measurable growth in the controls after 24 hours. The 24-hour growth was determined by photodensitometer measurements. In some instances centrifugation in Hopkins' vaccine tubes was also employed for growth estimates.

In the quantitative assays, a growth unit was used which may be defined as the potency capable of doubling the 24-hour crop of 1 ml. of microorganism culture as compared with controls, under the conditions of the experiments. This unit is smaller than Norris' unit (Norris and Kreke, 1937) which we used in previous papers (Loofbourow, Norris and Morgan, 1937; Loofbourow and Morgan, 1938). It serves better for assays on bacteria, however, because it obviates many extrapolations from the experimental data, which would otherwise be necessary. A potency of "one growth unit per milligram" as used here means that the addition of 1 mg. of the material per ml. of culture is sufficient to double the twenty-four hour crop as compared with controls, the crop being measured on a weight basis rather than on the basis of population count.

Since the production of irradiation products seems to result
frequently in an increased weight yield in the filtrates, as well as an increased potency per unit weight of the material obtained, expression of growth potency in terms of growth units per ml. of original filtrate gives a more accurate picture of the relative yield of growth factors than expression of potencies in terms of growth units per unit weight. When possible, potencies are, therefore, presented both on the unit weight and unit filtrate volume basis.

**TABLE 1**

*Assays on yeast of yeast preparations, including mixtures used for tests on bacteria*

<table>
<thead>
<tr>
<th>PREPARATION</th>
<th>CONCENTRATION OF SUSPENSIONS</th>
<th>IRRADIATION</th>
<th>YIELD</th>
<th>GROWTH UNITS PER ML. OF FILTRATE</th>
<th>RATIO OF IRRADIATED TO NON-IRRADIATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>mgm. per ml.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.5</td>
<td>60 m.</td>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.5</td>
<td>Control not irradiated</td>
<td>Distance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.5</td>
<td>25</td>
<td></td>
<td>1.59</td>
<td>13.3</td>
<td>21.2</td>
</tr>
<tr>
<td>17.5</td>
<td>Control not irradiated</td>
<td></td>
<td>0.77</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

| 2           | 17.5                          |             |       |                                  |                                      |
| 17.5        | 55 m.                         |             |       |                                  |                                      |
| 17.5        | Control not irradiated        |             | 1.78  | 20.8                             | 37.0                                 |
| 17.5        | 25                            |             | 0.255 | *                                | *                                    |

Mixture A

| Average 20  | Average 60 m.                 |             |       |                                  |                                      |
| Average 20  | Control not irradiated        |             | 2.88  | 5.0                              | 14.4                                 |
| Average 20  | Control not irradiated        |             | 2.71  | 0.76                             | 2.06                                 |

Mixture B

| 100         | 6 hrs.                        |             |       |                                  |                                      |
| 100         | Control not irradiated        |             | 11.26 | 3.05                             | 34.3                                 |
| 100         | Control not irradiated        |             | 0.88  | 0.85                             | 0.75                                 |

* These samples not assayed in sufficient quantities to determine their potency quantitatively. In the concentrations used, they showed no measurable potency.

**RESULTS**

*Preparations from S. cerevisiae*

In all, seven preparations were used. Table 1 shows examples of assays on yeast, details of preparation, etc. Figure 1 shows an individual assay on yeast. In order to obtain sufficient material for testing on various bacteria, it was necessary to mix several preparations. The assays of the mixture used in the experiments on bacteria are shown in the table.

As in previous investigations, the potencies of preparations
from irradiated yeast (in growth units per cubic centimeter of filtrate) ranged upwards from seven times as great as the potencies of filtrates from non-irradiated suspensions. The greatest ratio thus far reported is approximately ninety to one (Loofbourow, Dwyer and Morgan, 1938). Thus, the effect for yeast is well outside the range of experimental error and is easily
duplicated provided conditions are so chosen that an extended period is required to kill most of the cells.

Results of assays of the yeast preparations on bacteria are summarized in table 2. Figure 2 shows examples of the assays.

