

MIXED CULTURES OF BACTERIA AND FUNGI

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The bacteriologists rightfully condemn the fungi as frequent and undesirable weeds contaminating their cultures. It is equally true, however, that those working with fungi must exercise every precaution to keep bacterial weeds from their fungus cultures.

In attempting to make pure culture isolations of fungi from almost any plant material but more especially from material such as roots with adhering soil, bacteria are the most troublesome components of the mixed culture which often results from the first attempt at isolation.

Plate cultures of fungi often become contaminated with various organisms either because of the ill fitting lids of the petri plates or through careless manipulation. Bacteria constitute one of the most frequent contaminations; possibly coming in on dust particles.

Separation of the components of a mixed culture may be easy or difficult depending both upon the nature of the components and upon their distribution. It is not especially difficult to secure a pure culture from a mixture of two or more fungi. Differential growth and mutual antagonisms usually separate colonies of fungi in such a manner that pure cultures may be made by transferring the appropriate mycelium from its delimited region of growth in the culture. Many times bacteria remain in separate colonies sharply differentiated from the surrounding fungi. In such cases pure cultures of the fungi are as readily obtained as when fungi alone may be present in the culture. A more difficult situation arises when one has to contend with a bacterium of the "spreader" type. Such an organism grows so rapidly that it crowds into and under the fungus growth. Even with such a bacterial contamination the cultures may be differentiated by careful manipulation. The most difficult type of bacterial contamination however is one which tends to keep pace with the growth of the individual hyphae of the fungus. The hyphae growing next to the surface of the substrate often lie in minute grooves in the substrate. These grooves are doubtlessly produced by the enzymatic activities of the fungus. The bacteria inhabit these grooves probably gathering nutrition from the fungus digested materials of the medium. It would seem as if a symbiotic relationship existed between the two organisms. Such combinations cannot be separated by differential growth and other more strenuous means such as the use of an acidified medium must be adopted. An intimate association of fungus and bacteria such as just described produces a marked effect upon the nature of the fungus development. In most cases there is a complete suppression of aerial mycelium. The hyphae take on a greasy appearance as viewed by the naked eye and frequently the presence of the bacteria cannot be detected except by the most careful observation with the highest power of the microscope.

When such contaminations exist in fungus cultures little attention is paid to the bacterial components except to get rid of them. No

attempt is made to identify the bacterial organism or to otherwise study it. That such study would yield profitable information must be apparent from the standpoint of the antagonisms that may exist, from the standpoint of the possible symbiotic relationships, and from the effect on the physiology and the morphology of the fungus. In many cases we might regard our fungi as being diseased by the bacteria. The first step in the conquest of any disease is to understand the causal agent and its relationship to the organism diseased.

The research recorded in this paper is an attempt to analyze the relationships that exist between known bacteria and known fungi and the effect of such relationships upon the growth of the fungus.

A number of pure cultures of bacteria were secured from the Department of Bacteriology at Purdue University.

Potato dextrose agar was used as the medium upon which to carry out the experiments. This was chosen because most fungi grow well upon this medium and we carry our stock cultures of fungi upon potato dextrose agar. It is true that potato dextrose agar is not the most desirable medium upon which to grow some of the bacterial cultures used but the problem is being studied from the standpoint of routine fungus technique and not from the standpoint of the best technique for bacterial culture.

Tubes of P. D. Agar were melted and permitted to cool to 45°C. At this temperature they were inoculated with the appropriate bacterial culture and the inoculum was mixed with the agar of the tube by rotation of the tube between the hands. Plates were poured from the agar so inoculated. Six hours after pouring the plates each plate was inocu-

TABLE I
Experiments Upon the Growth Rate of *Basisporum Gallarum* as Affected
by Various Bacteria. Growth of Fungus
Colony Recorded in mm.

Combined with:	48 hrs.	72 hrs.	Increm't increase	96 hrs.	Increm't increase	Remarks
Ck.	36 mm.	74	38	90	16	
B. mycoides	37 mm.	72	25	90	18	
B. subtilis	33	60	27	78	18	Bacteria follow hyphal growth
B. mesentericus vulgatus	2	2	0	4	2	
Orange sarcina	43	63	20	83	20	
Yellow sarcina	44	70	26	90	20	
Strep. lactis	26	51	25	82	31	Bacteria follow hyphal growth
Ps. campestris	0	57	57	90	33	Growth scanty
B. carotovorus	18	29	9	42	13	Fungus excessively branched and somewhat distorted
B. megaterium	27	45	18	64	19	Bacteria follow hyphal growth
Inhibitor*	0	0	0	0	0	

TABLE II

Experiments Upon the Growth Rate of *Sclerotium Rolfsii* as Affected
by Various Bacteria. Growth of Fungus
Colony Recorded in mm.

