Sodium Chloride Tolerance of Terrestrial Fungi

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A survey was made of the NaCl tolerance of 975 species of terrestrial fungi selected from the major taxonomic classes. The penicillia and aspergilli were notably the most resistant with the majority of their species able to grow in the presence of 20% NaCl. The Basidiomycetes, as a class, were decidedly the least tolerant with over half the species unable to withstand more than 2% NaCl. Uniformity of tolerance by multiple strains of various species suggests that this may provide a useful taxonomic criterion.

Among the features that promote the survival of many higher fungi is their capacity to tolerate the harsh environment created by the presence of high osmotic substances. At the same time, others are equally influenced by their innate intolerance of physiological dryness. It is probable, therefore, that the distribution of at least some terrestrial and marine fungi is influenced by the salinity of their surroundings.

Much has been written regarding the fungal tolerance of high-salt environments, especially with respect to marine organisms. Johnson and Sparrow (1) comprehensively reviewed much of this literature. Comparatively few studies, however, have been made of the salt tolerance of terrestrial fungi and only a limited segment of taxa have been treated.

The present work was undertaken to define more precisely the relative salt tolerance of a wide diversity of terrestrial fungi by using the single salt sodium chloride.

MATERIALS AND METHODS

Cultures. Organisms tested during the survey were primarily from a large reference collection of fungi accumulated from various culture repositories. Multiple strains of some species originated as isolates for our antibiotic screening program.

Medium and cultural conditions. The basal test medium was a potato-dextrose-agar (PDA) consisting of instant potatoes (Kraft Foods, Dist., Chicago, Ill.), 40.0 g; dextrose, 15.0 g; MgSO4·7H2O. 0.2 g; agar (Difco), 20.0 g; water (distilled), 1,000 ml. All ingredients were combined, heated in a steamer until the agar melted, dispensed, and then sterilized by autoclaving (121°C for 15 min). No pH adjustment was necessary. The PDA was supplemented with a graded series of NaCl concentrations (5, 10, 15, 20, and 25%). Organisms not able to tolerate 5% NaCl were subsequently tested on 1, 2, 3, and 4%. Saline suspensions (0.85% NaCl) of the organisms washed from 14-day-old agar slant cultures were used to inoculate 7-ml slants of the PDA-NaCl medium in 16-mm screw-capped tubes. Cultures were incubated for 10 days at 24°C, at which time determinations were made of the maximum level that any growth occurred as well as any unusual physiological or morphological effects.

RESULTS AND DISCUSSION

Approximately 1,500 strains of 975 species comprising 351 genera, selected from the major taxonomic classes of fungi, were tested during the survey. An analysis of the accumulated data showed extremes in NaCl tolerance from <1% with certain basidiomycete species to >30% among several of the penicillia and aspergilli. When tolerance was assessed according to systematic groupings, it was noted that certain fungal classes, as a whole, differed from others. The data are summarized in Table 1 with the classes arranged in their ascending order of tolerance. The penicillia and aspergilli normally would be included in the Deuteromycetes, with ascocarpic forms assigned to the Ascomycetes. In this investigation, they are treated separately, however, because their generally high tolerance and the large sampling tested would have unrealistically influenced figures for these classes.

The Basidiomycetes were outstandingly intolerant, with 90.3% of the species unable to grow in the presence of 5% NaCl. When retested on concentrations from 1 to 10%, it was discovered that over half the species would not tolerate more than 2% (at <1% NaCl, 5.8%); at 1% NaCl, 21.2%); at 2% NaCl, 27.0%); at 3% NaCl, 19.2%); at 4% NaCl, 13.4%); at 5% NaCl, 11.5%); at 10% NaCl, 19.9%). To emphasize further the NaCl sensitivity of the Basidiomycetes, the maximum level of tolerance
was 10%, and only 1.9% of the species could withstand this concentration. In each of the other fungal classes, however, half or more of the species could survive this amount.