In every case, the preparations from irradiated yeast stimulated the growth of the organisms tested. Quantitatively, the
### TABLE 2

**Summary of assays of irradiated and non-irradiated yeast preparations on bacteria**

<table>
<thead>
<tr>
<th>PREPARATION</th>
<th>POTENCY, GROWTH UNITS, ON VARIOUS ORGANISMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. cerevisiae</td>
</tr>
<tr>
<td>Irradiated mixture A</td>
<td>5.0 /mgm.</td>
</tr>
<tr>
<td></td>
<td>14.4/ml.</td>
</tr>
<tr>
<td>Irradiated mixture B</td>
<td>3.05/mgm.</td>
</tr>
<tr>
<td></td>
<td>34.3/ml.</td>
</tr>
<tr>
<td>Non-irradiated mixture A</td>
<td>0.76/mgm.</td>
</tr>
<tr>
<td></td>
<td>2.06/ml.</td>
</tr>
<tr>
<td>Non-irradiated mixture B</td>
<td>0.85/mgm.</td>
</tr>
<tr>
<td></td>
<td>0.75/ml.</td>
</tr>
</tbody>
</table>

Irradiated mixture A:
- Potency: 5.0 /mgm., 14.4/ml.
- Growth units: 14.4 ml.
- Additional inhibition:
  - Inhibition: < 6 mgm. per ml.
  - Inhibition: > 6 mgm. per ml.

Irradiated mixture B:
- Potency: 3.05/mgm., 34.3/ml.
- Growth units: 34.3 ml.
- Additional inhibition:
  - Inhibition: < 6 mgm. per ml.
  - Inhibition: > 6 mgm. per ml.

Non-irradiated mixture A:
- Potency: 0.76/mgm., 2.06/ml.
- Growth units: 2.06 ml.
- Additional inhibition:
  - Inhibition: < 6 mgm. per ml.

Non-irradiated mixture B:
- Potency: 0.85/mgm., 0.75/ml.
- Growth units: 0.75 ml.
- Additional inhibition:
  - Inhibition: < 6 mgm. per ml.
effect was much less than in the case of growth tests on yeast, however. It is unfortunate that one preparation could not be used throughout, because of insufficient quantity, but comparison of the effects of the irradiation product mixtures A and B on *Aerobacter aerogenes* indicates that the potencies of these two mixtures for bacteria were roughly parallel to their potencies for yeast. If this is assumed, one may arrange the microorganisms as follows in order of decreasing activity of the irradiated yeast preparations on them. (1) *S. cerevisiae*, (2) *E. coli*, (3) *S. aureus*, (4) *D. pneumoniae*, (5) *A. aerogenes*.

In the case of the preparations from non-irradiated yeast, both stimulation and inhibition were found. In two instances (*E. coli* and *A. aerogenes*) low concentrations appeared to stimulate while higher concentrations inhibited.

Comparison of the effects of preparations from irradiated and non-irradiated suspensions showed, in all instances, that irradiation led to the production of a growth factor effective on the microorganisms tested. The growth factor (or factors) in the irradiation products was evidently heat stable, since the assays were made after drying the filtrates in an oven and autoclaving the concentrates. This confirms previous results (Loofbourow, Dwyer and Morgan, 1938).

**Preparations from S. aureus**

Twenty-one preparations from *Staphylococcus aureus* were assayed on *S. aureus* or *Saccharomyces cerevisiae*. The results were quantitatively much more variable than in the case of preparations from yeast.

At low concentrations the irradiation products appeared to stimulate *S. aureus*, while at high concentrations they inhibited it. The control concentrates usually inhibited throughout the entire range, but to a lesser extent than the irradiation products at high concentration. The results are summarized in table 3. The examples of results at low concentration in figure 3 show the extreme range of variations obtained with different preparations.

When the concentrates from *S. aureus* were tested on yeast, stimulation was obtained throughout the entire range of con-
## Summary of effects on S. aureus of preparations from S. aureus

**TABLE 3**

<table>
<thead>
<tr>
<th>Number of Preparations</th>
<th>Number of Assays</th>
<th>Concentration of Suspensions from Which Filtrates Were Prepared</th>
<th>Irradiation</th>
<th>Growth Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/m. per ml.</td>
<td>Time (m)</td>
<td>Distance (cm)</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>1.25*</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>5</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washed organisms</td>
<td>1</td>
<td>10</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>Repeated assays on mixtures to get large quantities</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Washed organisms, Filtrate dried and then made up to desired concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Wet weight:
centrations employed, both with control materials and irradiation products (fig. 4). The growth-concentration curves were dis-
tinctly different from those for yeast products tested on yeast, however. In the case of irradiation products, there was a steep rise of stimulation with concentration to a maximum, then a decline, and a later more gradual rise. In the case of control concentrates, the rise was more gradual at low concentrations but greater at high concentrations, the stimulation by control concentrates overtaking that by irradiation products in the middle concentration range and exceeding it for higher concentrations.

