The genetical control of osmotolerance in fungi: a mutation analysis in the ascomycete

_Aspergillus nidulans_

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The ascomycete _Aspergillus nidulans_ is remarkably osmotolerant and the wealth of genetical and physiological techniques available for this species make it an attractive model for a study of the basis of this character. A mutation assay estimates that around 200 genes can mutate to osmosensitivity and a number of these have now been identified as contributing to the ability to grow at reduced water potentials.

**Keywords:** _Aspergillus nidulans_, osmosensitivity, mutations.

Fungi adapted for growth and survival in a range of habitats have necessarily evolved mechanisms to overcome the effects of a range of deleterious factors which may vary globally and locally. Such growth constraints may include reduced nutrient availability, unfavourable pH, extremes of temperature and the occurrence of particular toxins or growth inhibitors such as high salt concentrations. However, a ubiquitous requirement is the presence of water. Many fungi are renowned for their capabilities to survive under extreme conditions of drought, not least as symbiotic partners in lichens. The important role of some species in post-harvest losses of stored crops such as grains depends upon the ability of members of genera like _Aspergillus_ to grow when reduced availability of water excludes most bacteria. A fungus must maintain a more negative internal water potential than that outside the cell or hypha to allow continued influx of osmotically-driven water. Whilst marine habitats present the most obvious and extensive osmotic challenges, other environments, including animal and plant hosts, also put demands on the potential pathogen to acquire sufficient water for growth.

Recently the genetical and molecular basis of osmotolerance has been studied in a number of fungi, notably in yeasts (Garcia _et al._, 1997). These organisms, particularly the baker’s yeast _Saccharomyces cerevisiae_ Hansen, bequeath an impressive legacy of techniques which make them attractive laboratory subjects. However, such unicellular species may have a limited application as models for multicellular or compartmentalised filamentous fungi that thrive upon solid substrates rather than in liquid media. Consequently, the ascomycete _Aspergillus nidulans_ (Edam) Winter, which also provides a wealth of physiological and molecular methods (Clutterbuck, 1974), has emerged as an interesting subject for the study of the survival and growth of fungi in osmotically-challenging environments.

How many genes control the ability to grow in an environment where the availability of water is reduced perhaps by salt or sugars and the fungus is osmotically stressed? Traditionally, geneticists have recognised genes by the effects that mutations have upon the behaviour or appearance (phenotype) of the organism. We can estimate the number of genes involved in controlling osmotolerance by comparing the number of “osmotic” and auxotrophic mutations obtained in the same experiment, assuming that each group of genes has a roughly equivalent rate of mutation. The number of gene loci that can produce nutritional dependence or auxotrophic mutations in _A. nidulans_ has been estimated to be 61 (Dorn _et al._, 1987). Although the Fungal Genetics Stock Centre (http://www.kumc.edu/research/fgsc/main.html) currently lists strains carrying around 135 different mutations that could conceivably lead to auxotrophic phenotypes on specific media, these would not all be detectable...
in conventional mutation screens.

Ascanial spores (conidia) from \textit{A. nidaulans} were exposed separately to four different mutagens each with a different mode of action: ultraviolet radiation, the denaturing chemical nitrous acid, a reducing agent hydroxylamine, and the alkylating agent MNNG (N-methyl N-nitro N'-nitrosoguanidine). Dose response or ‘kill’ curves were obtained for each mutagen and exposures that caused 99% mortality were chosen for the treatment of large numbers of spores. Survivors were allowed to grow on a rich or ‘complete’ medium and then screened for enhanced sensitivity to NaCl and their ability to grow on a starvation or ‘minimal’ medium (Clutterbuck 1974). Putative mutants were subcultured and single spore isolates taken before testing their stability. These isolates were then classified as salt sensitive if they showed enhanced sensitivity just to ionic compounds (NaCl or KCl) or as osmosensitive if they also showed sensitivity to glycerol. Fig 1 shows an example of such a simple plate growth test showing the reduced colony size and sporulation of sensitive strains compared with controls. In sexual crosses, salt/osmo-sensitivity segregated as a single gene marker for each of five different mutants tested.

Nitrous acid proved most successful in producing osmo/salt sensitive mutants (0.56% of survivors), compared to 0.37% for ultra violet light, 0.09% for MNNG and zero for hydroxyl-amine. Spathas (1978) produced a comparable frequency of salt/osmo-sensitive mutants of 0.12% of survivors following MNNG exposure of conidia from the same species. In total 9787 survivors of the four types of mutagenesis were screened and 32 stable salt/osmo-sensitive mutants were generated compared with 9 auxotrophic mutants. If 61 gene loci produce auxotrophic mutations then around 200 (217) genes can be estimated to produce salt/osmo-sensitive phenotypes (Clement, 1997). Half of the mutants were sensitive just to salt with their responses to glycerol unaffected. Using 2D polyacrylamide gel electrophoresis (2D-PAGE), Redkar et al. (1996) recognised around 50 novel or induced proteins produced by salt-adapted cultures of \textit{A. nidaulans} with 24 further \textit{de novo} synthesised or up-regulated proteins being identified in salt-shocked cultures. Blomberg (1995) used the same approach to estimate that around 200 genes and/or proteins respond to salt stress in \textit{S. cerevisiae}. This 2D-PAGE method is likely to produce an underestimate of the number of “osmotic genes”, partly because some gene products may be expressed at undetectable levels and also because genes that can mutate to give an osmosensitive phenotype need not be salt - inducible. The number of “osmotic” genes estimated for \textit{A. nidaulans} appears to be lower than the 300 to 1000 gene loci judged as involved in conidiation and produced by the random mutation method (Martinelli and Clutterbuck, 1971). A molecular estimate of the number of conidiation genes based upon the diversity of mRNA molecules at

