SALINITY-CELLULOLYTIC ACTIVITY RELATIONSHIP OF SOME ASPERGILLI ISOLATED FROM MUDDY SOILS*

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ABSTRACT

Cellulolytic activity of six isolates of each of the four Aspergilli, viz. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, and *A. terreus* was studied. There was a correlation between cellulolytic activity of various isolates and the organic matter content of the mud of their habitat. Isolates of *A. terreus* showed maximum cellulolytic activity of the four species followed by *A. flavus*. Relatively poor activity was exhibited by isolates of *A. fumigatus* and *A. niger*. The salinity of the culture medium affected the cellulolytic activity of all the forms tried, but to varying degrees. Most of the isolates, obtained from mud having a high salinity, showed their peak cellulolytic activity at higher salinity levels than did those isolated from mud of low salinity. This suggests certain potentialities to eco-physiological adjustments. Of the four Aspergilli, isolates of *A. terreus* has a greater tolerance to salinity in relation to cellulolytic activity than did the other species.

INTRODUCTION

Cellulose, one of the polysaccharides and a major structural component of plants, is of paramount importance in being continuously cycled organic material on the earth. In a pond ecosystem, where aquatic plants and animals die, a large number of micro-organisms including fungi attack on their residues to convert complex organic compounds locked up in them into simpler ones in order to draw their nourishment and also to replenish the environment. HOFSTEN AND EDBERG (1972) have pointed out the importance of cellulosic materials in aquatic as well as terrestrial ecosystems. They have also emphasized the lack of information on cellulose degradation in water courses where its addition is comparatively manifold than the terrestrial counterparts. The significance of ecological factors, viz. temperature, pH, humidity, etc. on the cellulose degrading ability of some fungal forms has been worked out and is well documented by SIU (1951). In addition, the effect of certain nutritional factors, such as carbon and nitrogen on cellulolytic activity of some fungi, has also been studied (SCHNITZ & KAUFERT, 1934 ; TRIBE, 1960 ; MERRILL & COWLING, 1966 ; LEVI *et al.*, 1968 ; LEVI & COWLING, 1969 ; PARK, 1976a-c). Except for papers by RAI AND CHOUDHERY (1976) and MALIK *et al.* (1979), little is known about the effect of salinity, an inimical factor of the soil environment, on the cellulolytic activity of fungi inhabiting muddy soils. Consequently, a study using six isolates of each of the four Aspergilli isolated from different ponds was undertaken to evaluate the influence of salinity of the medium on their cellulolytic activity.

MATERIAL AND METHOD

Six isolates of each of the four Aspergilli, viz. *A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus*, most commonly isolated during the microbial analysis of mud of six different ponds having varied salinity and organic matter, were used. These were grown at 28 ± 1°C on modified Richard's medium (pH 6.5) containing 1 per cent cellulose powder

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as the sole carbon source. To study the effect of varying salinity on the cellulolytic activity of these isolates, appropriate quantities of sodium chloride were added to the culture medium (before autoclaving) to obtain the final concentration of 1.5 per cent, 3.5 per cent, 5.5 per cent and 7.5 per cent of NaCl in the medium. Flasks having no NaCl in the medium served as controls. After 10 days of growth, cultures were filtered and filtrates were used as crude enzyme preparations.

Enzyme Assay

For assaying the cellulolytic activity 1 ml of enzyme preparation was mixed with 5 ml of 1 per cent carboxymethyl cellulose (CMC) in 0.1 M citrate buffer (pH 4.5) and incubated for 1 h at 30°C. Viscosity of the reaction mixture after an hour of incubation was measured with the Fenske Ostwald Viscometer. Relative enzyme activity was expressed as percentage reduction in the viscosity of reaction mixture/ml of enzyme/h at 30°C. It was calculated by the formula of Morrall *et al.* (1972).

