SporeWeb: an interactive journey through the complete sporulation cycle of *Bacillus subtilis*

Robyn T. Eijlander¹,²*, Anne de Jong¹,², Antonina O. Krawczyk¹,², Siger Holsappel² and Oscar P. Kuipers²,*

¹Top Institute Food and Nutrition (TIFN), Nieuwe Kanaal 9A, 6709 PA Wageningen, The Netherlands and ²Department of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9747 AG Groningen, The Netherlands

Received July 26, 2013; Revised and Accepted October 4, 2013

**ABSTRACT**

Bacterial spores are a continuous problem for both food-based and health-related industries. Decades of scientific research dedicated towards understanding molecular and gene regulatory aspects of sporulation, spore germination and spore properties have resulted in a wealth of data and information. To facilitate obtaining a complete overview as well as new insights concerning this complex and tightly regulated process, we have developed a database-driven knowledge platform called SporeWeb (http://sporeweb.molgenrug.nl) that focuses on gene regulatory networks during sporulation in the Gram-positive bacterium *Bacillus subtilis*. Dynamic features allow the user to navigate through all stages of sporulation with review-like descriptions, schematic overviews on transcriptional regulation and detailed information on all regulators and the genes under their control. The Web site supports data acquisition on sporulation genes and their expression, regulon network interactions and direct links to other knowledge platforms or relevant literature. The information found on SporeWeb (including figures and tables) can and will be updated as new information becomes available in the literature. In this way, SporeWeb offers a novel, convenient and timely reference, an information source and a data acquisition tool that will aid in the general understanding of the dynamics of the complete sporulation cycle.

**INTRODUCTION**

During adverse environmental conditions, bacterial cells adopt developmental strategies, such as endospore formation, to ensure their survival. Bacterial spores are a continuous problem in food- and health-related industries because of their persistence after treatments and their ability to revert to vegetative cells through the process of germination (1,2). For instance, the disease of anthrax can persevere through the ingestion of spores that are able to survive the gastrointestinal tract and germinate to vegetative cells that produce lethal toxins (3). On the other hand, use of bacterial spores in the form of bioinsecticides (4), antigen delivery systems and vaccines (5) or probiotics (6,7) are upcoming fields that offer attractive applications. Therefore, a better understanding of the sporulation and germination processes, the level of heterogeneity therein, all genes and proteins involved, as well as influential effects of environmental factors have formed important fields of study for the past decades (8) and have provided a wealth of knowledge (9–12).

Most of the work on sporulation has been performed using the Gram-positive non-pathogenic organism *Bacillus subtilis*. The obtained data are extremely valuable and are often used as a reference model in sporulation research concerning other (pathogenic) bacteria, including *Bacillus anthracis* (13,14), *Bacillus cereus* (15,16) and various *Clostridium* species and strains that are of both medical and industrial importance (including *Clostridium difficile*, *Clostridium perfringens* and *Clostridium botulinum*) (17–22). This results in even more data and information, with various theories and speculations on molecular mechanisms, conservation of ‘core’ sporulation genes and emergence of evolutionary foundations (23,24). Sporulation of *B. subtilis* is an extremely complex cellular developmental process (11,25,26).

New technological advances such as RNA sequencing, identification of small non-coding RNAs and increased understanding of processes through mathematical modelling allow us to answer questions beyond previous expectations (27–31), but simultaneously add to the complexity. Newly sequenced bacterial genomes of other...
spore-formers are increasingly available due to faster and cheaper methodologies and demand efficient analyses and readily available databases for comparison purposes (32). Therefore, a general overview on how spore formation is established (including which genes and regulatory pathways are involved) is very valuable to the field, but due to the complexity and dynamics increasingly difficult to obtain.

In this work, we describe a novel knowledge and data acquisition platform called SporeWeb (http://sporeweb.molgenrug.nl), which focuses on all developmental stages of sporulation of \textit{B. subtilis} from a gene regulatory point of view. Through an interactive web interface querying the SporeWeb database (details available in Supplementary Material), it offers both a textual description and a graphical representation of the sequence of events throughout sporulation (Figure 1). Importantly, it easily links to more catalogued information present on other knowledge Web sites, such as SubtiWiki (http://subtiwiki.uni-goettingen.de/) (33). This allows the reader to grasp what happens inside the cell on the regulatory level, with additional detailed information on key regulatory proteins involved. The database-driven SporeWeb Web site is dynamic and will be updated and extended when novel scientific data become available in the future. We believe that SporeWeb will be a continuous valuable asset to the research field of bacterial sporulation and will aid in our overall understanding of this complex developmental process.

An interactive journey through all stages of the bacterial sporulation process

Commitment to sporulation is characterized by asymmetric cell division and expression of dedicated gene sets (34). This expression is tightly regulated in various sequential developmental stages and governed by complex biochemical communication between the two compartments of the cell (11). SporeWeb offers an interactive review of this complete process, which is the result of an extensive literature study. The homepage serves as a starting point for any sporulation stage of interest, which can be accessed by clicking on the homepage image or by selecting the ‘State’ in the menu bar. Subsequent pages offer both detailed descriptions and schematic representations of development. The figures are interactive and dynamic: they contain clickable items of interest and ensure updated information as genes are added to or edited in the database. Legends to the figures are described in the vertical grey bar on the right of the web page, whereas a review-like description of the sporulation state is shown below the figure, with direct links to relevant literature references.

