

## Studies on Choanephoraceae. II. Effect of Hydrogen-ion Concentration

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With 2 fig. in the text.

Hydrogen and hydroxyl-ions are present in all media and they exert a decided effect on the growth and sporulation of fungi. Lilly and Barnett (1951) have pointed out that pH also influences many other life processes of a fungus. Foster (1949) states that pH of the medium plays a part in determining whether a particular amino acid can be utilized. Besides, utilization of sugars has also been reported to be influenced by the pH of the culture medium. Raper et al. (1953) reported that some fungi utilize glucose only when the pH of the medium is below 6, best pH for the same being 5.5. Further, they reported that the utilization of fructose takes place only in acidic medium and no growth occurs on a neutral fructose medium within two weeks. Fungi differ considerably in their tolerance to different pH values. The growth of fungi may be completely inhibited in media, which are either too acidic or too alkaline. The growth on the acidic side has been recorded up to as low as pH 3 and on the alkaline side up to pH 8.0—9.0 by several investigators. On the other hand, various fungi have been reported to grow on media having pH even below and above these pH levels. As for instances some species of *Fusarium* and *Penicillium* attacking citrus fruits, acid producing strains of *Aspergillus niger*, *Gloeosporium papayae* and *G. musarum* have been reported to grow even below pH 3. Similarly, some fungi, viz., *Penicillium variabile*, *Alternaria tenuis*, *Alternaria capsici*, *Cercosporina ricinella*, *Colletotrichum gloeosporioides* and *Curvularia penniseti* have been reported to grow above pH 9 by several workers (Johnson, 1923; Chaturvedi, 1961; Chandra, 1961).

From the above, it is clear that fungi tolerate a wide range of pH for growth. For investigating the nutritional needs of a fungus it is essential, that optimum pH for its growth must be investigated first. To better understand the biology of these fungi, comparative studies have been made on their nutrition. The immediate goal of these studies has been threefold: (1) to establish the effect of different pH values on the growth and sporulation; (2) to determine what, if any differences exist among the closely related species; and (3)

Table L

Effect of various hydrogen-ion concentrations on growth and sporulation of Choanephoraceae\*.

pH	<i>B. trispora</i> <i>C. cucurbitarum</i>						<i>C. infundibulifera</i>			<i>C. conjuncta</i>			<i>C. heterospora</i>			<i>C. circinans</i>			
	G	S		Final pH	G	S	Final pH	G	S	Final pH	G	S	Final pH	G	S	Final pH	G	S	Final pH
1.	2.0	—	—	2.2	—	—	2.2	—	—	2.2	—	—	2.2	—	—	2.2	—	—	2.2
2.	3.0	8.7	+	4.0	8.0	—	3.7	8.0	—	3.9	11.8	—	4.5	9.2	—	4.0	12.2	—	4.6
3.	4.0	17.1	+++	6.4	16.1	—	6.5	20.0	+++	6.7	16.7	—	6.0	14.6	—	5.5	17.6	+	6.0
4.	5.0	17.5	+++	6.5	20.7	—	7.0	25.7	++++	7.8	21.0	—	6.5	16.6	—	6.7	22.1	+	6.5
5.	5.5	21.4	+++	7.0	26.6	+	7.6	29.5	+++	7.6	24.3	—	6.9	18.6	+	7.0	26.4	—	6.7
6.	6.0	26.5	+++	6.9	24.8	+	7.8	32.2	+++	7.8	26.9	—	7.2	26.5	+	7.3	29.0	—	6.7
7.	7.0	35.5	+++	7.5	18.2	+++	7.6	25.4	++	8.0	23.5	—	7.6	22.5	+++	7.6	23.2	—	7.6
8.	7.5	24.5	++++	7.7	17.9	++++	7.8	21.9	++	8.2	22.5	—	7.7	18.4	++++	7.7	18.0	—	7.8
9.	8.0	21.0	++++	8.1	16.3	++	7.8	20.4	+	8.0	22.0	—	7.8	14.0	++	8.2	26.1	—	7.8
10.	9.0	20.0	++++	8.5	11.6	—	8.3	14.3	—	8.7	21.0	—	8.4	13.0	—	8.6	18.2	—	8.6
11.	11.0	14.0	—	9.6	10.9	—	9.7	9.1	—	9.4	12.4	—	9.6	4.0	—	9.6	9.4	—	9.4

\* Key: G = Growth in milligrams; — = No growth; S = Sporulation; — = No sporulation;  
 + = Poor sporulation; ++ = Fair sporulation; +++ = Good sporulation;  
 ++++ = Excellent sporulation.

to compare the behaviour of the present species with that of other members of the Mucorales and also other fungi.

