Baughman, W., and Hajjeh, R.A. (2003). Clin. Infect. Dis. *36*, 789–794.

Mylonakis, E., Ausubel, F.M., Perfect, J.R., Heitman, J., and Calderwood, S.B. (2002). Proc. Natl. Acad. Sci. USA *99*, 15675–15680.

Schmit, J.P., and Mueller, G.M. (2007). Biodivers. Conserv. *16*, 99–111.

Sorrell, T.C. (2001). Med. Mycol. *39*, 155–168.

Xue, C., Tada, Y., Dong, X., and Heitman, J. (2007). Cell Host Microbe *1*, this issue, 263–273.

Bacillus subtilis: A Shocking Message from a Probiotic

Paul Williams[1,*]
[1] Institute of Infection, Immunity and Inflammation, Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD, UK
*Correspondence: paul.williams@nottingham.ac.uk
DOI 10.1016/j.chom.2007.05.010

How probiotics such as *Bacillus subtilis* exert a protective effect has been much debated. In this issue of *Cell Host & Microbe*, Fujiya et al. reveal that a *B. subtilis* quorum-sensing signal molecule, the competence- and sporulation-stimulating factor (CSF), is internalized via the mammalian oligopeptide transporter OCTN2, where it induces the heat shock protein Hsp27, which protects intestinal cells against oxidant-mediated tissue damage and loss of barrier function.

Over the last decade or so, an important paradigm shift has been our changing perspective of bacteria as unicellular and noncooperative to socially interactive and capable of multicellular behavior. This has largely been driven by the discovery of quorum-sensing systems that facilitate bacterial cell-to-cell communication via the production and sensing of small diffusible signal molecules that facilitate the regulation of gene expression primarily when the population has reached a sufficient cell density (Williams et al., 2007).

Quorum-sensing signal molecules are chemically diverse, and in general, Gram-negative bacteria employ small organic molecules such as the *N*-acylhomoserine lactones (AHLs) and the 2-alkyl-4-quinolones (AHQs), while Gram-positives prefer peptides (Williams et al., 2007; Lazazzera, 2001). The latter may be modified to form cyclic structures such as the peptide lactones and thiolactones of *Enterococcus faecalis* and *Staphylococcus aureus,* the isoprenylated ComX peptide of *Bacillus subtilis*, or unmodified peptides such as CSF (competence- and sporulation-stimulating factor), also from *B. subtilis.* They can also be further subdivided according to whether they interact with receptors at the cell surface or are internalized. The intracellularly functioning quorum-sensing peptides are exemplified by the Phr peptides of *B. subtilis* and the mating pheromones of *E. faecalis*.

In *B. subtilis* the Phr peptides together with ComX regulate multiple processes including the initiation of genetic competence, sporulation, and antibiotic and exopolysaccharide synthesis, as well as the production of degradative enzymes (Lazazzera, 2001; Auchtung et al., 2006). Eight Phr pentapeptides have been identified, which are generated from pre-Phr peptides. These are exported and cleaved extracellularly and then transported back into the cell via an oligopeptide permease (Opp) where they interact with, and inhibit the activity of, the Rap proteins, a family of eleven different intracellular receptors. Opp belongs to the large family of ABC transporters, which hydrolyze ATP to drive Phr transport, and consequently *opp* mutants are unable to respond to Phr peptides. The intracellular Rap proteins act by antagonizing the functions of response regulator proteins such as ComA through interference with DNA binding or by promoting dephosphorylation. PhrC (also known as CSF), PhrF, and PhrK, together with ComX, synergistically stimulate the ComA-dependent gene expression at high cell population densities (Lazazzera, 2001; Auchtung et al., 2006).

As our knowledge and understanding of the molecular intricacies of quorum-sensing systems have developed, many questions have arisen with respect to the impact of quorum-sensing signal molecules produced by one bacterial species not only on other bacteria occupying the same ecological niche but also on higher organisms. Conversely, the latter may also manipulate quorum-sensing pathways by producing signal molecule mimics; by stimulating quorum-sensing pathways through the direct action of cytokines, hormones, or neurotransmitters on the bacterial cell; or alternatively, by blocking quorum sensing through the enzymatic inactivation of quorum-sensing signal molecules or through the deployment of compounds that block quorum-sensing signal transduction (Bauer and Mathesisus, 2004; Kendall and Sperandio, 2007;

Rasmussen and Givskov, 2006; Pritchard, 2006). Consequently, we now appreciate that quorum-sensing molecules display broad biological activities well beyond their role in bacterial cell-to-cell communication. For example, AHLs such as *N*-(3-oxododecanoyl)homoserine lactone (3-oxo-C12-HSL) produced by *Pseudomonas aeruginosa* exhibit antibacterial, pharmacological, and immune modulatory activities. Similarly, AHQs such as 2-heptyl-3-hydroxy-4-quinolone (PQS) chelate iron and are also potent immune modulators (Diggle et al., 2007; Pritchard, 2006). However, our appreciation of the biochemical basis of the biological response of eukaryotes to bacterial quorum-sensing signal molecules is still at an early stage, and virtually nothing is known about the impact of Gram-positive peptide signals on host cells. In this issue of *Cell Host & Microbe*, Fujiya et al. (2007) present an intriguing discovery with respect to the *B. subtilis* pentapeptide quorum-sensing signal molecule CSF (PhrC).

