

EFFECT OF HYDROGEN-ION CONCENTRATION ON GROWTH AND PROTEIN PRODUCTION BY THE MYCELIA OF *GYMNOPILUS CHRYSOMYCES*, *LEUCOCOPRINUS BIRNBAUMII* AND *LEUCOCOPRINUS CEPAESTIPES* UNDER SUBMERGED CONDITIONS

By

MITRA BASU AND N. SAMAJPATI

Department of Botany, Calcutta University
Calcutta-700 019

The effect of hydrogen-ion-concentration on growth and production of protein by *Gymnopilus chrysomyces*, *Leucocoprinus birnbaumii* and *Leucocoprinus cepaestipes* were studied. The data revealed that optimum pH for growth and protein production for *G. chrysomyces*, *L. cepaestipes* is 5.5 while it is 6.0 for *L. birnbaumii*.

INTRODUCTION

Under given conditions, a fungus grows maximally over a certain range of initial pH values of the medium, and fails to grow at high and low extremes. So hydrogen-ion concentration (i. e. pH) of any substratum plays an important role in growth and production of metabolites of any fungus. Several workers have found that a certain optimum pH or pH range favoured growth of fungi above or below of which the growth response is not very well. Most of pH optima reported in the literature are less than 7.0. Meacham (1918) has reported pH 3.0 to be optimum for *Lenzites saepiaria*, *Fomes roseus*, *Merulius lacrymans* and *Coniophora cerebella*. Wolpert (1924) has found pH optima of 8 wood-destroying fungi to be in the neighbourhood of 5.5. He has shown that temperature and incubation period might influence the optimum pH range as has been found in *Armillaria mellea*. The optimum pH range for growth of *Fomes fraxineus* has been obtained at 6.0—7—0 (Montgomery, 1936). *Coprinus* requires alkaline condition for its growth (Treschow, 1944; Fries, 1956). Block *et al* (1953) have reported the pH range 3.5 to 7.5 as favourable for growth of *Agaricus blazei* mycelium. Etheridge (1955) has reported that pH range 4.6 to 4.9 is most favourable for growth of *Fomes annosus*. Jennison *et al* (1955) have found pH 5.0—5.5. optimum for most of 42 wood-rotting species of fungi except *Poria radiculosa*. Reusser *et al* (1958) have stated that for *Tricholoma nudum* optimum pH and pH range for mycelial growth are 4.5 and 2.0—6.5 respectively. Litchfield *et al* (1963) have found that optimum pH for *Morchella hortensis* is 6.5. A high optimal degree of acidity for *Piptoporus*

betulinus and *Fomitopsis officinalis* and low optimal acidity for *Innotus glomeratus* and *Chimacodon septentrionalis* are observed by Nizkovskaya and Milova (1966). Varadi and Lumomir (1968) have obtained extensive growth of *Schizophyllum commune* between pH 5.9–6.0. Shrivastava and Bano (1970) have observed a broad pH range lying between 4.0 to 7.5 for *Pleurotus flabellatus*. Johri (1972) has obtained best pH range 5.0–7.0 for growth of *Cyathus helenae*. Brodziak (1980) has noticed intensive growth of *Lentinus edodes* at pH 3.5. Recently Kosaric and Nabuo (1981) have shown optimum pH to be 5.0–5.5 for morel mushrooms. Hong *et al* (1981) have obtained optimum pH 6.0 to 6.5 for *Agaricus bitorquis* and 5.0 to 6.5 for *Pleurotus ostreatus*.

Most common and generally useful is phosphate buffer to keep the pH steady in fungal medium. Concentration may be as low as 0.003 M (Creaseman, 1954) or as high as 0.2 M (Darby, 1954). Usefulness of phosphate in buffering has also been noted by Child *et al* (1973).

In this experiment necessary studies are made to find out the optimum pH for growth and protein production by mycelia of *G. chrysomyces*, *L. birnbaumii* and *L. cepaestipes*.

MATERIALS AND METHODS

Test organisms

The tissue cultures of *Gymnopilus chrysomyces* (Berk.) Sacc., *Leucocoprinus birnbaumii* (Corda) Sing. and *Leucocoprinus cepaestipes* (Sow. ex. Fr.) Pat. were made. Cultures were maintained in 3% malt extract agar medium by subculturing at regular intervals of 15 days and keeping at 25°C in complete darkness.

Preparation of inoculum

A small portion of actively growing mycelium of each test-fungus from agar slants was transferred separately to a sterilized 250 ml Erlenmeyer flask containing 50 ml of glucose-asparagine synthetic medium of Lilly and Barnett (1951) and incubated in a shaking incubator (120 r.p.m.) at 30°C ($\pm 0.5^\circ\text{C}$) for seven days in complete darkness. After the incubation period the mycelium was fragmented into small pieces with the help of a waring blender. Then the fragmented mycelial mass was washed a number of times with sterile distilled water to remove all traces of medium and then suspended in a phosphate buffer-medium (pH 5.5) for 24 hrs to overcome the shock encountered during blending. One ml of the mycelial cell suspension thus prepared was used as inoculum in case of all the three mushroom species used.