\begin{table}
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\begin{tabular}{|l|l|l|}
\hline
Gene/Mutation & Phenotype & Author \\
\hline
\textit{bin} G & osmotic remedial phosphatase & Borgia & Dodge, 1992 \\
\textit{chl} A & osmosensitivity & Chabani & Grindle, 1990 \\
\textit{myo} A & myosin 1 & McGoldrick & et al., 1995 \\
\textit{nfs} A & sodium fluoride sensitivity & Shawcross & et al., 1994 \\
\textit{orl} A,B & osmotic remedial chain formation & Borgia & Dodge, 1992 \\
\textit{orl} C,D & osmotic remedial β 1,3 glucan development & Borgia & Dodge, 1992 \\
\textit{slt} A & NaCl/KCI sensitivity (arginase) & Spathas, 1978 \\
\textit{sor} A,B & sorbose resistance (\textit{sor} B = phosphoglcomutase) & Clement & et al., 1996 \\
\textit{tsb}E & temperature sensitive glucosamine synthesis & Elorza & Arst, 1971 \\
\hline
\end{tabular}
\caption{Examples of gene mutations in \textit{Aspergillus nidaulans} with “osmotic” phenotypes.}
\end{table}
different stages of the life cycle reached a similar figure (Timberlake and Clutterbuck, 1994).

What types of genes can mutate to salt/osmosensitivity in A. nidulans? The literature carries a number of reports of mutations that respond to osmotic stimuli, although not all of these give sensitive phenotypes (Table 1). One category of mutations affects the formation of the hyphal wall, for example tsE and orfA,B,C,D. Some mutations allow the recovery of a particular activity in the presence of elevated salt. The orfA gene encodes a trehalose - 6 - phosphate phosphatase which, when defective, lowers the temperature stability of an enzyme involved with aminosugar synthesis. This mutational defect can be remedied by osmotic stabilisers (Borgia et al., 1996). Interestingly a high proportion of isolates identified as being defective in sporulation fall into this latter class of “osmotic remedial” mutants (Martinielli and Clutterbuck, 1971). Defects in myosin (myoA) can lead to osmosensitivity, suggesting that the cytoskeleton may act as an osmosensor to rapidly respond to changes in hyphal turgor or ion content as in S. cerevisiae (Blomberg, 1995). Other mutations in A. nidulans appear to control salt - sensitive processes or enzyme activities which may include mitosis (bin1), DNA repair (nsfA) and amino acid catabolism (sitA). Membrane transport proteins involved in ion transport are yet to be detailed by mutant analysis in A. nidulans although altered membrane permeability or transport processes may explain resistance to toxic sugars (sorA) and the fungicide resistance of some osmosensitive mutants (ohlA). When stressed with high concentrations of NaCl, both glycerol and erythritol normally accumulate as compatible solutes to allow continued water uptake (Beever and Laracy, 1988) and yet a number of regulatory mutations that affect glycerol production show no disturbance of osmotic relations (Hondmann and Visser, 1984).

In conclusion, a large number of genes appear to be able to mutate to osmosensitivity in A. nidulans, although this is somewhat fewer than the number involved in the complex process of conidiation. In some cases mutational analysis has allowed us to identify and clone genes which are involved in osmoregulation whilst other genes have less obvious roles. An understanding of the functions of these genes will greatly advance our perceptions of the factors controlling the ecological distribution of fungi.

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References


Cookery Corner

LANGERMANNIA ESCALOPE
(WITH LEMON & TARRAGON SAUCE)

Giant puffballs are so delicious and something I always look forward to. Combined with this sauce these escalopes make a wonderful and unusual addition to a dinner party menu.

Ingredients

Eight 2 cm thick slices of Langemannia gigantea.
2 large beaten eggs.
225 g fresh white breadcrumbs.
Little oil for frying.

Sauce

25 g butter.
50 g plain flour.
300 ml milk.
Juice & rind 1 lemon.
1-2 tsp. dried tarragon.
150 ml double cream (optional).
Seasoning salt & pepper.

Method

Take the slices of puffball and remove the outside cuticle which is like a rind. Dip the slices in the beaten egg and then in the breadcrumbs until they are completely coated.

To make the sauce, melt the butter and then beat in the flour. Whisk in the milk a little at a time gently bringing to the boil. Allow to simmer until the sauce thickens, constantly whisking. Add the lemon juice, zest, tarragon and (if preferred) cream for a more luxurious taste. Season with salt and pepper.

Heat a little oil in a frying pan and fry the slices of puffball, a few at a time, on both sides until golden brown.

Serve two slices per person with the sauce poured over the top

Diana Bateman

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