The greater growth stimulation of irradiation products than of control concentrates at low concentration, both when tested on *S. aureus* and on *S. cerevisiae*, indicates that irradiation leads to the release of growth promoters into the intercellular fluid. The greater growth inhibition of irradiation products at high concentration, as tested on *S. aureus*, and their lesser stimulation of yeast growth as compared with control filtrates at high concentrations, suggests that irradiation also leads to the production of a growth-inhibiting or toxic factor, the effect of which becomes evident only at the higher concentrations. This inhibiting, or toxic, factor seems to be more active on the homologous organism than on yeast, since it simply resulted in decreased stimulation in the case of yeast, whereas actual inhibition of growth was obtained in the case of *S. aureus*.

Repeated washing and centrifugation of the organisms before suspending them in saline for irradiation was somewhat effective in reducing the growth-inhibiting action of the control concentrates of *S. aureus*, but did not eliminate it entirely in the case of the most concentrated suspensions which stood for long times. We are inclined to believe, therefore, that the inhibition of the control concentrates on *S. aureus* cannot be attributed in its entirety to toxins carried over from the culture medium. Attempts to inactivate the growth inhibitor in the filtrates from irradiated suspensions by heating at 60°C. for one hour in a water bath were ineffective.

The increased toxic effect of the irradiation products for *S. aureus*, at high concentrations, as compared with control concentrates, the evidence for the production of toxic factors by
thoroughly washed organisms standing for long periods in the control suspensions, and the fact that (under the conditions of the experiments) the inhibiting factors were not capable of actually decreasing yeast growth but only of reducing the extent of stimulation obtained are observations especially interesting in view of Besredka’s reports (1923, 1925, 1930) that broth-culture filtrates of Staphylococcus and other organisms contain substances (which he attributed to cell disintegration products) that inhibit the growth of homologous strains but not of heterologous strains. Besredka’s results have been questioned by Barnes (1931) who failed to duplicate them. Palgen (1935), on the other hand, published evidence supporting Besredka’s findings.

**Preparations from A. aerogenes**

Ten preparations from *A. aerogenes* were used. These were all irradiated at concentrations of 15 to 17 mgm. per milliliter for 2.5 hours at 25 cm., a longer irradiation time being necessary than in the case of *S. aureus* in order to bring about practically complete killing in comparable suspensions of this organism.

The assays of preparations from *A. aerogenes* on yeast were quite similar to those of preparations from *S. aureus* (fig. 4), irradiation products stimulating more than control concentrates at low concentrations and less than control concentrates at high concentrations.

The assays on *A. aerogenes* showed inhibition of growth by irradiation products at all the concentrations employed and by the control concentrates at the higher concentrations. The control concentrates stimulated growth at the lower concentrations (fig. 5). The inhibition obtained was consistently greater in the case of irradiation products than in the case of controls.

These data seem to indicate that irradiation leads to the increased production of an inhibiting factor effective in inhibiting growth of *A. aerogenes* and effective in reducing the growth-stimulating action on yeast of other factors present, and that irradiation leads to the production of a growth-stimulating factor.
for yeast. As in the case of tests of *S. aureus* preparations, these results suggest a relationship to Besredka’s work.

Whether lower concentrations of the irradiation products than those employed would stimulate *A. aerogenes* can only be determined by further experiment. If so, and if the stimulation obtained at these concentration levels should prove to be greater than that caused by control concentrates, the effects would be quite analogous to those obtained with preparations from *S. aureus*.

![Graph showing growth response of yeast](http://jb.asm.org/download)

**Fig. 5**

**Preparations from E. coli (communior)**

Seven preparations from *E. coli* were used. All were irradiated 1.25 hours under the conditions employed for *A. aerogenes*. These preparations gave much more regular growth response with yeast (fig. 1) than did those from *A. aerogenes* or *S. aureus*. Their yeast growth curves resemble most closely those of the preparations from yeast, both in the relationship of growth to concentration and in the magnitude of the effects obtained.

Unfortunately these preparations were tried on *E. coli* only in high concentrations, in which inhibition occurred, but it should be noted that the inhibition at these high concentrations was less with the concentrates from irradiated organisms than with those from non-irradiated ones (fig. 5).

No evidence for an increase in production of toxic factors by irradiation was found. Rather, the results are most easily
interpreted as showing that the effect of irradiation on *E. coli* is to cause it to produce growth-stimulating factors only.

Hollaender and Duggar (1938) state that the production of growth factors released into the suspending medium by ultra-violet irradiated *E. coli* cannot explain the increased proliferation of this organism which they noted among survivors in suspensions irradiated until about 80 per cent of the organisms were killed. They cite experiments in which ultra-violet killed organisms, added to suspensions, failed to produce stimulation.

