Relevance of Trehalose in Pathogenicity: Some General Rules, Yet Many Exceptions

Hélène Tournu1,2*, Alessandro Fiori1,2*, Patrick Van Dijck1,2*

1Department of Molecular Microbiology, VIB, Leuven, Belgium, 2Laboratory of Molecular Cell Biology, Katholieke Universiteit Leuven, Leuven, Belgium

Trehalose: A Supporting Act More than a Starring Role

Trehalose, a natural disaccharide, consists of two glucose molecules linked by an α, α-1,1-glucoside bond. Apart from its function as a reserve carbohydrate, trehalose is known for its role as a stress protectant in many organisms across kingdoms. It is present in plants, invertebrates, fungi, and prokaryotes, but not in mammals. Despite its well-known protective roles against desiccation, freezing, starvation, and osmotic stress [1], trehalose has escaped categorization into a specific biological pathway. Interestingly, a role for its metabolism is emerging in the establishment of virulence traits in distantly related microbial species.

In general, although notable exceptions exist, the absence of a complete trehalose metabolism apparatus is associated with a lower pathogenic potential. The mechanisms involved, however, are less clear. Pathogens have engineered several distinct trehalose-associated mechanisms that contribute to cell morphogenesis, cell wall integrity, regulation of metabolism, and evasion from the host immune response.

Trehalose Synthesis Pathways and Virulence: E Pluribus Unum?

Trehalose can be synthesized via different metabolic pathways [2], sometimes coexisting, with both shared and specific functions relevant to pathogenicity (Table 1). Of the three potential trehalose biosynthesis pathways differently contributing to Mycobacterium tuberculosis virulence, the OtsAB pathway (Figure 1) is the dominant one [3]: deletion of OtsA results in severe growth defects, whereas the OtsB gene is essential for viability. Of the two additional pathways, the TreYZ pathway and the TreS pathway, only the latter seems necessary for late development of chronic infections in mice, while absence of the first doesn’t cause any apparent defect in microbial growth or virulence.

Only the TreS and TreYZ pathways are present in the plant pathogen Pseudomonas syringae, and their absence leads to reduced survival on host tomato leaves [4], an effect that may be attributed, at least in part, to a fitness defect of the corresponding mutants in low-water environments. Inactivation of the same two pathways reduces the pathogenicity of Pseudomonas aeruginosa against plants, restored by co-inoculation of exogenous trehalose, but not against worms, insects, and mice [5].

In fungal pathogens, trehalose is mainly synthesized via the TPS/TPP pathway (Figure 1). Originally demonstrated in Candida albicans [6], the abolishment of trehalose production has a significant negative impact on the in vitro survival of several plant and human pathogens, including Magnaporthe oryzae [7], Sclerotinia nodorum [8], Cryptococcus neoformans [9], and Cryptococcus gattii [10]. Aspergillus fumigatus, the causative agent of invasive pulmonary disease in immunocompromised patients, represents an exception to this pattern. In this organism, disruption of the two trehalose 6-phosphate synthase genes, tspA and tspB, surprisingly leads to higher resistance to phagocytosis and hypervirulence in mice, despite the complete absence of trehalose accumulation and severely reduced fitness of these mutants [11]. Conversely, deletion of the trehalose 6-phosphate phosphatase OrlA (Figure 1) results in avirulence, despite the absence of growth defects in vitro [12].

Trehalose Is Associated with Several Biological Processes: Sporulation, Germination, Metabolism, and Morphogenesis

Trehalose and trehalose biosynthetic enzymes have emerged as essential players in virulence-associated phenotypes. Interestingly, the hierarchy of trehalose biosynthetic pathways appears to be species-specific. Contrary to the case of M. tuberculosis, described above, in the closely related M. smegmatis the OtsAB, TreYZ, and TreS pathways show a great level of redundancy [13], whereas osmotically regulated trehalose synthesis in Corynebacterium glutamicum is predominantly mediated by the TreYZ pathway (Table 1) [14,15]. Independently from these differences in metabolic routes, however, impairment in trehalose production results in severe growth defects in all mentioned species. In Pseudomonas syringae, trehalose accumulation is abolished in mutants of either producing pathways, with consequences on growth in hyperosmotic but not in stress-free conditions.