### Materials and Methods

The cultures used throughout this investigation were: *Blakeslea trispora* Thaxter, *Choanephora cucurbitarum* (Berk. and Rav.) Thaxter, *C. infundibulifera* (Currey) Saccardo, *C. conjuncta* Couch, *C. heterospora* Mehrotra and Mehrotra and *C. circinans* (Naganishi & Kawakami) Hesseltine and Benjamin.

A basal medium of the following composition was used: glucose, 3gm; asparagine, 1 gm;  $\text{KH}_2\text{PO}_4$ , 1gm;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5gm;  $\text{FeSO}_4$ , 0.2mg;  $\text{ZnSO}_4$ , 0.2mg;  $\text{MnSO}_4$ , 0.1mg; thiamine hydrochloride, 100 $\mu\text{g}$ ; distilled water, 1000ml. The hydrogen-ion concentration of the medium was varied from pH 2–11 and the medium was adjusted at eleven different pH values. Twenty five ml of the medium was poured into 150 ml Erlenmeyer Pyrex flasks and the flasks were autoclaved at 15 lb pressure for 15 minutes. The growth records were maintained by weighing the fungal mat after 15 days. Change in the pH of the medium as well as degree of sporulation of the species was also noted.

### Results

The results of the effect of different hydrogen-ion concentrations of the medium, on the growth and sporulation of Choanephoraceae are given in Table 1 and statistically represented in Table 2.

Table II.

Summary of dry weight results and conclusions at 5% level.

1. *Blakeslea trispora*  
S. E. = 1.76; C. D. =  $\pm 3.69$   
7 > 6 8 5 9 10 4 3 11 > 2 > 1
2. *Choanephora cucurbitarum*  
S. E. = 1.24; C. D. =  $\pm 4.67$   
5 6 4 7 8 9 3 10 11 2 > 1
3. *Choanephora infundibulifera*  
S. E. = 1.76; C. D. =  $\pm 3.69$   
6 5 > 4 7 8 9 3 > 10 > 11 2 > 1
4. *Choanephora conjuncta*  
S. E. = 1.71; C. D. =  $\pm 3.58$   
6 5 7 8 9 4 10 > 3 > 11 2 > 1
5. *Choanephora heterospora*  
S. E. = 1.63; C. D. =  $\pm 3.4$   
6 > 7 > 5 8 4 3 9 10 > 2 > 11 > 1
6. *Choanephora circinans*  
S. E. = 1.44; C. D. =  $\pm 3.02$   
6 5 9 7 4 > 10 8 3 > 2 11 > 1

It is evident from the results that the present species could grow within a wide range of pH, viz., 3—11. pH within 5—7 was generally found to be suitable for the growth of all the species. Good growth of *C. circinans*, *C. cucurbitarum* and *C. infundibulifera* was obtained at pH 5, while the rest of the species grew moderately at this pH value. On the other hand, pH 7 supported good growth of *C. hetero-*

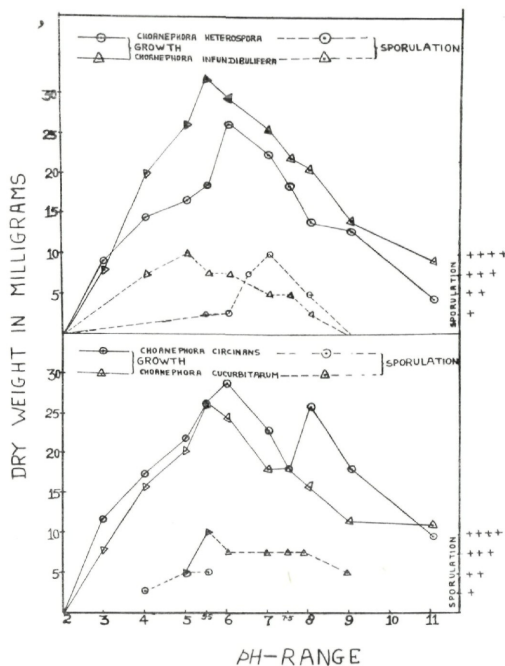


Fig. 1.

*spora*, *C. conjuncta* and *B. trispora* and moderate of the rest of the species. At pH 8, all the species grew moderately except *C. conjuncta* and *C. circinans*, of which good growth was obtained at this pH value. All the species grew moderately at pH 9.0, except *C. infundibulifera*, which grew poorly at this pH value. pH values below 4.0 and above 9.0 proved to be useless for all the species.

