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EFFECT OF SODIUM CHLORIDE ON THE CELLULOLYTIC ABILITY OF SOME ASPERGILLI

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SUMMARY

The effect of sodium chloride on the cellulolytic ability of 16 species of *Aspergillus* isolated from salt-affected areas of Punjab, Pakistan was studied. Sodium chloride had, in general, an inhibitory effect on cellulolytic ability. However, *A. ochraceus* showed a marked increase in cellulolytic ability and *A. oryzae*, *A. nidulans* and *A. sydowi* showed a slight increase in cellulolytic ability at lower concentrations of NaCl. *Aspergillus terreus* was the most salt tolerant, showing the maximum cellulolytic ability at levels of NaCl tested. However, *A. luchuensis* was the most efficient cellulose decomposer in the absence of NaCl.

Large tracts of arable land in Pakistan have gone out of cultivation due to the advant of salinity and sodicity. Such salt-affected soils have a preponderance of sodium ions in the soil complex which results in the compaction of soil. Such conditions lead to decreased water permeability of soil and exclusion of air from the soil pores. This makes the environment quite unfavorable for microbial growth which is so essential for maintaining soil productivity. Several methods have been recommended by different workers for the amelioration of such soils (3, 5), and work on the reclamation of these soils through biological means has been going on in this laboratory (12). It essentially involves raising the biological activity of soil by in situ production of organic materials. This process is a part of an ecological succession starting with the highly salt-tolerant grass *Diplachne fusca* (L.) Beauv. followed by a relatively less salt-tolerant legume *Sesbania aculeata* Pers. and ending with an economic crop. Green manuring with both these plants and their subsequent decomposition by the soil microflora results in increased CO_2 pressure which helps in the solubilization of the $CaCO_3$ already present in our soils (7). The calcium thus released goes onto the soil colloidal complex, replacing sodium which can then be leached out easily.

Because cellulose is the most abundant constituent of the plant residues in the soil, a survey of the cellulolytic soil mycoflora inhabiting salt-affected soils was made which indicated the common occurrence of several species of *Aspergillus*. Since NaCl is the most common salt in saline soils, the present study was undertaken to elucidate the effect of NaCl on the cellulolytic ability of *Aspergillus* species isolated from salt-affected soils.

MATERIALS AND METHODS

Cultures.—The species of *Aspergillus* were isolated from salt-affected soils having a *p*H ranging from 8–10, an electrical conductivity (EC) from 0.8–4.0 Sm^{-1}, and an exchangeable sodium percentage (ESP) from 8–30 using Warcup's soil plate method (**17**). All of the species were cultured on Eggins and Pugh's (E & P) cellulose agar (**1**). These fungi were used as mass isolates.

Cellulolytic ability.—For the estimation of cellulolytic ability at different salt concentrations, Garrett's method (**2**) was used with some modifications. Whatman's No. 1 filter paper, 8 cm diam, was made up into wads of five papers and dried to a constant weight at 80 C. These preweighed and dried wads were placed in Petri plates and moistened with 10 ml E & P nutrient solution of the following composition: $(NH_4)_2SO_4$, 0.5 g; KH_2PO_4, 1.0 g; KCl, 0.5 g; yeast extract, 0.5 g; asparagine, 0.5 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; distilled H_2O, 1 liter (**5**). Various concentrations of NaCl were made by including 0, 3, 5, 10, 16 or 20% NaCl (w/v) in the nutrient medium. After autoclaving, three replicate plates were used for each combination of NaCl concentration and fungal species. The inoculum was in the form of 11-mm-diam agar discs cut from the actively growing margins of fungal colonies grown on glucose-agar plates. Inoculated plates and a blank set (wads receiving agar discs without inoculum) for all NaCl concentrations were incubated for 30 da at 30 C in the dark. Then the filter-paper wads and the fungal mycelia were dried at 80 C to a constant weight, and the weight loss determined. This loss represents only a portion of the cellulose actually consumed in respiration by the test fungus and does not include the remainder of the cellulose that was converted into fungal substances.

