COMMENTARY

Compatible solutes and fungal development

Jan DIJKSTERHUIS*[1] and Ronald P. DE VRIES†[1]

*Applied and Industrial Mycology, Centraalbureau voor Schimmelcultures, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands, and †Microbiology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

Compatible solutes are components that can be quickly accumulated and degraded inside fungal cells. They do not disturb the functioning of proteins and protect the cell under adverse conditions. In this issue of the *Biochemical Journal*, Solomon and co-workers evaluate the role of mannitol, one of these components, in *Stagonospora nodorum*, a plant-pathogenic fungus, and find surprising effects on the development of spores and spore-forming structures.

Key words: compatible solute, conidium, mannitol, plant-pathogenic fungus, spore formation.

SPORES AND SPORE FORMATION

In the fungal Kingdom, spores are a major vehicle of distribution. The variation in shape and the number of different names given to these structures is bewildering. Some spores have complex morphologies and ornamentations that reflect very specialized functions. Spores of the fungus *Harposporium*, for example, have a shape that resembles 'newly hatched chickens', and precisely fit into the buccal cavity of nematodes (eelworms), which are subsequently penetrated and digested. Other spores are simply unicellular and spherical and are dispersed into the air, before they reach a novel substrate. When these cells encounter conducive conditions for growth, they start to germinate and form a germ tube and a mycelium (hyphal network), which penetrates and dissolves the nutrient matrix. At a certain time, for instance when a shortage of nutrients occurs, spore-forming structures are formed. Again, the variation of spore-forming structures is enormous, ranging from simple, very small open tubes to intricate three-dimensional fruit bodies that can be beautifully ornamented.

After formation on, or inside, the spore-forming structure (fruit bodies), spores are released into the environment by different mechanisms. Some spores are released by the action of water droplets that splash on spore-bearing structures with the result that the contents of the fruit bodies are propelled into the air. In contrast, conidiophores of *Penicillium* gently lift the spores into the air, in the zone where turbulent movement of the air occurs, and as such the spores travel considerable distances before landing on new substrate. In fact conidia (spores) of *Penicillium*, *Cladosporium* and *Aspergillus* release spores so profusely and effectively that they are present everywhere on Earth. Every cubic metre of air contains fungal spores, and the three genera mentioned above are identified in every sample. Alternatively, spores just remain where they are, encased in a thick wall and surviving for long periods of time. These spores disperse in time and literally await better times.

Many fungi form different types of spores at the same time. These can be sexual or asexual types of spores, but also 'space'-dispersed and 'time'-dispersed.

LEAF-PATHOGENIC FUNGI

The combined surface area of plant leaves on earth must be tremendous, and this green 'continent' provides an enormous area that can be colonized by living organisms. However, especially for micro-organisms, the leaf surface is a hostile area with nearly desert-like characteristics. A single growing fungal hypha on the leaf surface will encounter transient periods of water stress during the day. The leaves are covered with a wax layer that keeps the water away, and when the sun is shining on the leaves, the temperature and UV-radiance are relatively high. In order to survive these conditions, many leaf-inhabiting or epiphytic fungi are clearly pigmented, and they are able to survive transient periods of drought with remarkable efficiency [1]. Often fungi that grow on the surface of leaves will try to enter the inside where sufficient carbohydrates and water are to be found, but where they will have to withstand the plant's defences against fungal attack, such as oxidative stress. *Stagonospora nodorum* is such a fungus, and an important pathogen on wheat and the major cause of leaf and glume blotch disease in Western Australia and Ohio [2]. When conidia (spores) of this fungus land on leaves, they germinate to form thin germ tubes that grow over the leaf surface. A wet period of 6 h is required for effective infection, which occurs when germ tubes sense the opening of the stomata and grow inside the leaf. Inside, they start to kill and destroy the tissues and grow on the remnants. This defines this fungus as a necrotrophic pathogen. When enough biomass of the fungus is formed, a spore-forming structure called a pycnidium is formed inside the cavity underneath the stomata. These structures can be regarded as a fungal tissue forming a globose structure with an opening. As a result of rainfall on the leaves, the conidia are released from the structure.

COMPATIBLE SOLUTES IN FUNGAL CELLS

To counteract osmotic stress or other stressors, fungi accumulate so-called 'compatible' solutes inside living cells. These are named as such because they do not disturb the functioning of proteins and other biomolecules, and the complexes formed by them, when they are present in high amounts inside the cell. Widely spread compounds are glycerol, mannitol, trehalose, arabitol and erythritol. All these compounds are observed in various growth stages and under different environmental conditions. Is there a preference for a certain solute in relation to a certain environmental condition? In fungi, one could state that glycerol (and erythritol) is linked to osmotic stress and growth at low water

[1] Correspondence may be addressed to either of the authors (email dijksterhuis@cbs.knaw.nl or r.p.devries@bio.uu.nl).

activity. Mannitol and trehalose provide protection against heat, cold and drought, and trehalose is linked to longevity. For instance, spores of the fungus *Talaromyces macrosporus* are packed with trehalose and can survive many years of storage [3]. Remarkably, two papers provide information that the combination of trehalose and mannitol is important in fungal cells and their protection [4,5].