All the species of *Choanephora* sporulated within a wide range of pH except *C. conjuncta*, which failed to sporulate at any of the pH values. *Blakeslea trispora* sporulated within the widest range of

pH, viz., 3—9. Excellent sporulation of this fungus was obtained at pH values from 7.5—9. On the other hand, *C. infundibulifera*, *C. heterospora* and *C. cucurbitarum* sporulated within pH ranges 4—8, 5.5—8 and 5—9 respectively. Excellent sporulation of *C. infundibulifera* was obtained at pH 7.5 and *C. cucurbitarum* at pH 5.5. A very narrow range of pH for sporulation was obtained in case of *C. circinans*, which was 4—5.5.

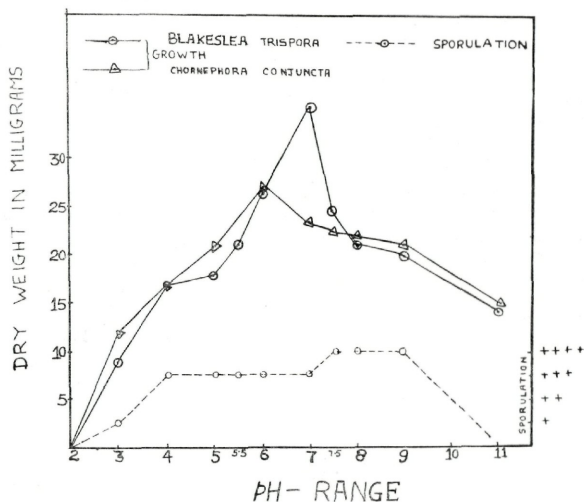


Fig. 2.

### Discussion

From the above results, it is clear, that pH of the medium had a marked effect on the growth and sporulation of these fungi. All the members of the family are very similar in their behaviour, and the differences which have been demonstrated are of a quantitative rather than a qualitative nature. The dry weight results show that the growth of the present species was better either in the slightly acidic side or at the neutral point; generally alkaline media were not favourable. *Blakeslea trispora* was found to be different in its behaviour than the rest of the species. Its best growth was noticed at pH 7.0, while the rest of the species grew best between 5.5—6.0. Similarly Johnson (1923), Brancato and Golding (1955) and Agnihotri (1962) also found that the growth of their fungi was more satisfactory on the acidic side than on the alkaline side. On the

other hand, Mehrotra and Mehrotra (1962) found better growth of *Helicostylum piriforme* on alkaline media than acidic ones. Hesselatine *et al.* (1953), reported maximum growth of *Pilobolus kleinii* at a pH above 7. O. Similar report has been made by Ellis (1963) for *Rhopalomyces elegans*.

Too acidic or alkaline media were found to be not suitable for the species, because in such media the proteins have a tendency to develop lesser viscosity. Simultaneously, its colloidal behaviour is changed in the sense that more hydrolysis of the proteins, to form simpler products, results in the formation of colloidal particles of smaller dimensions. This disturbance in the proper colloidal state of the cytoplasm hinders its normal function. Under such circumstances, the formation of complex protein molecules becomes difficult resulting in the retardation of growth.

The growth curves of all the species of *Choanephora* plotted against several pH values, show a single maximum except that of *C. circinans*, where the pH growth curve rises to a maximum at 6.0, drops to a minimum at 7.5 and rises to another maximum at 8.0. Above pH 8.0, the curve drops off rapidly. This type of bimodal curve has been found in several other fungi (Hopkins, 1922; Lindfors, 1924; Tilford, 1936; Webb, 1921; Saksena, 1936; Mehrotra, 1949; Mehrotra and Mehrotra, 1962). Arrhenius (1922), has suggested the cause of this double maxima curve with a minimum as being due to changed intensity of permeability or to the fact that the solubility of salts differs at different hydrogen-ion concentrations.

### Summary

Effect of different hydrogen-ion concentrations was studied on the growth and sporulation of the different species of *Choanephora* and *Blakeslea trispora*. During the present investigation, it was found that the pH of the medium had a marked effect on the growth and sporulation of the species. Good growth of all the species was obtained within a pH range of 5.5—7.0 except *B. trispora*, which grew moderately at pH 5.5. pH values below 4.0 and above 9.0 proved to be useless for all the species. Sporulation was recorded within a wide range of pH in all the species of *Choanephora* except *C. conjuncta* which failed to sporulate at any of the pH values. *Blakeslea trispora* sporulated within the widest range of pH, viz., 3.0—9.0.

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\* Originals not seen.

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