Combined with:	48 hrs.	72 hrs.	Incre- ment increase	96 hrs.	Incre- ment increase	Remarks
Ck.	22	35	13	64	29	
<i>B. mycoides</i>	24	54	30	63	9	
<i>B. subtilis</i>	19	46	27	67	21	
<i>B. mesentericus vulgatus</i>	0	0	0	0	0	
Orange sarcina	0	16	16	34	18	
Yellow sarcina	0	28	28	42	14	
<i>Strep. lactis</i>	0	16	16	37	21	
<i>Ps. campestris</i>	0	10	10	32	22	Growth scanty and sub- merged
<i>B. carotovorus</i>	0	27	27	46	19	
<i>B. megaterium</i>	0	17	17	35	18	Bacteria follow hyphal growth
Inhibitor	0	0	0	5	5	

lated with a mycelial culture of a fungus. Plates were permitted to stand at room temperature (about 22°C.) for 48 hours and a growth reading of the fungus colony was taken. The colony was measured in mm. and the average of two diameters was taken as the correct measurement of the colony. Three readings were taken at subsequent 24-hour intervals. Tables I, II, and III, designate the bacterial and fungus cultures used and show the total growth and increment of growth of the various checks and fungus cultures grown in intimate contact with bacteria.

The inhibitor mentioned in the above table is a bacterial spreader of the *Proteus* type having exhibited strong inhibitory effects when grown with fungi.

All cultures including checks were in duplicate or triplicate. Since there was a general agreement in growth rates, figures presented in the column show the average growth rate for all the cultures of any particular combination.

A study of Tables I, II, and III indicates that most of the bacteria used did not materially check the growth rate of the fungus. In fact, stimulatory effects may be indicated in some cases. There was a definite checking of growth whenever *B. mesentericus vulgatus* or the "inhibitor" was used. In order to determine if this effect was general for fungi the experiment was repeated using *B. mesentericus vulgatus* and the "inhibitor" in plate cultures inoculated with the following fungi: *Colletotrichum nigrum*, *Brachysporium* sp., *Helminthosporium gramineum*, *Helminthosporium inaequalis*, *Physalospora cydoniae*, *Botrytis alii*, *Botrytis paeoniae*, *Botrytis tulipae*, *Rhizoctonia* isolated from potatoes, *Fusarium niveum*, *Thielavia basicola*, *Sclerotinia fructigena*, *Pythium deBaryanum*, *Cephalothecium roseum*, and *Penicillium* sp. All of the

TABLE III
Experiments Upon the Growth Rate of *Glomerella Cingulata* as Affected
by Various Bacteria. Growth of Fungus
Colony Recorded in mm.

Combined with:	48 hrs.	72 hrs.	90 inc.	96 hrs.	90 inc.	Remarks
Ck.	34	39	5	47	8	
<i>B. mycoides</i>	32	41	9	50	9	Bacteria follow hyphal growth
<i>B. subtilis</i>	14	23	9	34	11	Bacteria follow hyphal growth
<i>B. mesentericus vulgatus</i>	4	4	0	5	1	
Orange sarcina	25	34	9	54	20	
Yellow sarcina	26	40	14	54	14	
<i>Strep. lactis</i>	25	39	14	52	13	
<i>Ps. campestris</i>	23	36	13	47	11	Growth scanty and mycelium apparently submerged
<i>B. carotovorus</i>	14	22	8	29	7	Growth restricted and appressed
<i>B. megaterium</i>	19	28	9	37	9	Bacteria follow hyphal growth
Inhibitor	0	0	0	20	20	Growth extremely scanty and hyphae closely appressed to the medium

fungi failed to grow in plates of potato dextrose agar which had been inoculated six hours previously with either *B. mesentericus vulgatus* or with the "inhibitor."

In order to determine whether *B. mesentericus vulgatus* and the inhibitor were capable of inhibiting fungi at a distance, plates were poured with potato dextrose agar medium and were inoculated at their centers with fungi. When the fungus growth was well established, i. e., when the colonies were about the size of a ten cent piece inocula of *B. mesentericus vulgatus* and the "inhibitor" were placed in the same plate on opposite sides of the fungus colony and as far away from the fungus colony as the size of the plate would permit. Three fungi were used in this experiment, viz. *Glomerella cingulata*, *Sclerotium rolfsii*, and *Basisporum gallarum*. The results were evident after 24 hours. *Basisporum* was but slightly inhibited by either bacterial organism, the fungus growing over the bacterial colonies. Both the *Glomerella* and the *Sclerotium* were inhibited sharply by both organisms. *B. mesentericus vulgatus* proved to be the better inhibitor and exercised its inhibitory influence at a considerable distance from the fungus.

The results of the experiments thus far conducted demonstrate that bacteria vary widely in their powers to check the growth of a fungus. Those bacterial organisms which tend to spread rapidly over the medium produce the greater effects in inhibiting growth. Most bacteria apparently have little effect upon the development of the fungus colony. A limited number, however, are capable of preventing the growth of most fungi.