In both the fungal species *Aspergillus nidulans* and *Aspergillus niger*, conidia contain mannitol and trehalose. For *A. niger*, these compounds are present at concentrations of 10.9 % and 1.8 % dry weight. Mannitol is produced by the action of two enzymes mediating a reduction step and a phosphatase step respectively, from fructose 6-phosphate via mannitol 1-phosphate by the action of MPD (mannitol-1-phosphate dehydrogenase). The Δ*mpdA* strain of *A. niger* that is deficient for the MPD has increased trehalose and reduced mannitol levels. Mutant conidia show 90 % viability loss after nearly 1 h of heating at 50 °C, whereas the wild-type survives easily for 2 h at this temperature. A Δ*tpsA* strain of *A. nidulans* was unable to produce trehalose, and germinating conidia of the mutant showed very low colony formation when stored for approx. 15 h at 44 °C, whereas the wild-type showed no significant decrease. These data may suggest that a combination of trehalose and mannitol gives protection against different stressors.

MANNITOL IN *STAGONOSPORA NODORUM*

In the paper by Solomon and co-workers in this issue of the *Biochemical Journal* [6] the role of mannitol in spore formation by the fungus *S. nodorum* is evaluated. Two synthetic routes are hypothesized in mannitol synthesis: one as described above, and another via fructose by the action of MDH (mannitol dehydrogenase). MDH uses NADH as a cofactor; MPD uses NADPH. Together, they form a putative mannitol cycle, which may ensure rapid production and degradation of mannitol and enables the production of NADPH at the expense of NADH and ATP. The authors constructed different deletion mutants of *mdh*, *mpd* and *mdh*/*mpd*, and studied the resistance against stressors, spore formation and pathogenicity. In addition, they analysed the metabolites present inside fungal cells during different stages of growth in an artificial medium under laboratory conditions and in plants.

In both wild-type hyphae and the *mdh* mutant, mannitol levels were the highest; these were lowered in the *mpd* mutant, and nearly absent in the double mutant. Trehalose was highest in the *mpd* mutant, and its level was also increased in the double mutant, but to a lesser extent. In contrast, *in planta* the same levels were observed for mannitol in the different mutants, and trehalose was present to a considerable extent, although levels were slightly lowered in the *mpd* single and double mutants. The sensitivity of all the strains to oxidative (H_2O_2) or osmotic (NaCl) stress was similar, thus no protective effect of mannitol was clear in this case. Given that glycerol is also low in these strains, another factor must be involved in protection against these stresses. Heat protection in yeast cells, for example, is dependent on both the presence of compatible solutes and heat-shock proteins [7].

The authors conclude that a mannitol cycle probably does not occur in *S. nodorum*, but that there is a reversible route via MPD (and thus via the NADH-dependent reduction of fructose 6-phosphate), and that the MDH part of the route is of minor importance for both mannitol synthesis and catabolism.

Interestingly, the study in *S. nodorum* reveals significant differences from a previous study with *A. niger* [5]. The *S. nodorum* Δ*mpd* strain was unable to grow on mannitol as the sole carbon source, whereas the *A. niger* Δ*mpd* strain grew similarly to the wild-type. In addition, sporulation of *S. nodorum* Δ*mpd* was reduced compared with wild-type in the absence of mannitol, whereas no effect on sporulation could be observed in *A. niger* (R. P. de Vries, unpublished work). This indicates that MPD in *S. nodorum* is essential for mannitol utilization and significantly affects sporulation, whereas both phenomena are unaffected by the loss of MPD in *A. niger*. No comparison can be made between the two fungi with respect to the role of mannitol in ROS (reactive oxygen species) defence, as the conditions used to examine this phenomenon in *S. nodorum* were significantly different from those used with *A. niger*. The ability of *S. nodorum* to grow in the presence of H_2O_2 could be explained by the presence of catalases, and does not exclude the possibility that other ROS compounds could affect growth or spore viability of this fungus, or that the defence mechanism is (partially) dependent on mannitol.

MANNITOL, SPORULATION AND PYCNIDIA

The authors studied the formation of spores on minimal medium and without mannitol, and a 4- or 200-fold decrease in spore number was observed in the *mpd* and double mutant respectively. These observations were confirmed by experiments in which every possible internal store of mannitol in the fungal cells was depleted by repetitive inoculation of spores on medium containing or lacking mannitol. When wheat leaves were infected with conidia of the fungal strains (at least some spores were formed also by the double mutant), all were able to grow in the leaves and did harm to the tissue, but the *mpd*/(*mdh*) deletion mutants did not form fruit bodies (pycnidia). That *mpd* provided a major route here was also confirmed by means of quantitative PCR experiments. Interestingly, the addition of drops of external mannitol (3 mM) restored the capability of the fungus to form pycnidia.

