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Comparative Physiological Studies of *Scopulariopsis breviaulis* and *Stemphylium piriforme*

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Abstract

Response of *Scopulariopsis brevicaulis* (alkalotolerant) and *Stemphylium piriforme* (alkalosensitive) towards pH range between 6.5 to 11.5 were investigated. Sugar uptake was maximum at pH 9.5 and 7.5 for *S. brevicaulis* and *S. piriforme* respectively. Amino-n content showed significant decrease in the case of *S. piriforme* with the increase in pH value, while differences were not significant in the case of *S. brevicaulis*. Polysaccharide content of *S. brevicaulis* significantly decreased with the increase in pH value, while that of *S. piriforme* was insignificantly affected. Considering nitrogenous contents, there was a clear relation in the case of *S. brevicaulis*, as the total insoluble nitrogen increased by the rise in pH value, total soluble nitrogen decreased. On the other hand, significant decrease of both components was observed in the case of *S. piriforme*.

Introduction

Alkalophilic microorganisms are those which having growth optima at pH 9-10.5 and badly grown near the neutral range (Horikoshi and Akiba, 1982). On the other hand, alkalotolerant organisms grow well at pH 8.5-9, but also grow at neutral pH values (Guffanti *et al*.,1980).

Alkalophilic microorganisms especially bacteria received the attention of many microbiologists in the last few years. However, growth and physiological activities on alkalophilic alkalosensitive fungi received less attention. Thompson *et al*. (1993) studied the effect of pH on mycelial growth and conidial germination in *Fusarium* spp and *Penicillium* spp. All tested fungi were able to grow at pH 4.0 to 10.0. in a study on *Aspergillus versicolor* strain, Wang and Sun (1988) reported that, mycelial weights were greater at pH 6.5 and 8.0 than that at pH 1.0 and 3.0. Bansod *et al*. (1993) reported that extracellular xylanases isolated from *Cephalosporium* species were active and stable at high alkaline pH (8-9.5). Also, Kim *et al*. (1996) found that beta-carotene synthesis in *Blakeslea trispora* increased under strong alkaline culture conditions.

The present investigation carried out to study some physiological activities in two fungal species. *Scopulariopsis brevicaulis* and *Stemphylium piriforme*, the first one reported to be alkalo,.totlerant, while the second considered as alkalosensitive (Geweely, 1997).

Materials and Methods

Two fungi were used in the present study. *Scopulariopsis brevicaulis* and *Stemphylium piriforme*. The medium used to maintain growth was Horikoshi (2) (Horikoshi and Akiba, 1982). It is composed of soluble starch, 10.0, peptone, 5.0, years extract, 5.0, potassium dihydrogen phosphate, 1.0, magnesium sulphate.7 H_2O, 0.20, sodium carbonate, 10.0, distilled water, 1000 ml, rosebengal, 1:30000, streptomycin, 30 μg/ml, initial pH 10.0. Sodium carbonate must be sterilized separately.

Effect of pH on growth media and mycelial mats: Inorder to find out the response of alkalotolerant fungus and the alkalosensitive towards variable pH values especially those occur in the alkaline side, the following parameters were measured at the investigated pH values (6.5, 7.5, 8.5, 9.5 10.5 and 11.5) direct reducing sugar value (D.R.V.), sugar uptake percentage, total soluble nitrogen, total insoluble nitrogen and polysaccharides.

Horikoshi (2) medium was prepared and sterilized by autoclaving. Two buffer system, sodium carbonate-sodium bicarbonate and sodium dihydrogen phosphate-sodium hydroxide (Okada *et al*., 1993) were used to buffer the medium over a pH range 6.5-11.5. The pH from 6.5-8.5 was adjusted by 2 M phosphate buffer and from 9.5-11.5 by 2 M carbonate buffer. The buffers were separately sterilized by filtration before addition in suitable aliquots to the basal medium to give pHs: 6.5, 7.5, 8.5, 9.5, 10.5 and 11.5 (Khodair *et al*., 1991).