RESULTS

The per cent salinity and organic matter of the six ponds studied have been given in tables 1 and 2, respectively. As seen in table 1, salinity of the muddy soil of all the six ponds ranged from 0.1-2.7 per cent throughout the year. On the basis of salinity, ponds numbering 1, 3 and 6 can be placed in one group having salinity ranging from 0.1-1.7 per cent, whereas the other three ponds numbering 2, 4 and 5 in the other group where salinity ranged between 0.2-2.7 per cent. The organic matter of the mud of all

Table 1—Variation in per cent salinity of soils of six ponds in different months

Pond number / Months	1	2	3	4	5	6
January	0.4	0.3	0.1	0.2	0.3	0.1
February	0.4	0.3	0.1	0.2	0.3	0.1
March	0.4	0.3	0.1	0.2	0.3	0.1
April	0.2	0.6	1.0	0.9	0.9	0.4
May	1.0	2.7	1.4	2.5	2.5	1.7
June	0.1	1.6	1.0	1.0	0.9	0.6
July	0.2	2.1	1.3	1.3	0.6	0.5
August	0.1	2.0	1.3	1.0	0.6	0.5
September	0.3	1.5	1.3	0.4	0.6	0.4
October	0.3	2.0	1.7	0.8	0.6	0.6
November	0.3	1.5	1.6	0.7	0.5	0.4
December	0.2	0.7	1.3	0.6	0.2	0.4

the six ponds ranged between 0.1-3.7 per cent, and on the basis of the average organic matter the ponds can be numbered in their descending order as 5, 1, 6, 2, 4 and 3 (Table 2).

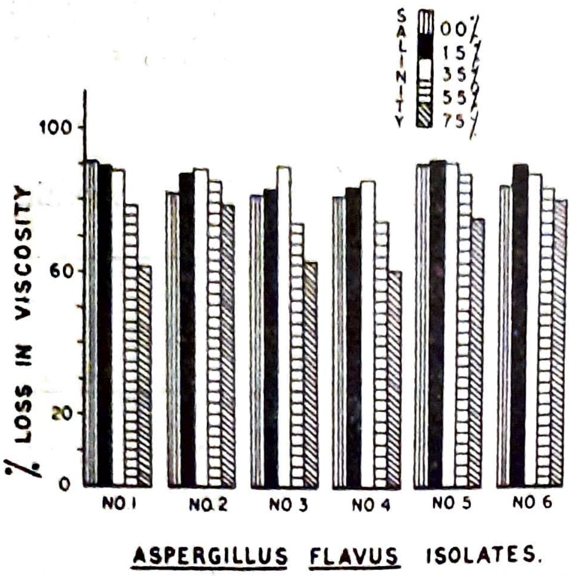
Table 2—Variation in per cent organic matter of soil of six ponds in different months

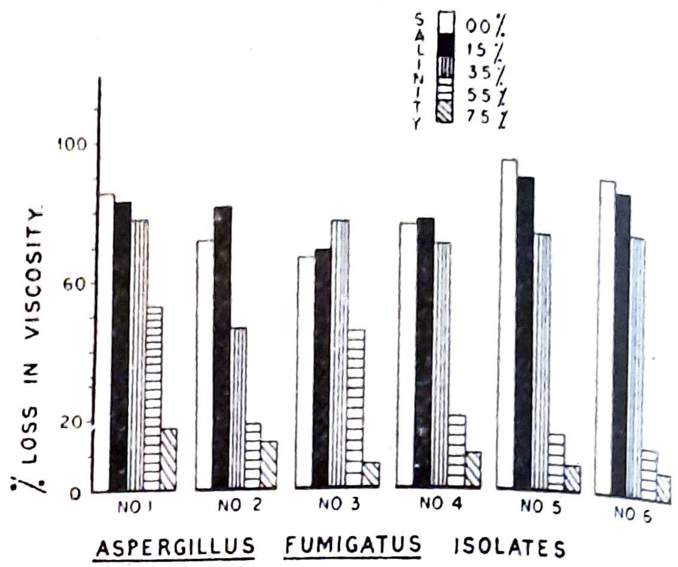
Pond number / Months	1	2	3	4	5	6
January	1.0	2.4	1.8	1.6	3.2	2.4
February	1.0	2.1	1.6	1.3	3.0	2.1
March	1.0	2.0	1.3	1.2	2.9	2.0
April	3.4	2.0	0.2	0.6	2.9	2.1
May	2.8	2.2	0.2	0.4	2.1	1.2
June	1.7	1.2	0.5	0.8	2.1	1.2
July	1.6	1.3	0.2	1.0	2.4	0.9
August	1.3	0.6	0.4	0.5	0.5	2.4
September	2.8	0.9	0.1	0.3	2.8	2.9
October	2.2	0.8	0.8	0.9	3.0	1.2
November	3.7	0.8	0.6	0.4	2.9	2.9
December	1.2	0.9	0.5	0.3	1.6	0.8
Average	1.97	1.40	0.60	0.77	2.30	1.80