Johnson and Sparrow (1) reported that the only basidiomycete known to occur submerged in seawater is *Melanotaenium ruppiae*, a smut fungus growing on *Ruppia maritima*. Our findings that smuts were among the most salt-tolerant of the basidiomycetes is in accord with this observation. It is not surprising that *Basidiomycetes* as a class are poorly represented in the oceans, since a large percentage of the species would not likely survive the 3% salinity of the marine environment.

If the NaCl tolerance pattern for members of the *Deuteromycetes* is considered a normal distribution, then the *Ascomycetes* as a group tend to be somewhat less tolerant. The *Phycomycetes*, on the other hand, appear to be more tolerant. This may result, however, from a disproportionately large number of Mucorales in our sample that commonly exhibit relatively high tolerances.

Sodium chloride tolerance was studied in 124 species (273 strains) of *Penicillium* and in 81 species (196 strains) of *Aspergillus*. These two genera, as a whole, were outstandingly more resistant to NaCl than any of the other organisms studied. Over three-fourths of the penicillia (Table 2) could tolerate 20% NaCl and more than half survived at 25% or greater concentrations. The aspergilli (Table 3) appeared to be somewhat less tolerant; nevertheless, about 70% could withstand 20% NaCl and nearly half survived at 25%. No other fungi studied were able to grow at the 25% level. Tolerance data for the penicillia are arranged according to the "sections" of Raper and Thom (3) and the data for aspergilli are arranged according to the "groups" established by Raper and Fennell (2). An examination of the tolerances of the penicillia indicates that certain sections were exceptionally high, e.g., the *Asymetrica-Velutina*. Conversely, a large part of the *Biverticillata* were considerably less tolerant. This was particularly noticeable in the *P. funiculosum* series. Similarly, of the aspergilli, certain groups were notably tolerant (namely, *A. restrictus, A. glaucus, A. ochraceus*), whereas others (*A. cervinus, A. fumigatus, A. ornatus*) apparently were generally more sensitive.

Sodium chloride tolerance was assessed at the species level to determine the uniformity of response from strain to strain. Table 4 contains

### Table 1. Sodium chloride tolerance of major fungal classes

| Class            | No. of | Percentage NaCl |
|------------------|--------|-----------------|
|                  | Strains| Species| Genera| <5| 5| 10| 15| 20 |
| Basidiomycetes   | 162    | 104     | 47    | 90.3| 8.6| 1.1|
| Ascomycetes      | 196    | 160     | 87    | 17.5| 33.1| 35.6| 11.9| 1.9 |
| Deuteromycetes   | 428    | 358     | 182   | 8.4 | 22.9| 41.1| 21.5| 6.1 |
| Phycomycetes     | 330    | 148     | 33    | 12.8| 9.5 | 40.5| 34.5| 2.7 |

* Values are expressed as per cent of species.

### Table 2. Sodium chloride tolerance by taxonomic "sections" (3) of the penicillia

| Section          | Total no. species tested | NaCl (%) |
|------------------|--------------------------|----------|
|                  |                          | 5 | 10 | 15 | 20 | 25 or > |
| Monoverticillata | 31                       | 1a| 1  | 2  | 7  | 20  |
| Asymetrica       | 23                       | 6 | 6  | 11 |
| Divaricata       | 14                       | 2 | 12 |
| Velutina         | 9                        | 1 | 2  | 6  |
| Lanata           | 8                        | 1 | 1  | 5  |
| Funiculosa       | 18                       | 2 | 7  | 9  |
| Fasciculata      | 20                       | 1 | 4  | 2  | 7  |
| Biverticillata   | 1                        | 1 |
| Polyverticillata |                          | 1.6| 5.7| 14.5| 21.8| 56.4 |

* Numbers of species.
a representative sampling of species for which multiple strains were available for testing. A considerable degree of constancy in NaCl tolerance was found within species, suggesting that the physiological measurement might offer a useful taxonomic criterion as was found with the *Streptomyces* (4).