Conical flasks contained 50 ml medium were inoculated with fungal discs (1 cm in diameter) taken from colony margin of 2-4 days-old cultures. Three replicate flasks for each pH-values were used. The flasks were incubated at 28°C for 10 days, then filtered. The mycelium washes several times with distilled water and then dried at 80°C til constant weight. Filtrates and dry biomass were used further analysis. The initial pH of the culture media was measured using Beckman-pH-meter to ensure that there is no change in the adjusted pH values.

Carbohydrate analysis: Extraction of mats for carbohydrate analysis was carried out according to the procedure recommended by Naguib (1963). A modification of Nelson's solution (Clark, 1964) was used in which 36.8 potassium oxalate replaced the sodium sulfate of the original solution of Nelson (1944).

Reducing monosaccharides (DRV) determination: The total monosaccharides content was measured as glucose using the Nelson's test described by Clark (1964).

Table 1: Effect of pH variation on the uptake of reducing sugars (%) from the culture media and direct reducing sugar values (D.R.V.) expressed as glucose units in the culture media of *S. brevicaulis* and *S. piriforme* after 7 days growth on starch-containing Horikoshi medium (2)

	Sugar uptake (%)				D.R.V. (Ug/ml medium)			
Fungal species	*S. brevicaulis*		*S. piriforme*		*S. piriforme*		*S. brevicaulis*	
pH values								
6.5	60.0	B	60.0	B	269	CD	321	B
7.5	83.0	A	65.0	C	476	A	343	D
8.5	72.0	B	56.0	E	413	AB	376	B
9.5	90.0	A	51.0	E	553	A	453	AB
10.5	88.0	B	57.0	E	475	A	419	AB
11.5	88.0	B	48.0	E	457	BA	377	C

Means followed by the same letters are statistically non-significant

Table 2: Effect of pH variation on the Total Soluble Nitrogen (T.S.N.) and amino acid contents in the culture media of *S. brevicaulis* and *S. piriforme* after 7 days growth on starch-containing Horikoshi medium (2), (peptone is the N source)

	T.S.N. (μg/ml medium)				Amino-N content (μg/ml medium)			
Fungal species	*S. brevicaulis*		*S. piriforme*		*S. piriforme*		*S. brevicaulis*	
pH values								
6.5	520	C	306	D	33	C	127	A
7.5	530	B	270	C	43	DC	56	BC
8.5	430	B	306	C	66	B	37	C
9.5	500	B	320	D	43	C	43	C
10.5	480	B	320	C	73	C	22	D
11.5	430	B	340	C	29	B	4	D

Means followed by the same letters are statistically non-significant

Table 3: Polysaccharide Direct Reducing Sugar content (D.R.S.) in the mycelial mats of *S. brevicaulis* and *S. piriforme* after 7 days growth on starch-containing Horikoshi medium (2) adjusted to different pH values

	Polysaccharides (μg/g dry biomass)				D.R.S. (μg/g dry mass)			
Fungal species	*S. brevicaulis*		*S. piriforme*		*S. piriforme*		*S. brevicaulis*	
pH values								
6.5	925	B	406	F	494	B	344	D
7.5	806	A	347	D	466	B	348	DC
8.5	794	B	391	D	430	CD	406	DE
9.5	713	A	317	D	620	A	242	D
10.5	670	B	416	E	585	AB	378	C
11.5	619	A	452	B	429	B	219	D

Means followed by the same letters are statistically non-significant

Table 4: Total insoluble nitrogen contents (protein), Total Soluble Nitrogen (T.S.N.) contents and amino acid contents in the mycelial mats of *S. brevicaulis* and *S. piriforme* after 7 days growth on starch-containing Horikoshi medium (2) adjusted to different pH values

	Total insoluble nitrogen (μg/g dry biomass)				T.S.N. (μg/g dry biomass) (μg/g dry biomass)				Amino acids content (μg/g dry biomass)			
Fungal species	*S. brevicaulis*		*S. piriforme*		*S. brevicaulis*		*S. piriforme*		*S. brevicaulis*		*S. piriforme*	
pH values												
6.5	90	D	387	CB	830	A	480	CB	57	A	19	BC
7.5	92	E	300	D	770	A	390	CD	47	A	20	C
8.5	141	E	311	C	730	A	300	E	54	A	20	CD
9.5	129	CD	196	B	630	A	280	D	43	B	49	AB
10.5	181	B	118	D	590	B	290	D	41	B	39	CB
11.5	182	B	102	B	580	B	170	E	33	B	54	A

