

RESISTANCE OF MUCOR ZYGOTES.

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In the fall of 1916, while attending Indiana University, various experiments were begun to test out the resistance of *Mucor* zygotes and spores to desiccation, to heat, and to different chemicals.

Fortunately the writer had a good culture of zygote material from which fresh zygotes could always be raised. Since the zygotes are supposed to be more resistant than the asexual spores, the experiments were made with the former in order to make them more conclusive.

After sterilizing the bread, inoculating it with zygotes, placing in a dark place, room temperatures, zygotes in unlimited number would be found in 5 to 7 days.

The work was carried out along several lines, and in all cases, unless otherwise stated, zygotes that had been just freshly matured, and those a year old, were used in order to make comparison.

OUTLINE OF WORK.

1. Resistance of zygotes to desiccation.
2. Resistance to heat of zygotes in the desiccator.
3. Resistance to heat of zygotes upon oven-dried bread.
4. Resistance to heat of zygotes placed upon bread with its normal amount of moisture present.
5. Resistance to heat of zygotes in presence of large amount of moisture.
6. Resistance of zygotes to various chemicals.

In all the experiments the utmost care was used to have everything sterile and, in case water or nutrient material had to be added, every precaution was taken so that spores from the outside would not be introduced. Control experiments were run for the purpose of checking.

1. *Resistance of Zygotes to Desiccation.*—Into sulphuric acid desiccators were placed numerous cultures of the one-year zygotes as well as the freshly matured zygotes with no nutrient material. These cultures

were left in this environment for various lengths of time ranging from one week to one year. At the end of these respective periods the small dish with the mucor within it was removed, and with the utmost care a piece of moist, sterilized bread was introduced, after which it was set aside in a warm, dark place.

In all cases but the last one a vigorous growth was made within seventy-two (72) hours and in many cases zygotes were found within a week.

The results of the cultures remaining in the desiccator for one year were not very conclusive, due to a slight accident. The culture of the zygotes, that was freshly matured when it was placed in the desiccator, produced growth within twenty-four (24) hours, and sporangia within forty-eight (48) hours, but the culture with the older zygotes in it failed to grow within two (2) weeks after being removed from the desiccator and moistened, though upon further moistening a vigorous growth was produced. Unfortunately, though, when the culture was being moistened the second time the lid slid off for an instant and there is a slight possibility of spores from the outside gaining entrance.

In one of the first experiments performed, growth failed to take place until further moistening, and it is the belief of the author that such was the case in this last experiment.

2. *Resistance to Heat of Zygotes in Desiccator.*—Zygotes were placed upon oven-dried bread, put in a sulphuric acid desiccator, and then placed in an oven at 60 degrees centigrade for various lengths of time, ranging from seventy-two (72) hours to five (5) weeks. At the end of these periods a culture would be removed and the bread moistened with sterile water. In all the cultures the zygotes survived the heat, and within forty-eight (48) hours after being removed there was a vigorous growth, in many cases zygotes being formed within a week.

Another set of experiments was run along similar lines, through in this case the temperature was raised to seventy (70) degrees centigrade, the time ranging from one week to one month. In the case of the freshly matured zygotes, or as will hereafter be termed *New Zygotes*, a culture was able to survive two (2) weeks of heat and desiccation, though at the end of three (3) weeks, no growth took place when placed in favorable environment. The one year old zygotes were not

able to withstand the heat and desiccation for two (2) weeks, though the culture that had been in the heat for one week germinated readily.

3. *Resistance to Heat of Zygotes Upon Oven-dried Bread.*—The bread was first dried in an oven, the temperature of which was kept at 110-120 degrees centigrade for several hours. In each test tube was placed a small cube of this bread, which had been inoculated with zygotes; the test tubes were plugged with cotton, and then placed in the oven at 100 degrees centigrade for different lengths of time ranging in close series from 1 min. to 25 min. After the cultures were removed and allowed to cool the bread was moistened with sterile water. In every instance, up to and including those remaining in the heat for 17½ minutes, zygotes were produced within a week; but in those cultures remaining in the heat 20, 22½, and 25 minutes, no zygotes were formed, though there was a vigorous growth.