Medium and growth conditions

Glucose-asparagine basal medium was prepared. Different sets of flasks for different pH grades were prepared with the help of 0.2 M phosphate buffer.

Nine pH grades used in the experiment were 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. 50 ml of medium was taken in each of 250 ml Erlenmeyer flasks. Flasks were then properly plugged and sterilized at 10 p.s.i. for 20 minutes. Each set of flasks with one pH grade was then separately inoculated with 1 ml suspension of the mycelium of each mushroom species. Flasks were then incubated at 30°C for 20 days in case of *G. chrysomyces* and for 16 days in case of *L. birnbaumii* and *L. cepaestipes* according to the optimum temperatures and incubation periods obtained in the previous experiments. Five flasks were taken for each treatment or each test-fungus.

Measurement of Growth

After the optimum incubation period, five flasks for each pH treatment of each species were harvested. Medium and mycelium were separated by filtration through a tarred sintered funnel (Jena IG-3). Filtered mycelium was washed repeatedly with distilled water to remove any trace of medium in it, dried to constant weight at 60°C. The dry weight of mycelium thus obtained was taken as an index of growth.

Estimation of Protein

The total nitrogen content of the dried mycelium was determined following the colorimetric method of Folin and Wu (1919) and the method of Vogel (1961) using photoelectric colorimeter (Model AE-11, Tokyo Erma Optical Works Ltd, Japan). On the basis of 16 percent nitrogen content of protein, a factor of 6.25 was used to convert the nitrogen values to crude protein content. The complete set of experiment was done in triplicate.

RESULTS AND DISCUSSION

The experimental data thus obtained are given in Table—1

The data in Table 1 reveal that in case of *G. chrysomyces*, optimum pH for the growth and protein production is 5.5. In growth it is followed by pH 5.0, 6.0, 4.5, 4.0, 6.5, 7.0, 7.5 and 8.0 and in protein production it is followed by 5.0, 6.0, 4.0, 4.0, 4.5, 6.5, 7.0, 7.5 and 8.0. So, optimum pH and pH range are 5.5 and 5.0—6.5 respectively for the test-fungus.

For *L. birnbaumii*, growth and protein production is best at 6.0 (Table 1), which is being followed, in growth by 5.5, 5.0, 6.5, 7.0, 7.5, 8.0, 4.5 and is followed in protein production by 5.5, 5.0, 4.5, 6.5, 8.0, 4.0, 7.0 and 7.5. So, the optimum pH is 6.0 and pH range 5.0—6.0 is suitable for the test-fungus.

In case of *L. cepaestipes*, growth and protein production is found to be best at 5.5 (Table 1). In growth, this is followed by 6.0, 6.5, 7.5, 4.5, 8.0 and 4.0 and in protein production, it is followed by 6.0, 6.5, 5.0, 7.0, 7.5, 4.5, 4.0 and 8.0. The optimum pH is 5.5 and the test-fungus responds well in pH range 5.0—6.5.

TABLE 1. Data (mean*) showing the effect of hydrogen-ion-concentrations on the growth (g/l) and yield of protein (%) by the mycelia of *G. chrysomyces*, *L. birnbaumii* and *L. cepaestipes* at respective optimum incubation and temperature under submerged-condition.

pH	<i>G. chrysomyces</i>		<i>L. birnbaumii</i>		<i>L. cepaestipes</i>	
	Dry Wt. of mycelium (g/l)	Protein content (%)	Dry Wt. of mycelium (g/l)	Protein content (%)	Dry Wt. of mycelium (g/l)	Protein content (%)
4.0	4.38 ±0.26	22.97 ±0.03	2.85 ±0.13	21.87 ±0.02	3.90 ±0.16	18.28 ±0.03
4.5	4.63 ±0.23	22.60 ±0.02	3.19 ±0.13	22.34 ±0.06	4.34 ±0.20	18.40 ±0.06
5.0	4.96 ±0.07	24.38 ±0.02	3.85 ±0.14	22.81 ±0.04	6.11 ±0.13	21.25 ±0.02
5.5	5.61 ±0.14	26.50 ±0.04	4.14 ±0.05	23.90 ±0.05	7.88 ±0.13	27.80 ±0.07
6.0	4.96 ±0.11	23.69 ±0.06	4.48 ±0.12	24.37 ±0.05	7.65 ±0.24	24.53 ±0.06
6.5	3.90 ±0.21	20.21 ±0.05	3.84 ±0.04	22.18 ±0.06	7.31 ±0.16	21.25 ±0.01
7.0	3.37 ±0.09	19.08 ±0.04	3.43 ±0.21	18.50 ±0.02	6.96 ±0.21	20.47 ±0.02
7.5	3.05 ±0.13	17.06 ±0.04	3.31 ±0.18	17.18 ±0.03	5.57 ±0.18	18.60 ±0.04
8.0	2.78 ±0.08	16.56 ±0.02	3.23 ±0.12	22.18 ±0.08	4.22 ±0.20	18.28 ±0.05

* Average mean data of five replicates for growth and three replicates for protein yield.