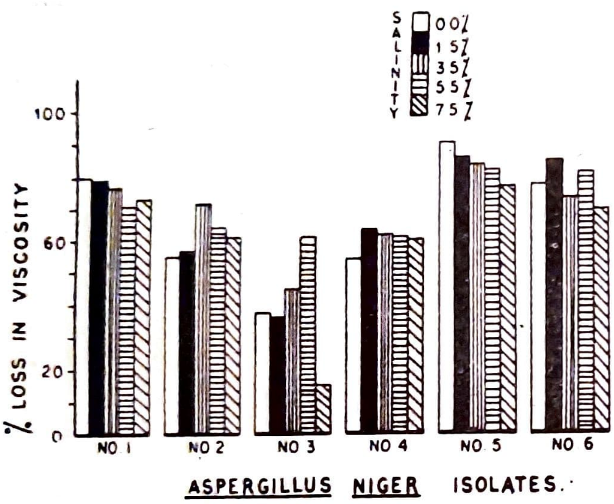
The cellulolytic activity of six isolates of each of the four Aspergilli at different salinities of the culture medium is shown in Figs. 1-4. All isolates showed varying degrees of cellulolytic activity at different salinities. Isolates from pond number 3, 2 and 4 of all the four Aspergilli showed the peak activity at relatively higher salinity levels than 5 and 6. Isolates 1, for all the fungi studied, showed peak activity at 00.0 per cent salinity.

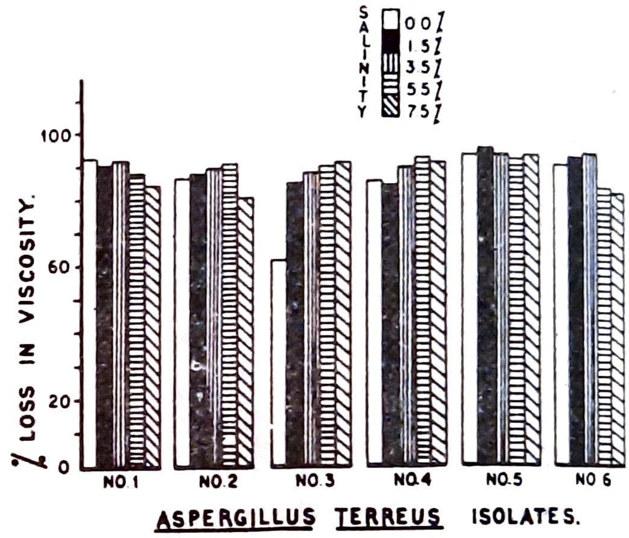
Aspergillus terreus alone exhibited comparatively higher cellulolytic activity at the higher levels of salinity than did the other species. Its isolates numbering 2, 3, 4, 5 and 6 exhibited optimum activity at 5.5, 7.5, 5.5, 1.5 and 3.5 per cent salinity, respectively. However, isolate number 1 had its highest activity at 0.0 per cent salinity.