Media containing high NaCl concentrations frequently produced unusual and interesting physiological and morphological effects. Many

### Table 3. Sodium chloride tolerance by taxonomic “groups” (2) of the aspergilli

| Group             | Total no. of species tested | NaCl (%) |
|-------------------|-----------------------------|----------|
|                   | 5 | 10 | 15 | 20 | 25 or > |
| *A. candidus*     | 1 | 1  |    | 1  |         |
| *A. cervinus*     | 3 | 1a | 2  |    | 1       |
| *A. clavatus*     | 3 | 1  | 1  | 4  | 1       |
| *A. fumigatus*    | 7 | 1  | 1  | 4  | 1       |
| *A. flavipes*     | 2 |    |    |    | 2       |
| *A. flavus*       | 5 |    |    | 3  | 2       |
| *A. glaucus*      | 10| 2  | 2  | 6  |         |
| *A. nidulans*     | 9 | 3  | 4  | 2  |         |
| *A. niger*        | 9 | 1  | 4  | 2  |         |
| *A. niveus*       | 1 |    |    |    | 1       |
| *A. ochraceus*    | 5 | 2  | 1  | 4  |         |
| *A. ornatus*      | 5 | 1  | 1  | 1  |         |
| *A. restrictus*   | 4 |    |    |    | 4       |
| *A. terreus*      | 2 |    |    |    | 2       |
| *A. ustus*        | 2 |    |    |    | 2       |
| *A. wentii*       | 3 | 1  | 2  | 7  |         |
| *A. versicolor*   | 10| 1  | 2  | 7  |         |
| Per cent of total species tested... | 4.9 | 6.2 | 19.8 | 25.9 | 43.2 |

*a Numbers of species.

### Table 4. Sodium chloride tolerance of multiple strains of several diverse fungi

| Organism                  | Total no. of strains tested | NaCl (%) |
|---------------------------|----------------------------|----------|
|                           | <5 | 5 | 10 | 15 | 20 | 25 or > |
| *Poria ambigu*a*          | 6 | 6a| 2  |    |    |         |
| *Polyporus versicolor*    | 29| 27| 2  |    |    |         |
| *Mortierella candidabrum* | 5 | 5 |    |    |    |         |
| *Xylaria polymorpha*      | 5 | 1 | 4  |    |    |         |
| *Dactylium dendroides*    | 3 | 3 |    |    |    |         |
| *Phycomyces blakesleeanus*| 5 |    |    |    |    |         |
| *Mortierella isabellina*  | 12|    |    | 12 |    |         |
| *Spicaria violacea*       | 8 |    |    | 8  |    |         |
| *Trichoderma viride*      | 6 |    |    | 6  |    |         |
| *Mucor ramannianus*       | 10|    |    | 10 |    |         |
| *Neurospora crassa*       | 3 |    |    | 3  |    |         |
| *Gibberella fujikuroi*    | 4 |    |    | 4  |    |         |
| *Absidia glauca*          | 6 |    |    | 6  |    |         |
| *Penicillium lilacinum*   | 10|    |    | 10 |    |         |
| *Mucor circinelloides*    | 15|    |    | 15 |    |         |
| *Paecilomyces variotii*   | 8 |    |    | 6  | 2  |         |
| *Aspergillus clavatus*    | 16|    |    | 2  | 14 |         |
| *Penicillium chrysogenum* | 15|    |    |    | 15 |         |
| *P. herquei*              | 9 |    |    | 9  |    |         |
| *A. ochraceus*            | 7 |    |    |    | 7  |         |

*a Numbers of strains.*
members of the *Mucorales* and several species of *Aspergillus* produced abnormally elongated sporophores, and an increase in size and number of spore heads was sometimes noted. Various species of *Mucor, Rhizopus, Absidia, Cunninghamella,* and other *Mucorales* developed as yeastlike colonies on media containing 10 to 15% NaCl. Brightly colored pigments, normally not evident, were produced commonly by certain of the aspergilli and penicillia; intensification of pigmentation frequently also occurred with other organisms at particular NaCl levels. Enhanced mycelial growth was occasionally observed. Increased sporulation sometimes was encountered, especially among the penicillia and aspergilli at 5 to 10% NaCl. Zygospore formation was greatly stimulated in *Sporodinia grandis* by 5% NaCl. These and many other physiological and morphological changes reflect the markedly altered metabolic patterns of the organisms brought about by the harsh environment of the high salinity.

**LITERATURE CITED**

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