Impairment of trehalose homeostasis results in increased susceptibility to oxidative stress in Aspergillus nidulans [16], defects in melanin synthesis, capsule production, mating, and cell wall integrity in Cryptococcus gattii but not in C. neoformans [9,10], and in poor sporulation in S. nodorum and M. oryzae [7,8]. In the latter species, Tps1 governs the process of carbon catabolite repression, via glucose 6-phosphate sensing, favouring the utilization of glucose over other carbon sources [17]. Multiple functions for the trehalose 6-phosphate synthase Tps1 were uncovered in M. oryzae. Besides trehalose biosynthesis, Tps1 regulates nitrogen source utilization, hence contributing to adaptation of the pathogen to the plant host [18]. Recently, a similar process has been identified in...
P. aeruginosa, in which trehalose itself was shown to promote the acquisition of nitrogen-containing nutrients upon infection of plants [5]. M. oryzae Tps1 also controls transcriptional effectors linked to virulence factors via NADPH-binding during appressorium-mediated rice infection [19].

In A. fumigatus, disruption of the two trehalose 6-phosphate synthase genes, treA and treB, leads to the complete absence of trehalose accumulation, and to defects in spore germination, growth at high temperature, and susceptibility to oxidative stress [11]. Deletion of the trehalose 6-phosphate phosphatase gene OrlA in this organism results in unaltered levels of trehalose and increased levels of trehalose 6-phosphate. Two putative trehalose phosphorylases (TreP) (Figure 1) were found to be encoded by the genome of A. fumigatus [12]. Functionality of the TreP pathway in this organism might explain the unexpected accumulation of trehalose and trehalose 6-phosphate in orlA mutants. However, this hypothesis is awaiting experimental validation.

In C. albicans, absence of a functional trehalose synthesis machinery causes defects in the yeast to hyphal transition [6], whereas high levels of trehalose correlate with deficient Hsp90-dependent cell elongation in response to elevated temperature [20]. Trehalose accumulation is induced by addition of amphotericin B in C. albicans [21]. This ergosterol-targeting antifungal promotes trehalose synthesis activity and deactivation of the neutral trehalase; however, it is not known whether high trehalose can reduce the fungal susceptibility to amphotericin B.

**Fluctuations and Turnover of Trehalose Content during Infection Processes: It’s All about Timing**

Trehalose is essential at certain stages of development or in certain environmental conditions, and its abundance varies from undetectable levels to up to 10% of dry weight in response to stress, in late phases of the life cycle, or during the infection process. Increased trehalose contents were suggested to indicate adaptation of microorganisms to adverse or stressful conditions. Recycling of released trehalose is essential for virulence of M. tuberculosis [22]. The trehalose moiety of the glycolipid trehalose monomycolate is released extracellularly, and its re-uptake, mediated via an ABC transporter, is essential to virulence. In C. albicans, mutants with a defect in trehalose biosynthesis are still virulent. Interestingly, however, failure to import the compatible solute glycine betaine in ΔopuA-D mutants leads to a strong increase in trehalose biosynthesis under stress conditions, ultimately resulting in increased tolerance to stress conditions mimicking the host innate immunity. The trehalose-dependent higher proficiency of ΔopuA-D mutants to colonize host tissues, as compared to the wild type, indicate that the OpuA-D transporter represses trehalose biosynthesis, therefore limiting virulence in favour of chronic infections [23]. Thus, while the absence of trehalose has no known effect in vitro, high levels of the disaccharide may promote more aggressive infections. In P. aeruginosa, trehalose, like other metabolites such as amino acids and acetate, is differentially abundant during the evolution of lung infections in patients affected by cystic fibrosis [24]. In Klebsiella pneumoniae, expression of the treB and treC genes, involved in trehalose uptake and hydrolysis, respectively (Figure 1), is induced during early stages of biofilm formation, and the absence of either gene impairs biofilm development [25]. Proper trehalose utilization and catabolism into glucose and glucose 6-phosphate is required for capsule formation, known to play a role in biofilm formation in vitro as well as in a murine model of gastrointestinal tract colonization.