Aspergillus flavus ranks next to *A. terreus* as far as its cellulolytic activity is concerned. Isolates of *A. fumigatus* and *A. niger* showed relatively poor cellulolytic activity in comparison to other two Aspergilli tried. Most of their isolates exhibited optimum activity at lower salinities of the medium. All the six isolates of *A. fumigatus* had very poor activity at higher salinities, viz. 5.5 and 7.5 per cent. However, isolates of *A. niger* showed comparatively better tolerance than *A. fumigatus* to higher salinities.









Figs. 1-4. The effect of salinity on the cellulolytic activity of the isolates of *Aspergillus flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*, respectively.

DISCUSSION

During this study it was found that isolates belonging to the ponds having high organic matter showed higher cellulolytic activity than those isolated from ponds of lesser organic matter. This is perhaps due to the fact that inhabitants of a habitat, rich in organic content tend to develop better capacity to produce higher amount of cellulolytic enzymes to degrade the plant debris which constitutes the major part of the organic matter of both, the terrestrial as well as the aquatic ecosystems. This is in conformity with the findings of Rai and Chowdhery (1976) who in their study of comparative cellulolytic activity of some fungi isolated from mangrove swamps and fertile soils found that isolates from mangrove mud having high organic content showed higher cellulolytic activity than fertile soil counterparts.

Various isolates of the Aspergilli tried showed varying degree of cellulolytic activity—salinity relationship. This varying ability of different species and their isolates of the genus *Aspergillus* studied, may possibly be due to the varying efficacy of their enzymatic equipment and potentialities to adapt the habitats to which they were subjected. Except number 1, all the isolates of *A. terreus* could tolerate appreciably higher salinities in relation to cellulolytic activity. This indicates that the isolates of *A. terreus* have relatively higher endurance to salinity of the medium in relation to the activity and have better ability for saprophytic life. The higher tolerance to salinity of the culture medium with respect to cellulolytic activity of isolate number 3 of all the four Aspergilli can be attributed to the fact that pond number 3 has comparatively higher salinity than other ponds to which these isolates inhabit and, therefore, the inhabitants have developed certain degree of ecological specialization to tolerate higher salinity. Similarly, all the isolates obtained from pond 1, where minimum salinity was recorded throughout the course of the study, showed their highest activity at 0.0 per cent salinity of the medium. All this may further be indicative of the fact that the nature and quality of the habitat of the organisms play significant role on their physiological behaviour and also influence the degree of their adaptability to overcome varying environmental conditions. Such adaptive ability perhaps affects the fungal forms in competitive colonization and so also their survival potentialities over the cellulosic substrate under varying environmental stresses. MEYERS AND REYNOLDS (1959), while working on the effect of salinity on the hydrolysis of cellulose by some lignicolous fungi, found that the forms tried produced varying amount of reducing sugars in sea and distilled water media. Except for *Stachybotrys atra* and *Alternaria* sp., all other forms produced higher amount of reducing sugars in sea water media than in distilled water which partially indicate their adjustment or affinity to the marine environment. This finding strengthens the observations made during the present study regarding the eco-physiological specialization of some of the most frequently occurring Aspergilli.

MALIK *et al.* (1979), while studying the effect of soil salinity on decomposition and humification of organic matter by some cellulolytic fungi, found that the activity decreases with the increase in salinity levels, but the fungi they tried did not belong to different salinity regimes. However, they have noted the variability in the activities of different fungi in soil.

Such a high activity of *A. terreus* and *A. flavus* over a broader range of salinity may make them to be successful and better competitor in an adverse and rapidly changing conditions. Thus, these species may very well contribute to the recycling of carbon in their ecological niche. The possibility that these species may be suitable for the degradation of industrial organic wastes, particularly of cellulosic nature, should be explored.

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