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THE CARBON AND NITROGEN METABOLISM OF STEREUM GAUSAPATUM FRIES[1]

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The importance of *Stereum gausapatum* Fries as a heartrot organism has been well established (3, 10). As previously mentioned (5) this led the writer to carry on studies of the general biology of the organism. The present paper, as the title indicates, is a study of its ability to obtain carbon and nitrogen from various chemical sources.

The cultures used were established from specimens collected at various points in the Eastern United States. The collection data, together with a study of the cultural behavior and characteristics of some of the isolates, has been reported in an earlier paper (5).

CARBON METABOLISM

Bergenthal (1) and others (2, 8) have cultured this fungus on various media but no one has reported any investigation as to its ability to use various compounds as a source of carbon. In order to investigate the ability of this fungus to use various carbon compounds, it was grown on several synthetic media, each offering a different source of carbon.

The media were prepared by mixing, aseptically, a sterile base solution with an appropriate quantity of a sterile solution of the carbon

[1]This paper is a revision of a portion of a thesis submitted to the faculty of The Ohio State University as partial fulfillment of the requirements for the degree of Doctor of Philosophy plus some additional experimental work which was performed at Kent State University.

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compound to be investigated. The base solution was of the following composition:

900.0 cc. H_2O.
0.5 g. $MgSO_4$.
0.5 g. KH_2PO_4.
5.0 g. peptone.
1.0 drop of 10% aqueous solution $FeCl_2$.
Sufficient HCl to adjust acidity to pH 5.0.[2]

Forty-five cc. portions of the base solution were autoclaved for 20 minutes at 15 lbs. pressure. Five cc. portions of a 10% aqueous solution of the various carbon compounds were placed in 250 cc. Erlenmeyer flasks and autoclaved for 20 minutes at 15 lbs. pressure. When cool, 45 cc. of the sterile base solution was added, aseptically, to each of the flasks containing the carbon compounds. Each flask thus prepared contained 50 cc. of a 1% solution of the carbon compound in question. Five days were allowed for contaminations to manifest themselves, and then five flasks of each solution were uniformly inoculated with small pieces of mycelium cut from the margin of a vigorously growing malt agar culture of a monosporous mycelium.[3] A similar set of cultures was prepared using an isolate which was started from sporophore tissue.[4] The cultures were incubated at room temperature, in darkness, for 35 days (March 17 to April 21, 1939). The amount of growth was determined by filtering the five cultures of each set through a previously weighed filter paper, rinsing twice with distilled water, and drying at 100° C. Finally, the filter papers with the fungous mats were weighed and the dry weight of the mycelium calculated. The appearance of the cultures was such as to indicate a fair degree of uniformity of growth among the 5 cultures of each isolate growing on each medium.

The essential data of this experiment are presented in Table I.

The only source of carbon in the check was the peptone and a small amount of medium introduced with the inoculum. A comparison of the dry weights of the mycelia of the checks with that produced on the other media leaves no doubt that *S. gausapatum* is able to obtain carbon from all of the compounds tested. Glycerine is obviously of slight value but it did support sufficient growth to be readily observed before filtration and the dry weight is decidedly greater than that obtained on the check medium.

On account of the insoluble nature of cellulose, the above technique could not be employed. Cultures were prepared, however, using the same base solution plus filter paper, cotton cloth, and absorbent cotton, respectively. In every case there was a luxuriant mycelial growth which clearly demonstrated the ability of the organism to obtain sufficient carbon from nearly pure cellulose.

Pure lignin was not available for this study but the writer has observed decay produced by this fungus on several deciduous woods in culture and in every case it produced a typical white rot. In nature, the writer and others (3) have observed that this fungus produces a typical

[2]The optimum pH for growth lies slightly below this. (6).

[3]This isolate, designated as *isolate* S-12, is described in an earlier paper (5).

[4]This isolate, designated as *isolate* 1, is described in an earlier paper (5).

white rot of deciduous wood. White rots are generally believed to be due to the digestion of the lignin by the fungus (7).

Tannins are aromatic compounds which are abundant in oak wood and more so in oak bark. That tannins may serve as food for *S. gausapatum* was demonstrated by the following experiments.