Means followed by the same letters are statistically non-significant

Polysaccharide determination: Polysaccharide content was determined in the residue left after mat treatment according to Naguib (1963). Known weight of the residue was boiled with 1N HCL in a reflux condenser for 6 hours. The solution was then cooled, neutralized to phenol red cleared with basic lead acetate, deleaded by sodium dihydrogen phosphate and the cleared extract was made up to known volume and its reducing sugar in terms of glucose was measured spectrophotometery.

Nitrogen analysis: Total soluble nitrogen: Extraction of mats for nitrogen analysis was. carried out according to the procedures described by Naguib (1969). A known volume of the extract was digested using 1 ml 50 percent sulfuric acid and 1 ml 30 percent perchloric acid on a sand bath at low temperature until contents become concentrated and turn brown. The samples were kept on sand bath, at 180-200°C, till the mixture turned to a pale green, clear solution. After cooling the volume was completed to 10 ml by distilled water. The nitrogen content in the sample was determined as ammonia, by the Berthelot reaction (Fawcett and Scott, 1960; Chaney and Marbach, 1962).

Total insoluble nitrogen: The same procedures as described in total soluble nitrogen determination were applied to a known volume of the NaOH centrifugate to estimate total insoluble nitrogen.

Total Amino Acids (TAA): The procedure used was that adopted by Russell (1944).

Statistical analysis: Application of the least significant test (L.s.d) described by Duncan method, Sac Institute (1982).

Results

The results of Table 1 shows the effect of variable hydrogen ions concentration on the uptake of sugars as well as the direct reducing sugar value. It is clear that at pH 9.5 both fungi exhibited high enzymatic activity and the detected DRV in the case of *Scopulariopsis brevicaulis* was 553 μg/ml compared to 269 μg/ml medium of the control (pH 6.5), in the case of *Stemphylium piriforme*, DRV was 453 μg/ml medium compared to 321 μg/ml medium of the control (pH 6.5). On the other hand, the uptake of sugar was maximum at pH 9.5 for *S. brevicaulis*, but maximum uptake was at pH 7.5 for S. *piriforme* and then decreased significantly by the increase in pH value.

The most obvious observation in the results of Table 2 was that for amino-N content in *S. piriforme*, there was a significant decrease in the amino-N content with the rise in pH value and the least content (4 μg/ml medium) was observed at pH 11.5, while that of the control was 127 μg/ml medium. In case of *S. brevicaulis* the differences in the recorded values at PH'S between 6.5-11.5 were not significant.

Considering the Total Soluble Nitrogen (TSN), the results of Table 2 show a significant decline in the case of *S. brevicaulis* with the least content (430 μg/ml medium) at pH values 8.5 and 11.5. The trend was not the same in the case of *S. piriforme*, total soluble nitrogen significantly decreased at pH 7.5 to 270 μg/ml medium compared to 306 μg/ml medium and other pH values were not effective. Table 3 shows the effect of variable pH values on the mycelial mats contents of polysaccharides and reducing sugars (DRV). In the case of *S. brevicaulis*, polysaccharid content significantly decreased with the increase in p value and reached it's least amount (619 μg/ml medium) pH 11.5 compared to 925 μg/ml medium of the control. On the contrary DRV was insignificantly variable at all tests values. In case of *S. piriforme*, polysaccharide content was insignificantly affected by changing 'pH values from 5.51 11.5, but DRV value significantly increased at pH 8.5 (40 μg/g dry biomass), compared to 344 μg/g dry biomass, pH 6.5, then significantly decreased at higher pH values. pH 11.5 it was 219-μg/g dry biomass.