Probiotics are food supplements containing potentially beneficial bacteria such as lactobacilli and *B. subtilis* and have been promoted for the treatment or prevention of a number of diseases (Hong et al., 2005). The mechanism(s) by which they exert their protective effects in the gastrointestinal tract is not well understood but is likely to involve pathogen control or exclusion as well as protection of host tissues against inflammatory responses. When the human colonic epithelial cell line Caco2 was exposed to cell-free spent culture supernatants from several different probiotic bacteria including *B. subtilis*, Fujiya et al. (2007) observed that heat-shock-inducible protein 27 (Hsp27) was induced by the Gram-positive but not Gram-negative bacteria examined. Hsps confer protection against a wide variety of stresses and, when overexpressed, can protect intestinal epithelial cells from oxidative injury and hence contribute to the maintenance of intestinal homoeostasis (Tao et al., 2006). The extracellular *B. subtilis* factor involved was initially characterized as a 3 kDa, heat-stable pepsin-sensitive molecule, suggesting to the authors that it might be CSF (PhrC). Synthetic CSF (ERGMT) induced Hsp27 and also activated the Akt and p38 MAP kinase pathways, which are also known to be important in cytoprotection, since the former promotes Hsp25 (the murine equivalent of Hsp27) expression and the latter blocks apoptosis. This activity of CSF is within the physiological concentration range required for quorum-sensing activity in *B. subtilis* (10–100 nM), an important consideration given that some of observed activities of quorum-sensing molecules such as 3-oxo-C12-HSL on mammalian cells are only apparent at high, nonphysiological concentrations (Pritchard, 2006). Whether the other *B. subtilis* Phr peptides that are closely related to CSF are also active was not investigated, although the scrambled peptide EMTRG was inactive. However, the ease with which these pentapeptides can be synthesized should make a thorough structure activity relationship very straightforward to obtain.

The ability of CSF to induce Hsp27 raises questions with respect to the signal transduction pathway involved, whether the CSF is sensed intracellularly or extracellularly at the intestinal cell surface, and whether the response to CSF is sufficient to induce protection against oxidant-mediated stress. As noted above, the Phr peptides act intracellularly in *B. subtilis* after internalization via an Opp (Lazazzera, 2001; Auchtung et al., 2006). Consequently, Fujiya et al. (2007) speculated that CSF might be transported by an apical membrane oligopeptide transporter such as OCTN2, which is well expressed in Caco2 cells. CSF was readily taken up by Caco2 cells but not in cells where OCTN2 expression was silenced with siRNA. OCTN2 transports carnitine, which was also shown to compete with CSF for OCTN2 transport. Furthermore, CSF and OCTN2-mediated CSF transport were both required to protect Caco2 cells against monochloroamine-induced injury, while the silencing of Hsp27 reversed this protective effect (Fujiya et al., 2007).

Confirmation of the relevance of these cell culture experiments to the intestinal tract ex vivo was obtained by repeating the experiments using ligated murine intestinal loops. While the nature of the intestinal cell target through which CSF induces Hsp expression remains to be elucidated, the data obtained indicated that CSF-dependent Hsp induction rather than Akt or p38 MAPK pathway activation is primarily responsible for protection against oxidant-induced stress.

The small peptide-mediated induction of cytoprotective Hsps and the MAPK signaling pathways by other probiotics, notably *Lactobacillus* GG (Tao et al., 2006), as well as the inhibition of carnitine uptake by culture supernatants from a variety of Gram-positive bacteria, certainly implies a major role for these quorum-sensing peptides in the development and activities of the gut flora. This is especially noteworthy given the growth- and virulence-factor-inhibitory activities of many of these molecules. Clearly the work of Fujiya et al. (2007) establishes an important platform from which novel prophylactic, therapeutic, and growth-promoting agents could emerge as alternatives to antibiotics.

REFERENCES

Auchtung, J.M., Lee, C.A., and Grossman, A.D. (2006). J. Bacteriol. *188*, 5273–5285.

Bauer, W.D., and Mathesisus, U. (2004). Curr. Opin. Plant Biol. *7*, 429–433.

Diggle, S.P., Diggle, S.P., Matthijs, S., Wright, V.J., Fletcher, M.P., Chhabra, S.R., Lamont, I.L., Kong, X., Hider, R.C., Cornelis, P., et al. (2007). Chem. Biol. *14*, 87–96.

Fujiya, M., Musch, M.W., Nakagawa, Y., Hu, S., Alverdy, J., Kohgo, Y., Schneewind, O., Jabri, B., and Chang, E.B. (2007). Cell Host & Microbe *1*, this issue, 299–308.

Hong, H.A., Duc, L.H., and Cutting, S.M. (2005). FEMS Microbiol. Rev. *29*, 813–835.

Kendall, M.M., and Sperandio, V. (2007). Curr. Opin. Gastroenterol. *23*, 10–15.

Lazazzera, B.A. (2001). Peptides *22*, 1509–1519.

Pritchard, D.I. (2006). Int. J. Med. Microbiol. *296*, 111–116.

Rasmussen, T.B., and Givskov, M. (2006). Microbiol. *152*, 895–904.

Tao, Y., Drabnik, K.A., Waypa, T.S., Musch, M.W., Alverdy, J.C., Schneewind, O., Chang, E.B., and Petrof, E. (2006). Am. J. Physiol. Cell Physiol. *290*, C1018–C1030.

Williams, P., Winzer, K., Chan, W., and Cámara, M. (2007). Philos. Trans. R. Soc. Lond. B Biol. Sci. Published online March 13, 2007. 10.1098/rstb.2007.2039.