In fungi, while synthesis of trehalose is required for proficient initial plant infection by M. oryzae, trehalose mobilization plays a role in the colonization step [7]. Deletion of the neutral trehalase Nth1 in C. gattii has no effect on virulence [10], while in C. albicans, deletion of the cell wall-associated acid trehalase, but not of the neutral trehalase, has a negative impact on morphogenesis and virulence in mice [26]. Trehalose mobilization is required for efficient growth resumption in favourable conditions, and this may also be associated with the use of trehalose as a carbon source. Trehalose levels also vary in C. albicans biofilms, where its content increases dramatically in the first six hours of biofilm formation, followed by a decline in mature biofilms [27]. The exact role of these fluctuations remains elusive, however it is tempting to speculate on an antioxidative function of trehalose in the early stages of biofilm formation, and a role as energy supply in mature structures. Alternatively, it is also possible that in the initial phases of biofilm formation, high trehalose levels may prevent filamenta-

**Table 1. Divergence in the relevance of the three main trehalose biosynthetic pathways in the viability and pathogenicity of prokaryotes.**

| ORGANISM         | PATHWAY | OtsAB/TPS-TPP | TreY/Z | TreS |
|------------------|---------|---------------|--------|------|
| M. tuberculosis  | Δotb2   | Dominant OtsB2: essential OtsB1: pseudogene | No role in cell viability in vitro and in vivo | Role in prolonged infection |
| M. bovis         | Δotb2   | OtsB2 and OtsA are essential enzymes |         |      |
| M. smegmatis     | Δotb1   | Redundancy between the three pathways: single deletions have no apparent phenotypes, while triple deletions result in growth inhibition at high temperatures |         |      |
| M. leprae        | ΔtreA   | Unique intact pathway | Dominant pathway in osmotic conditions | Contributes to trehalose degradation |
| Corynebacterium glutanicum | ΔtreA   | Contributes to glycolipids synthesis together with the TreY2 pathway |         |      |
| E. coli          | opuA    | Sole pathway | none | none |
| Salmonella enterica | opuA    | Sole pathway involved in environmental survival but not in virulence | none | none |
| Pseudomonas syringae | opuA    | Both pathways are required for trehalose synthesis and depend on each other (the TreS-mediated trehalose synthesis may depend on maltose generated by the TreY/TreZ pathway) | |  |

doi:10.1371/journal.ppat.1003447.t001
tion [20] so yeast cells can colonize the whole substrate, and that at later stages, lower trehalose levels allow filament formation. The decline in trehalose at later stages may also indicate a shift in metabolic flux as more UDP-glucose is likely required for \(\beta\)-glucan synthesis during matrix formation, resulting in less substrate for trehalose biosynthesis.

Trehalose and Lipids: A Unique Collaboration

A wealth of information describes the unique relationship between trehalose and mycolic acids in Mycobacteria and the phylogenetically related Corynebacteria [28,29]. Trehalose and mycolic acid form trehalose 6,6-dimycolate (TDM), or cord factor, which is the most abundant glycolipid in Mycobacteria. Involvement of TDM in several aspects of pathogenicity has been demonstrated, including protection against phagocytes’ killing, evasion from the immune response, and reduction of antibiotic effectiveness. Tissue damage and necrosis caused by its association with host lipids were also reported. Recent findings point toward the recognition of TDM by the host innate immune cells via the Mincle C-type lectin receptor, which also recognizes other pathogens such as \(C.\ albicans\), \(M.\ pellucida\), and \(F.\ pedrosoi\).
in a TDM-independent manner [30]. Yet, the ligand activity of TDM requires both the sugar and lipid moieties, as both components separately do not activate Mincle-expressing cells [30]. Bacterial cell wall–associated lipids, and in particular TDM, are also important for the infection process in the Gram-positive bacterium Noasida brasiliensis. Absence of TDM and other hydrophobic compounds abolishes the infection without affecting the pathogen’s viability [31].