TABLE I

GROWTH, DURING a 35-DAY PERIOD, OF *Stereum gausapatum* ON SYNTHETIC MEDIA OFFERING DIFFERENT SOURCES OF CARBON, EXPRESSED IN TERMS OF DRY WEIGHT OF THE MYCELIUM

CARBON SOURCE	TOTAL DRY WEIGHT OF THE MYCELIUM IN MG. PRODUCED IN FIVE FLASK CULTURES	
	Isolate S-12	Isolate 1
MONOSACCHARIDES:		
Pentoses—		
arabinose	144	155
rhamnose	177	147
xylose	477	438
Hexoses—		
dextrose	354	357
galactose	429	319
levulose	602	371
mannose	454	373
DISACCHARIDES:		
maltose	646	571
sucrose	671	565
TRISACCHARIDES:		
raffinose	439	451
POLYSACCHARIDES:		
glycogen	337	399
inulin	242	251
soluble starch	618	570
NON-CARBOHYDRATES:		
glycerine	89	87
Check: 45 cc. of base solution plus 5 cc. of distilled water	18	42

Tannins are easily extracted by means of hot water (4). To 400 gms. of finely chopped bark of *Quercus velutina* there was added 1500 cc. of tap water. This was heated to 50–80° C. for four hours, allowed to stand for two days, reheated, and then twice filtered. By evaporating 100 cc. of this solution to dryness 3.55 gms. of residue was obtained. A similar procedure was followed using heartwood of the same tree. This extract was found to yield 0.65 gms. of residue per 100 cc. of solution.

A third solution was prepared by adding 1% of tannic acid to a synthetic medium which was carbon free, except for 0.5% of peptone.

Two hundred and fifty cc. Erlemeyer flasks containing 50 cc. portions of the above solutions were sterilized in the autoclave and inoculated from agar cultures of *S. gausaptum.* The cultures were allowed to develop at room temperature. After two months three cultures of each were filtered through a previously weighed filter paper, dried, and weighed to determine the dry weight of the fungous mat. The results were as follows: hot water bark extract, 441 mg. per flask; hot water extract of heartwood, 88 mg. per flask. This technique could not be employed with the tannic acid cultures because of an abundant precipitate which was formed by the reaction of the tannic acid with the peptone. Growth in this culture medium was quite slow, possibly due to the slight availability of the peptone. Upon standing for four months there was considerable mycelium in the flasks. This evidence, in addition to the fact that the fungus grows well on sterile bark or heartwood of *Q. velutina*, strongly indicates that the tannins of oak may be utilized by the fungus. The effect of autoclaving the tannins has not been investigated.

NITROGEN METABOLISM

The ability of fungi to synthesize amino acids from inorganic nitrogen compounds has been under investigation for many years (9). Bergenthal (1) reports that several species of *Stereum*, including *S. gausapatum*, grow equally well on liquid media containing peptone or $(NH_4)_2SO_4$ as the only source of nitrogen. He concludes that the protein is not essential and that the fungus can utilize $(NH_4)_2SO_4$ as a source of nitrogen.

During the present study several attempts were made to culture *S. gausapatum* on media containing nitrogen only as inorganic salts. In every case growth was similar to that occurring on nitrogen-free media. Such experiences led to an attempt to duplicate Bergenthal's results.

Twenty-one 250 cc. Erlenmeyer flasks, each containing 50 cc. of Hagen's medium and a similar set of flasks containing the modified Brown's solution were prepared. The solutions were made up according to the formulae as given by Bergenthal (1). The two liquid media were of the following composition:

1. Hagen's solution.
   - 1000.00 cc. H_2O.
   - 5.0 g. glucose.
   - 0.5 g. $MgSO_4$.
   - 0.5 g. KH_2PO_4.
   - 0.5 g. $(NH_4)_2SO_4$.
   - 10.0 drops of 1% $FeCl_2$ solution.

2. Brown's solution (modified).
   - 1000.0 cc. H_2O.
   - 2.0 g. glucose.
   - 2.0 g. peptone.
   - 1.25 g. KH_2PO_4.
   - 0.75 g. $MgSO_4$.

The flasks of media were autoclaved for 20 minutes at 15 lbs. pressure. Twenty-four hours later the pH value of Hagen's solution was found to be 5.0, of Brown's solution, 5.2. In Bergenthal's media the acidities were reported as pH 6.3 and pH 6.1 respectively. The lower pH value reported here is possibly due to a more severe sterilizing process, but Bergenthal does not state how he sterilized his media. Each of the 21 flasks of Hagen's medium was inoculated with a different

isolate of *S. gausapatum.* The cultures used were isolates 1 to 21 inclusive, as described in an earlier paper (5). The 21 flasks of Brown's solution were inoculated in the same manner with the same 21 isolates. The cultures were then incubated in semi-darkness, at room temperature.