Why is mannitol so important for the formation of fruit bodies, and hence conidia? Pycnidia are formed in the substomatal cavity, which is a large space compared with the fungal hyphae. Pycnidia are also relatively elaborate structures, on a scale of several hundreds of micrometres, where fungal hyphae bundle together and form tissues. Entering the substomatal space may mean that fungal hyphae have to abandon their growth along the dead plant cells, and protrude into the air as aerial hyphae. In doing so, the fungal cell may encounter a certain type of drought stress (not salt stress), and mannitol may help to survive this. Further, the development of these pycnidia and subsequent massive spore formation may require large amounts of energy, and mannitol might be an important storage compound. That pycnidia formation under stomata is easily disturbed is shown by the observation that their formation is hampered in the case of different mutants of the fungus *Mycosphaerella graminicola* [8,9]. This fungus is the cause of *Septoria tritici* blotch on wheat, a similar disease.

Interestingly, trehalose is present inside cells of all strains used in this study. It would be interesting to establish to what extent trehalose and mannitol accumulate inside the hyphae, given that a certain minimal concentration seems to be needed for protective purposes. As stated above, in conidia levels of both components are approximately between 3 and 7 % wet weight (see [3,4]). With conidia, a mixture of mannitol and trehalose might be needed for optimal protection against heat stress. Pycnidia were not formed when trehalose was present and mannitol was absent, indicating that this might also be true in the *Stagonospora* model system. It would be interesting to see whether the disruption of trehalose synthesis would lead to a similar phenotype. Finally, the authors suggest that mannitol may play a role as a signalling molecule in this respect. It would be tantalizing to study the putative role of

this general accumulative compound as a signal for an important fungal development event.

REFERENCES

1 Park, D. (1982) Phylloplane fungi: tolerance of hyphal tips to drying. Trans. Br. Mycol. Soc. **79**, 174–178

2 Lipps, P. E. and Mills, D. *Septoria tritici* blotch and *Stagonospora nodorum* blotch. Extension fact sheet. The Ohio State University, http://ohioline.osu.edu/ac-fact/0002.html

3 Dijksterhuis, J., van Driel, K. G. A., Sanders, M. G., Molenaar, D., Houbraken, J. A. M. P., Samson, R. A. and Kets, E. P. W. (2002) Trehalose degradation and glucose efflux precede cell ejection during germination of heat-resistant ascospores of *Talaromyces macrosporus*. Arch. Microbiol. **178**, 1–7

4 Fillinger, S., Chaveroce, M.-K., van Dijck, P., de Vries, R., Ruijter, G., Thevelein, J. and d'Enfert, C. (2001) Trehalose is required for the acquisition of tolerance to a variety of stresses in the filamentous fungus *Aspergillus nidulans*. Microbiology **147**, 1851–1862

5 Ruijter, G. J. G., Bax, M., Patel, H., Flitter, S. J., van de Vondervoort, P. J. I., de Vries, R. P., van Kuyk, P. A. and Visser, J. (2003) Mannitol is required for stress tolerance in *Aspergillus niger* conidiospores. Eukaryotic Cell **4**, 690–698

6 Solomon, P. S., Waters, O. D. C., Jörgens, C. I., Lowe, R. G. T., Rechberger, J., Trengove, R. D. and Oliver, R. P. (2006) Mannitol is required for asexual sporulation in the wheat pathogen *Stagonospora nodorum* (glume blotch). Biochem. J. **399**, 231–239

7 Elliott, B., Haltiwanger, R. S. and Futcher, B. (1996) Synergy between trehalose and Hsp104 for thermotolerance in *Saccharomyces cerevisiae.* Genetics **144**, 923–933

8 Stergiopoulos, I., Zwiers, L.-H. and de Waard, M. (2003) The ABC transporter MgAtr4 is a virulence factor of *Mycosphaerella graminicola* that affects colonization of substomatal cavities in wheat leaves. Mol. Plant–Microbe Interact. **16**, 689–698

9 Mehrabi, R., van der Lee, T., Waalwijk, C. and Kema, G. H. J. (2006) MgSlt2 a cellular integrity MAP kinase gene of the fungal wheat pathogen *Mycosphaerella graminicola* is dispensable for penetration but essential for invasive growth. Mol. Plant–Microbe Interact. **19**, 389–398

Received 10 August 2006/15 August 2006; accepted 16 August 2006
Published on the Internet 27 September 2006, doi:10.1042/BJ20061229