Other cultures were placed in the oven at a temperature of 60 degrees centigrade. This experiment is scarcely complete, since the various lengths of time were not close enough together to warrant any conclusions. Cultures remaining in this heat for one week grew vigorously after being removed to suitable environment; but those remaining in the heat for five weeks failed to germinate after being removed to room temperature and moistened.

The third set of experiments under this heading was placed in an oven at seventy (70) degrees centigrade, the duration being from four (4) days to three (3) weeks. New zygotes produced growth after they had remained at seventy (70) degrees centigrade for two (2) weeks, though at the end of three (3) weeks there was no sign of germination. Old zygotes did not resist the heat as long, the longest duration being one week.

4. *Resistance to Heat of Zygotes in Presence of Small Amount of Moisture.*—In these cultures the amount of moisture was that which is ordinarily found in fresh bread. Experiments placed in the oven at sixty (60) degrees centigrade for one week showed no growth after being removed to favorable environment and neither did cultures after being in the oven for only forty-eight (48) hours at this temperature.

5. *Resistance to Heat of Zygotes in Presence of Large Amount of Moisture.*—These experiments were performed, first, by thoroughly soak-

ing small cubes of bread, placing one in each test tube, sterilizing them, and then inoculating the bread; after which the test tubes were tightly plugged and placed in warm water the temperature of which ranged from forty-five (45) to seventy (70) degrees centigrade.

The following table will give the temperature and the longest time for each of these temperatures that the zygotes were able to remain in it, and still retain the power of germination.

TABLE I.

	70°C.	65°C.	60°C.	55°C.	50°C.	45°C.	40°C.
1 yr., Zygotes	0 min.	1 min.	2 min.	4 min.	10 min.	30* min.	45* min.
New Zygotes.....	0 min.	3 min.	5 min.	10 min.	15 min.	30* min.	45* min.

*Experiments of longer duration were not made for this temperature.

6. *Resistance of Zygotes to Various Chemicals.*—The resistance of the zygote and the growing mycelium toward a few chemicals was tested out. Molecular solutions of NaCl (common salt), $\text{Fe}_2\text{Cl}_6 \cdot 12\text{H}_2\text{O}$, CuSO_4 , and $\text{C}_2\text{H}_5\text{OH}$ (ethyl alcohol) were the solutions used and were the only moisture that the germinating zygotes and growing mycelia received. Oven-dried bread was moistened with the chemical and then inoculated with zygotes after which the cultures were set aside in a warm, dark place to germinate. The first column of Table II indicates the highest molecular solution, or fraction of molecular solution, in which the zygotes and the mycelia would grow; while the second column shows the same in terms of per cent of the chemical in solution. Column three gives the highest molecular solution in which a vigorous growth took place, the last column indicating the same thing in per cent of the chemical in solution.

TABLE II.

	Highest Concentration in which Growth Occurred.		Highest Concentration in which a Vigorous Growth Occurred.	
	Mol. Sol.	% Sol.	Mol. Sol.	% Sol.
NaCl	Mol.	5.48%	M/10	.548%
$\text{Fe}_2\text{Cl}_6 \cdot 12\text{H}_2\text{O}$	M/11	1.2%	M/15	.808%
CuSO_4	M/70	.213%	M/150	.0994%
$\text{C}_2\text{H}_5\text{OH}$	3M+	13.8%+	2M	9.2%

DISCUSSION.

It has been generally thought that zygote material of *Mucor* would not retain the power of germination for more than one year, but the first experiment demonstrated that they retained this power for at least two years, one year of which they were entirely without moisture. Since this is the case one might expect to find the zygotes in the air for a longer period than that.

When heat was added as a factor, a remarkable power of resistance was still shown. How long the zygotes would be able to resist the sixty (60) degrees centigrade in a desiccator remains to be seen, as five (5) weeks was the longest period tried. When the temperature was raised to seventy (70) degrees centigrade the old zygotes showed the lesser resistance, not being able to withstand the heat for as long a period as the newly matured ones.