Of the three test-fungi under study, *G. chrysomyces* and *L. cepaestipes* show optimum pH 5.5 which is similar to the findings of Wolpert (1924) and that of Jennison *et al* (1955). The result also supports the pH 5.0–5.5 obtained by Kosaric and Nabuo (1981) working on morel mushrooms. *L. birnbaumii* shows optimum pH 6.0 which is similar to the observation of Varadi and Lumumir (1968) on *Schizophyllum commune*. All the three fungi favour the pH range 5.0–6.5. This is similar to the report obtained in case of *Pleurotus ostreatus* (Hong *et al*, 1981). It is evident from the findings that all the three test-fungi are acid-loving.

REFERENCES

- Block, S. S., Stearns, T. W., Stephens, R. L. and McCandless, R. F. J. (1953). Mushroom mycelium. Experiments with submerged culture. *A. Agr. Food Chem.* Vol. 1: 890-893.

- Brodziak, L. (1980). Selected aspects of *Lentinus edodes* biology in Poland. *Acta Mycol* 16 (1) : 43-54.
- Child, J. J., Kapp, C. and Eveleigh D. E. (1973). Improved pH control of fungal culture media. *Mycologia* 65 (5) : 1078-1086.
- Crasemann J. M. (1954). The nutrition of *Chytridium* and *Macrochytrium*. *Am J. Botany* 41 : 302-310.
- Darby, R. T. and Mandels, G. R. (1954). Inorganic nutrition of *Mycothecium verucaria*. *Mycologia* 46 : 276-288.
- Ethridge, D. E. (1955). Comparative studies of north American and European cultures of the root rot fungus. *Fomes annosus* (Fr.) Cooke, *Can J. Botany* 33 : 416-428.
- Folin, O. and Wu, H. (1919). Determination of non-protein nitrogen. *J. Biol. Chem* 36 : 87-90.
- Fries, L. (1956). Studies in the physiology of *Coprinus* II Influence of pH, Metal factors and temperature. *Svensk Botan Tidskr* 50 : 47-96.
- Hong, J., Kap, S. L. and Dong, S. C. (1981). Studies on Basidiomycetes I. Mycelial growth of *Agaricus bitorquis* and *Pleurotus ostreatus*. *Korean J. Mycol.* 9 (1) : 19-24.
- Jennison, M. W., Newcomb, M. D. and Henderson, R. (1955). Physiology of the wood-destroying Basidiomycetes. I. Growth and nutrition in submerged culture in synthetic media. *Mycologia* 47 (3) : 275-304.
- Johri B. N. (1972). Nutritional study of *Cyathus helenae* and related species. *Mycologia* 64 (2) : 298-303.
- Kosaric, N. and Nabuo M. (1931). Growth of Morel mushroom mycelium in cheese whey. *J. Dairy Res.* 48 (1) : 149-162.
- Litchfield, J. H., Overbeck, R. C. and Davidson, R. S. (1963). Mushroom culture : Factors affecting the growth of morel mushroom mycelium in submerged culture *J. Agr. Food. Chem.* 11 : 158-162.
- Lilly, V. G. and Barnett, H. L. (1951). Physiology of fungi, McGraw Hill Book Co, London. pp. 427.
- Meachum, M. R. (1918). Note upon the hydrogen-ion concentration necessary to inhibit the growth of four wood-destroying fungi. *Science* 48 : 499-500.
- Montgomery, M. B. S. (1936). A study of *Fomes fraxineus* and its effects on ashwood. *Ann. Appl. Biol.* 23 : 465-486.
- Nizkovskaya, O. P. and Milova, N. M. (1966). Comparative physiological description of Aphyllophorales and Agaricales fungi in culture. Nauka : Moscow—Leningrad 6-11. From FEF ZH BIOL 1966, No. 10 VI 25.
- Reusser, F., Spencer, J. F. T. and Sallans, H. R. (1951). *Tricholoma nudum* as a source of microbiological protein. *Applied Microbiology* 6 : 5-8.
- Shrivastava, H. C. and Bano, Z. (1970). Nutrition requirements of *Pleurotus flabellatus*. *Applied Microbiology* 19 (1) : 166-169.
- Treschow, C. (1944). Nutrition of the cultivated mushroom. *Dansk. Bot Arch* 11 : 1-180.
- Varadi, J. and Lumumir, J. (1968). Effect of pH on mycelium, CM—cellulase and xylanase formation in submerged condition of *Schizophyllum commune*. *DREV VYSK* 4 : 193-202.
- Vogel, A. I. (1961). *A Text Book of Quantitative Inorganic Analysis*. 3rd. Edition. The English Language Book Society and Longmann. pp. 783-784.
- Wolpert, F. S. (1924). Studies on the physiology of fungi XVII. The growth of certain wooddestroying fungi in relation to the hydrogen-ion-concentration of the media. *Ann. Missouri Botan. garden* 11 : 43-97.