**TABLE 4**

*Comparison of potencies as determined on yeast, of preparations from S. cerevisiae and E. coli*

<table>
<thead>
<tr>
<th>DETAILS OF PREPARATION</th>
<th>YIELDS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Irradiation</strong></td>
<td><strong>Mgm. G. U.</strong></td>
</tr>
<tr>
<td><strong>Organism</strong></td>
<td><strong>Conc.</strong></td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>20</td>
</tr>
<tr>
<td>Mixture &quot;A&quot;</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>16</td>
</tr>
<tr>
<td>Mixture &quot;A&quot;</td>
<td></td>
</tr>
</tbody>
</table>

Our experience with yeast shows that killed cells do not carry over sufficient growth factors produced by them to give the order of stimulation obtained with cell-free filtrates, so that the negative results they obtained on adding killed cells would not, in our opinion necessarily invalidate the possibility that growth factors released by the injured cells might account for the observed stimulation. They state further that factors in the suspending fluids do not account for their results, but they do not give details of their tests of this point, so that it is difficult for us to compare their results with ours. From the fact that our concentrates from irradiated *E. coli* gave such clear-cut stimulation of yeast growth as compared with controls, and from the fact that inhibition was less in the cultures of *E. coli* to which irradiation concentrates were added, we are inclined to believe, contrary to their
conclusions, that the results of Hollaender's and Duggar's experiments may quite possibly be explainable on the basis of proliferation-promoting factors produced by *E. coli* on irradiation with lethal ultra-violet and released into the intercellular fluid.

Table 4 is a comparison of typical preparations from *E. coli* and *S. cerevisiae* as tested on *S. cerevisiae*. It is not possible to compare in this same way the preparations from the other organisms, as their irregular growth curves preclude the possibility of quantitative representation of their potencies in terms of growth units.

**SUMMARY AND CONCLUSION**

Suspensions of *Saccharomyces cerevisiae, Aerobacter aerogenes, Escherichia coli* and *Staphylococcus aureus* were irradiated with lethal ultra-violet and the concentrates of cell-free filtrates from these suspensions and from the non-irradiated control suspensions were tested for growth-promoting effect. The concentrates from bacteria were tested on the homologous organisms and on *S. cerevisiae*. The concentrates from *S. cerevisiae* were tested on all of the organisms used in the preparation of irradiation products and on *Diplococcus pneumoniae*.

The preparations from ultra-violet irradiated yeast stimulated the growth of all organisms. The control concentrates stimulated *S. cerevisiae* and *D. pneumoniae*, but to a lesser extent than the irradiation products. They inhibited the growth of *S. aureus* slightly. In low concentrations, they slightly stimulated the growth of *E. coli* and *A. aerogenes*, but in higher concentrations inhibited their growth. The evidence indicates the production by ultra-violet irradiated yeast of a factor which is growth-stimulating for all of the organisms tested.

The preparations from *E. coli* most closely resembled those from yeast. Both irradiation products and control concentrates stimulated yeast growth, but the stimulation by irradiation products was greater, both on a filtrate volume and weight basis. The relation of growth to concentration in the tests on yeast was nearly linear, as it was in the case of the yeast products. The irradiation products and the control concentrates both inhibited
the growth of *E. coli*, but the inhibition was clearly less in the case of the irradiation products. It is concluded that irradiation leads to the production of a growth-stimulating factor by *E. coli*, assay of which on the organisms itself, under the conditions of these experiments, is complicated by the presence, in both the control and the irradiation concentrates, of materials inhibiting the growth of *E. coli*.

Irradiation products from *S. aureus* slightly stimulated *S. aureus* growth at low concentrations but inhibited it at high concentrations to a greater extent than control concentrates (which generally inhibited throughout the range tested). Tests of these products on yeast showed growth stimulation at all concentrations, but irradiation products stimulated more than control concentrates at low concentrations and less than control concentrates at high concentrations.

Irradiation and control concentrates from *A. aerogenes* behaved similarly to those from *S. aureus* when tested on yeast. In the tests on *A. aerogenes* only inhibition was obtained with irradiation products, and this was greater at high concentrations than the inhibition produced by the control concentrates. It is concluded that in the case of *A. aerogenes* and *S. aureus*, irradiation leads to the production of an inhibitory, or toxic, factor which is more effective on the homologous organism than on yeast, and to the production of a growth stimulating factor effective on yeast.

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