A casual periodic observation of the cultures revealed a much better growth on the Brown's medium than on the Hagen's medium. The difference was constant for all of the 21 isolates used. After three months the 21 cultures grown in each medium were all filtered through one filter paper, dried, and the weight of the fungus determined. The total dry weight of the 21 mycelia grown on Brown's medium was 565 mg., while the total dry weight of the 21 mycelia grown on Hagen's medium was only 90 mg. As will be seen later, mycelia grown on media containing no source of nitrogen may show a greater dry weight than was shown by the mycelia growing on Hagen's medium. It is obvious that the isolates of *S. gausapatum* which the author has studied are not able to obtain nitrogen from $(NH_4)_2SO_4$ as effectively as they can from peptone. It does not seem possible that Bergenthal had a strain which differed so greatly in its physiology.

The results presented above together with the fact that no previous work has been reported led to further study of the the ability of *S. gausapatum* to obtain nitrogen from various sources. The fungus was grown on synthetic media using various compounds as the only source of nitrogen. A base solution was made up as follows:

1000.0 cc. H_2O.
5.0 g. KH_2PO_4.
2.5 g. $MgSO_4$.
0.2 g. $FeCl_2$.
50.0 g. sucrose.

To one lot of this solution 1% of peptone was added. To other lots appropriate quantities of other nitrogen compounds (Table II) were added. The amount used in each case was such that the solution would contain the same percentage of atomic nitrogen as the 1% peptone solution. The actual quantities added are shown in Table II.

For each compound to be tested five 250 cc. Erlenmeyer flasks, each containing 50 cc. of solution were prepared and sterilized at 15 lbs. pressure for 20 minutes and then uniformly inoculated with a monosporous isolate. After a 30 day incubation period, at room temperature, in semi-darkness, the five flask cultures on each medium were filtered through a previously weighed filter paper and the dry weight of the mycelium determined. The average dry weight of the mycelia grown on the various media are shown in Table II.

The accompanying data indicate that peptone is the only compound tested from which *S. gausapatum* can readily obtain nitrogen. The mycelia growing on media containing asparagine, NH_4NO_3 and NH_4Cl show a slightly greater growth than the check. The difference, however, is not great, and the number of cultures employed is small. It does not, therefore, seem wise to attach any significance to the difference. The slight gain in dry weights of the check and of some others is probably due to (a) the weight of the agar and fungus introduced, (b) the slight growth allowed by the peptone present in the introduced bit of culture and (c) to precipitate formed in the solution. That the first

and third of these factors are of importance is indicated by the dry weight of the mycelia, etc., of the $NaNO_2$ cultures. $NaNO_2$ is so toxic, in the concentration used, that absolutely no growth took place, yet the dry weight of the mycelia, etc., compared favorably with that obtained on certain other media.

In order to investigate the toxicity of the nitrogen compounds a duplicate set of cultures was prepared, in which the media were the same as above except that 1% of peptone was present in addition to the other nitrogenous compound. The fungus appeared to be killed promptly by the solution containing $NaNO_2$, but a luxuriant growth occurred in the presence of all of the other compounds. It is obvious that $NaNO_2$ is very toxic, whereas, the other nitrogenous compounds are not toxic in the concentration used.

TABLE II

GROWTH OF *Stereum gausapatum* IN 30 DAYS ON SYNTHETIC MEDIA OFFERING VARIOUS COMPOUNDS AS SOURCES OF NITROGEN EXPRESSED IN TERMS OF DRY WEIGHT OF THE MYCELIA

Source of Nitrogen	Amount, in gms., of N-compound Added to 1 Liter of Base Solution	Average Dry Weight, in mgs., of Mycelium per Flask
Peptone	10.00	541
Asparagine	7.25	60
NH_4NO_3	4.50	55
NH_4Cl	6.75	59
$(NH_4)_2SO_4$	7.75	35
KNO_3	11.50	22
$NaNO_3$	9.75	36
$NaNO_2$	8.00	28
Check	0.00	35

SUMMARY AND CONCLUSIONS

The ability of *Stereum gausapatum* Fries, important cause of heartrot of oak, to use carbon and nitrogen from various sources has been investigated.

The fungus was found to grow well in culture with the following compounds as the only source of carbon: xylose, dextrose, galactose, levulose, mannose, maltose, sucrose, raffinose, glycogen, inulin and soluble starch. Rhamnose and arabinose were much less effective and glycerine was found to be of very little value but did support some growth. That lignin and tannins may serve as food for the fungus is indicated by various experiments and observations.

Synthetic media containing peptone supported a heavy growth of mycelium but when the nitrogen was furnished only

in the form of inorganic salts (NH_4NO_3, NH_4Cl, $(NH_4)_2SO_4$, KNO_3, $NaNO_3$, $NaNO_2$) or asparagine growth was not significantly greater than on a nitrogen-free medium. The fungus is therefore not able to use appreciable amounts of nitrogen from such inorganic compounds. $NaNO_2$ is definitely quite toxic. The other compounds were shown to be non-toxic.

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