Besides TDM, trehalose is the precursor of several metabolites and cell-wall glycolipids, the so-called “trehalome” of Mycobacteria. Detection and visualization of the cell surface trehalome and trafficking of the mycobacterial trehalome during the infection process is an important aspect of research in the field.

References

1. Iturriaga G, Suarez R, Novoa-Franco B (2009) Trehalose metabolism: from osmoprotection to signalling. Int J Mol Sci 10: 3793–3810.
2. Avonce N, Mendoza-Vargas A, Moret E, Iturriaga G (2006) Insights on the evaluation of trehalose biosynthesis. BMC Evol Biol 6: 109.
3. Murphy BN, Stewart GR, Mischenko VV, Apt AS, Harris R, et al. (2005) The OsnAB pathway is essential for trehalose biosynthesis in Mycobacterium tuberculosis. J Biol Chem 280: 14524–14529.
4. Freeman EC, Chen C, Beutre GA (2010) Identification of the trehalose biosynthetic loci of Pseudomonas syringae and their contribution to fitness in the phyllosphere. Environ Microbiol 12: 1486–1497.
5. Djonovic S, Urbach JM, Drenkard E, Bush J, Feinbaum R, et al. (2013) Trehalose biosynthesis promotes Pseudomonas aeruginosa pathogenicity in plants. PLoS Pathog 9: e1003217. doi:10.1371/journal.ppat.1003217.
6. Zaragoza O, Blazquez MA, Gancedo C (1998) Disruption of the Candida albicans TPS1 gene encoding trehalose-6-phosphate synthase impairs formation of hyphal and decreases infectivity. J Bacteriol 180: 3809–3815.
7. Foster AJ, Jenkinson JM, Talbot NJ (2003) Trehalose synthesis and metabolism are required at different stages of plant infection by Magnaporthe grisea. EMBO J 22: 2255–2265.
8. Lorenz RG, Lord M, Ryback K, Trengove RD, Oliver RP, et al. (2009) Trehalose biosynthesis is involved in sporulation of Stagonospora nodorum. Fungal Genet Biol 46: 381–389.
9. Pietzold EW, Himmedrich U, Mynolais E, Rude T, Teftelien D, et al. (2006) Characterization and regulation of the trehalose synthesis pathway and its importance in the pathogenicity of Cryptococcus neoformans. Infec Immunity 74: 5877–5887.
10. Ngamskulrungroj P, Himmelreich U, Breger JA, Wilson C, Chayakulkeeree M, et al. (2010) Bacterial cell wall–associated lipids, and in particular TDM, components separately do not activate Mincle-expressing cells in a TDM-independent manner [30]. Yet, the ligand activity of TDM requires both the sugar and lipid moieties, as both components separately do not activate Mincle-expressing cells [30]. Bacterial cell wall–associated lipids, and in particular TDM, are also important for the infection process in the Gram-positive bacterium Noasida brasiliensis. Absence of TDM and other hydrophobic compounds abolishes the infection without affecting the pathogen’s viability [31].

What does the future hold for trehalose and its synthesis, and in particular what does it hold for the field of antimicrobial research? No doubt that the beat goes on! The discovery of a novel pathway from trehalose to glycogen and β-glucans in M. tuberculosis leads the way. The GlgE enzyme is validated as a drug target candidate by the combination of its essentiality in a synthetic lethal pathway, and by the absence of GlgE homologs in humans and commensal gut microbiota [33]. Despite or perhaps due to its versatility, trehalose stands out as a major player in the lifestyle of pathogens. The challenge, however, remains in the identification of molecular links between trehalose or its metabolism and pathways regulating the ability of pathogens to infect their host and/or to hide from the host.

Acknowledgments

The authors kindly thank Prof. Filip Roland (KU Leuven) for constructive feedback on the manuscript and Nico Vangoethem for help with the figure.