RESULTS

The results of testing 16 species of *Aspergillus* for their cellulolytic ability as affected by increasing concentrations of sodium chloride are

TABLE I

EFFECT OF NaCl ON CELLULOLYTIC ABILITY OF *Aspergillus* SPECIES AS REPRESENTED BY PERCENTAGE WEIGHT LOSS IN FILTER PAPER WADS AFTER 30 DA AT 30 C

Fungi tested	Control (0 NaCl)	NaCl 3%	NaCl 5%	NaCl 10%	NaCl 16%	NaCl 20%
Aspergillus acristatus	7.75	3.23	2.34	1.53	0.85	0.50
A. candidus	9.75	8.06	2.23	1.96	1.45	0.54
A. columnaris	6.51	5.89	4.21	2.97	1.93	1.03
A. flavus	4.16	3.47	3.35	2.86	1.93	1.91
A. fumigatus	7.15	5.89	2.99	2.41	0.73	0.42
A. japonicus	7.58	4.11	3.59	2.63	1.11	0.54
A. luchuensis	15.64	9.72	4.90	3.2	1.11	0.45
A. nidulans	9.31	10.08	7.9	6.03	2.41	1.83
A. niger	9.57	5.24	1.33	1.07	0.68	0.35
A. ochraceus	5.84	10.41	11.91	9.3	4.13	0.82
A. oryzae	7.23	8.68	7.49	4.69	2.06	0.96
A. sulphureus	3.36	3.26	2.15	2.00	0.81	0.31
A. sydowi	12.16	12.52	10.19	7.40	2.73	0.51
A. tamarii	7.63	6.22	4.32	3.09	1.98	1.83
A. terreus	12.84	12.41	8.55	4.71	4.35	3.27
A. ustus	6.92	6.19	3.96	1.89	1.07	0.27

presented in TABLE I. All the species tested were able to produce a loss in weight of the filter paper. In nearly all cases, the addition of NaCl depressed cellulolytic ability but the degree of depression was different for different species. This is indicative of the degree of salt tolerance of these fungi. However, the results of the percentage-weight-loss determination indicate that when no NaCl was added, maximum cellulolytic ability was shown by *A. luchuensis* Inui which produced a 15.64% weight loss in 30 da. *Aspergillus terreus* Thom and *A. sydowi* (Bain. & Sart.) Thom & Church were the next most efficient cellulose decomposers, causing 12.84 and 12.16% weight loss, respectively. Next to these in decreasing order of cellulolytic ability were *A. candidus* Link, *A. niger* van Tieghem, *A. nidulans* (Eidam) Wint., *A. acristatus* Fennell & Raper, *A. tamarii* Kita, *A. japonicus* Saito, *A. oryzae* (Ahlburg) Cohn, *A. fumigatus* Fres., *A. ustus* (Bain.) Thom & Church, *A. columnaris* Link, *A. ochraceus* Wilhelm, *A. flavus* Link and *A. sulphureus* (Fres.) Thom & Church.

The addition of NaCl in general decreased cellulolytic ability. However, in some cases lower concentrations of NaCl in the nutrient medium exerted a favorable effect on the ability of certain species to decompose cellulose. In this respect, *A. ochraceus* showed an increase in cellulolytic ability with an increase in NaCl concentration up to 10%. Similarly, *A. oryzae, A. nidulans* and *A. sydowi* exhibited an increase in cellulolytic ability at 3% NaCl concentration only. Higher concentrations depressed cellulolytic ability as in the case of other species.

The magnitude of reduction of cellulolytic ability as compared to the treatments where no NaCl was added is presented in TABLE II. Though *A. luchuensis* produced maximum weight loss at no addition of NaCl, it proved to be relatively salt sensitive since the percentage reduction in cellululolytic ability was 37.85% at 3% NaCl which increased to 68.64, 79.65, 92.89 and 97.14% at NaCl concentrations of 5, 10, 16 and 20%, respectively. In comparison, *A. terreus* proved to be relatively more salt tolerant as percentage reduction in its cellulolytic ability was 3.93, 33.45, 63.3, 66.09 and 74.53 at NaCl concentrations of 3, 5, 10, 16 and 20%, respectively. *Aspergillus terreus* also produced a relatively high weight loss of filter paper at all NaCl concentrations tested. However, among the four fungi which showed an increase in percentage weight loss with 3% NaCl added, *A. ochraceus* exhibited the highest cellulolytic ability at the most (i.e. three) NaCl concentrations. Among the other *Aspergillus* species which did not show any increase in cellulolytic ability with the addition of NaCl, *A. flavus* seemed to be quite tolerant to increasing concentrations of NaCl as there was only little more than 50% reduction of cellulolytic ability at 16 and 20% NaCl concentrations. However, cellulolytic ability of *A. flavus* was quite low (4.16% weight loss) as compared to that of *A. terreus* (12.84% weight loss).