When the temperature was seventy (70) degrees centigrade the inoculated oven-dried bread resisted to the same extent as those in the desiccator, though when the temperature was sixty (60) degrees centigrade the inoculated oven-dried bread was not able to stand the heat as long as the zygotes in the desiccator. How near it would come to it was not ascertained. The only explanation that the author can give is that the amount of moisture that would be present at sixty (60) degrees centigrade in the oven would be sufficient to be detrimental to the zygotes.

Those experiments in which the zygotes were placed upon oven-dried bread in an oven at one hundred (100) degrees centigrade would have practically the same degree of desiccation as the three experiments that were placed in the desiccators. In this experiment there is shown the most remarkable case of resistance, twenty-five (25) minutes in this heat not being sufficient to kill the zygotes; but another interesting fact is brought out, that being, that the ability of the *mucor* to produce zygotes is gone from those cultures remaining in the heat over 17½ minutes.

According to the present understanding of the formation of zygotes, there must be what is termed "two strains." By the term "strain" the author means not different varieties, but what in higher plants would probably be called male and female plants. In other words, there is a differentiation of mycelial threads, the union of the two (2) being

TABLE III.

	20°C.	40°C.	45°C.	50°C.	55°C.	60°C.	65°C.	70°C.	100°C.
1 year zygotes in desiccator	(524,160) 1 yr.					(50,400) 5 wks. +		(10,080) 1 wk.	
New zygotes in desiccator	(521,160) 1 yr. +					(50,400) 5 wks. +		(20,160) 2 wks.	
1 year zygotes on oven-dried bread						(10,080) 1 wk. +		(10,080) 1 wk.	
New zygotes on oven-dried bread						(20,160) 2 wks. +		(20,160) 2 wks.	(25) 25 min.
1 year zygotes on moist bread		(45) 45 + min.	(30) 30 + min.	(10) 10 min.	(4) 4 min.	(2) 2 min.	(1) 1 min.	(0) 0 min.	(0) 0 min.
New zygotes on moist bread		(45) 45 + min.	(30) 30 + min.	(15) 15 min.	(10) 10 min.	(5) 5 min.	(3) 3 min.	(0) 0 min.	(0) 0 min.

NOTE.—The numbers within the parenthesis indicate the relationship between the various experiments as to the endurance in the heat and the desiccation.

necessary for the formation of the zygote. If this is the case, then one of the "strains" must be weaker than the other and killed out by the unfavorable conditions, since zygotes were not formed in those cultures that had remained in the one hundred (100) centigrade heat for more than 17½ minutes.

The difference in the resistance between the old and the new zygote material in this set of experiments was not ascertained, as only the new was used.

When moisture was added as a factor, even when the amount was small, the resistance of the zygotes to the heat declined rapidly. With the amount of moisture ordinarily found in bread it was found to be sufficient to kill the zygotes in less than forty-eight (48) hours, when the temperature was raised to seventy (70) degrees centigrade, the time probably being only a matter of minutes as can be seen from comparing the results of the different experiments as shown in Table III.

In case there was a large amount of moisture there was a very great dropping off of the power of resistance and also a marked difference in the resistant power of the old and the newly matured zygotes. The rapid decline is when the temperature reaches fifty (50) degrees centigrade. How long the zygotes would resist the temperature of forty-five (45) and forty (40) degrees centigrade was not ascertained.

From a general survey of all the experiments (See Table III) it will be seen that the zygotes are able to withstand a large amount of heat as long as no moisture is present; but the addition of only a slight amount causes the resistant power to fall off very rapidly. Also the factor of dessication is a very small factor, if any, in the lowering of the vitality of the zygote. On the other hand it is a decided factor in increasing the power of resistance to heat.

If, then, one wishes to kill mucor, the surest way to do so is to use heat and moisture, not much heat being necessary in this case; while if moisture is not present a high temperature and a long application will be required.

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