The relations among various nitrogenous contents in fungi under conditions of variable pH values were illustrated in Table 4. The most obvious trend was observed between insoluble nitrogen (protein) and total soluble nitrogen (TSN). In the mycelial mats of *S. brevicaulis*, total insoluble nitrogen significantly increased by the increase in pH value the highest observed value was at pH 11.5 (182 μg/g biomass) compared to 90 μg/g dry biomass at pH 6.5. On the contrary, total soluble nitrogen significantly decrease with the least amount of 580 μg/g dry biomass estimate at pH 11.5 compared to 830 μg/g dry biomass at pH 6. The whole trend was completely reversed in the mycelial mats of the alkalosensitive fungus *S. piriforme*, to insoluble nitrogen (protein) was at its highest rate at pH (387 μg/g dry biomass) and the insignificantly decreased the increase in pH value and reached its minimum amount (102 μg/g dry biomass) at pH 11.5. The same trend was observed in the case of total soluble nitrogen, the highest TSN value was recorded at pH 6.5 (480) μg/g dry biomass then started to decrease by changing pH towards more alkalinity with the least amount recorded at pH 11.5 (1 μg/g dry biomass).

Considering amino acid contents, there was a significant decrease in the .contents of the mycelial mats of previcaulis from 57 μg/g dry biomass at pH 6.5 to 33 μg/g dry biomass at pH 11.5. While, in the case of *S. piriforme* variable pH values showed insignificant effect on to amino acids contents.

Discussion

Fungi are known to grow well in acidic medium rather than alkaline one. However, few fungal species were reported maintain their optimal growth at alkaline medium.

In the present investigation, sugar uptake and DRV culture filtrate of both tested fungi under variable pH value between 6.5 and 11.5, revealed an increase in DRV in case of *S. piriforme* accompanied with a decline in sugar uptake, such observation could be attributed to poor su utilization at high levels of alkalinity. Geweely (1997) reported that *S. piriforme* produced optimal growth at 6.5 and the growth significantly decreased with increase in pH value and completely ceased at pH 11 This could be proved by the fact that pH values around neutral point (6.5-7.5) accelerated sugar uptake in *piriforme*.

The response of *S. brevicaulis* (which is alkalototlerant) high pH values, resulted in an increase in DRV, however

such increase is not attributed to low sugar uptake but most probable to the increased activity of amylases which have their optima at alkaline pH, this is confirmed by the good growth of the fungus at alkaline pH value 9.5 (Geweely, 1997).

The poor sugar uptake by the fungus *S. piriforme* was reflected on the mycelial content of polysaccharides, there was a marked decrease in polysaccharide contents as the pH value moved towards more alkalinity. On the contrary, in the alkalotolerant fungus (*S. brevicaulis*), the decrease in polysaccharide content cannot be attributed to the same reason, but such decrease may be attributed to the high growth activity which in turn required more energy obtained from the rapid incorporation of utilized glucose into glycolytic pathway. The results of the present study are in agreement with the work done with other microorganisms. Ohta *et al*. (1975), reported that respiration by cells of alkalophilic *Bacillus* sp. no. 8-1 increased with increase in the external pH values and was maximum at pH 9 in the presence of glucose or succinate. Also, the same trend observed by Ramos and Kaback (1977) with a study on alkalophilic Bacillus strain. Energy requirement by several species of alkalophiles was found to be greater than that required for non-alkalophiles (Rosing and Slater, 1972). The production of alkaline amylases was first achieved i an Alkalophilic Bacillus species strain No A-40-2 by Horikoshi and Akiba (1982).

Considering nitrogenous content, there was a marked decrease in both soluble and insoluble nitrogen in the mycelial mats of *S. piriforme*, which again confirmed the previous observation on growth by Geweely (1997). So, alkaline pH medium affected the ability of fungal cells to utilize nitrogenous sources in the culture medium and also may render them unavailable to fungal cells.

The observation with the alkalotolerant *S. brevicaulis* was different from that of the alkalosensitive *S. piriforme*. There was a decrease in the total soluble nitrogen as pH values increased to high alkalinity, but at the sometime, total insoluble nitrogen increased. This indicates that alkalotolerant fungi have got an enzymatic system that enables them to utilize nitrogen and produce more protein and other insoluble nitrogenous compounds. Koyama *et al*. (1976) reported that synthesis of protein by alkalophilic bacteria becomes active in an alkaline environment.

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