TABLE II

PERCENTAGE REDUCTION IN CELLULOLYTIC ABILITY OF 16 SPECIES OF *Aspergillus* DUE TO NACL

Fungi tested	NaCl				
	3%	5%	10%	16%	20%
Aspergillus acristatus	58.32	69.81	80.26	89.03	93.55
A. candidus	17.33	77.07	79.90	85.10	94.41
A. columnaris	9.54	35.32	54.39	70.38	84.15
A. flavus	16.58	19.51	31.12	53.57	54.12
A. fumigatus	17.63	58.19	66.30	89.80	94.13
A. japonicus	45.78	52.64	65.30	85.36	92.88
A. luchuensis	37.85	68.64	79.65	92.89	97.14
A. nidulans	8.27*	15.1	35.27	74.11	80.34
A. niger	45.29	86.16	88.78	92.94	96.36
A. ochraceus	78.20*	103.98*	59.18*	29.31	86.04
A. oryzae	20.06*	3.60	35.13	71.51	86.72
A. sulphureus	2.98	36.01	40.48	75.89	80.77
A. sydowi	2.93*	16.19	39.15	77.52	95.79
A. tamarii	18.48	43.23	59.51	74.05	76.02
A. terreus	3.93	33.45	63.30	66.09	74.53
A. ustus	10.55	42.77	72.69	84.54	96.10

* Percentage increase instead of reduction.

DISCUSSION

There have been numerous publications in the past regarding the cellulose-decomposing ability of members of the genus *Aspergillus* (11, 14, 18). Nearly all the species tested during the present study have been known to decompose cellulose. Simpson and Marsh (15) described their work on cellulose decomposition by the Aspergilli, with special reference to *A. niger,* by employing the strength loss of cotton fabric during incubation with the fungus as the criterion for cellulolytic ability. In addition to these studies, there have been some reports on the effect of salts on the fungi with special emphasis on stored grain fungi (6) or tolerance of high salt environments by marine organisms (4). Tresner and Hayes (16) studied the NaCl tolerance of terrestrial fungi by including NaCl in the culture medium. They found that the Penicillia and Aspergilli were notably the most resistant with the majority of their species able to grow in the presence of 20% or more NaCl. Similar studies have also been made by Kulik and Hanlin (6), Rai and Agarwal (9, 10) but there have been no reports regarding the effect of NaCl on the cellulolytic activity of various *Aspergillus* species. Previously Malik et al. (8) studied the effect of soil salinity on the mineralization and humification of organic matter by six cellulolytic fungi, including *A. terreus,* and found that *A. terreus* had a high humification and carbon-mineralization rate at all salinity levels, however, a general depression in CO_2 evolution and cellulase activity of soil was observed with the increase in salinity level.

A general depression in cellulolytic ability of the fungi on addition of salts may be due to the decrease in total growth of the species tested. When salts are added to the culture medium, the salts utilize some of the water that the microbial cells need for growth. In higher concentrations, the nutrient medium may become quite rich in salt (NaCl) molecules, rendering the contact of water molecules with the cells difficult if not impossible. Scott (13) has comprehensively reviewed the water relations of microorganisms with regard to food spoilage. Similar conditions of low water activity exist in saline soils where there is high osmotic pressure, low moisture content, and high *p*H, thus making conditions quite unfavorable for the growth of the normal soil mycoflora. However, it will be of interest to study such effects in order to differentiate between specific ion toxicity and the reduction of cellulolytic ability of soil fungi due to osmotic pressure.

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