Sporulation-specific regulators and their regulons are described on individual pages, which can be accessed by
clicking on the item in the schematic figures or via the menu bar. An example of such a page is shown in Figure 2. Genes under transcriptional control of a specific regulator have been indicated in blue or red boxes for transcriptional activation or repression, respectively. Additionally, there is a direct link to the SubtiWiki list of regulon members. Lists and descriptions of all genes within the regulon can be downloaded in the form of updatable Excel sheets by clicking on the coloured boxes or via the Excel icon in the top right corner (Figure 2).

Graphic representations of regulon interactions define subgroups of co-regulated genes

As sporulation progresses, sporulation-specific sigma factors are expressed and activated in a spatial and temporal manner (11,26). Together with secondary regulator proteins, they control the timing, sequence and level of gene expression that are necessary for formation, maturation and release of the endospore. Various transcriptomic studies in *B. subtilis* have led to the identification of genes controlled by these sigma factors and other regulators to map the sporulation gene regulatory networks (35–42). In SporeWeb, we have visualized these networks using Cytoscape-generated layouts for every sporulation-specific stage (details available in Supplementary Material) (43). These layouts can be accessed via the ‘Cytoscape’ option in the menu bar, or the Cytoscape icon on the top of every State page.

An example of such a layout is shown in Figure 3A. This representation immediately shows which genes are under single, dual or even triple or quadruple control and which genes are co-regulated during a specific sporulation stage. There is a zoom-in function that allows the user to identify genes or regulators of interest. The name and direct links to the gene SubtiWiki and MicroScope MaGe pages (http://www.genoscope.cns.fr/agc/microscope/home/) are provided when the node is clicked. Furthermore, recently published spore-specific gene classification of the minimal sporulation gene set by Galperin *et al.* (24) has been integrated to immediately appreciate similarities and differences in regulation of functional classes of genes. Additionally, a visualization tool called ‘User Subset’ has been implemented in the Cytoscape
Figure 3. Cytoscape-generated layouts on gene regulatory networks. (A) Active regulators during completion of engulfment are indicated by green squares (proteins) or blue hexagons (sigma factors). Positive or negative effects on the transcription of genes (coloured circles) are indicated by connecting blue and red lines, respectively. Important sporulation genes indicated as being part of the minimal sporulation gene set have been colour-coded according to their functional category as determined by Galperin and coworkers (24). Genes unassigned to these functional categories are indicated as yellow circles. These layouts are available on SporeWeb for five different stages during spore formation. (B) A personal Cytoscape layout on specific genes of interest can be generated using the ‘User Subset’ option in the ‘Cytoscape’ menu (indicated by a black arrow). Genes (separated by a comma)
Figure 4. Heatmap representation of gene expression values during the engulfment state of sporulation. Tables like these are available on SporeWeb for five different stages in spore formation. Expression value data were derived from Nicolas et al. (45). Activation of gene expression is indicated as positive values in blue boxes, whereas downregulation is indicated as negative values in red boxes. Genes are categorized in classes listed A–L according to their documented regulation. Expression values are shown throughout the complete sporulation process ($t=0$ – $t=8$) and for three time points taken during spore germination (ger).

Figure 3. Continued

only) should be typed in the white bar and will be organized in a graphical network via the ‘Add Cytoscape’ option. Coloured nodes (shapes) and edges (lines) represent genes and connections as described for Figure 3A. Three-letter abbreviations at the edges indicate during which stage in sporulation the particular regulation is relevant. For Spo0A regulation, thicker edges represent high-threshold genes, whereas thin edges represent low-threshold genes (44).
menu bar that will allow the users to generate their own Cytoscape interaction figure of specific sporulation genes of interest (Figure 3B).

**Gene expression values during sporulation are visualized in informative heatmaps**

A large group of important sporulation genes has already been identified and characterized, although many remain whose function and/or regulation is still unknown. To further visualize timing and co-regulation of gene expression during sporulation, we have displayed a recent sporulation-specific transcriptional dataset from Nicolas et al. (45) in colour-coded heatmaps. These heatmaps can be accessed via the ‘Heatmap’ option on the menu bar, or via the heatmap icon at the top of every State page. Genes are categorized in classes based on their previously documented regulation. Their expression values during the complete course of sporulation as well as during three time points of germination are shown in colour-coded boxes (Figure 4). In this way, differences and similarities in expression between co-regulated genes are visible and can provide clues about possible function and/or regulation of uncharacterized genes. The heatmaps can be downloaded via the Excel icon on the top right of the page.

**Concluding remarks and perspectives**

Knowledge on bacterial sporulation is rapidly growing, partly due to novel technological developments. This progress also reveals additional levels of complexity and makes it increasingly difficult to obtain a general understanding, especially for non-specialists in the field. Furthermore, rapid advances in DNA and RNA sequencing technologies have enabled faster and cheaper access to genomic- and transcriptional data of a large number of bacterial species. This leads to an expansion of our knowledge from laboratory-adapted model bacteria, such as *B. subtilis*, to more industrially or medically relevant species and strains. The data reveal high levels of conservation of certain genes or regulatory modules on the one hand and highlight important differences in gene presence/absence and regulatory events on the other, which have significant implications for the overall process of spore formation in specific groups of bacteria (23,24).

The wealth of information that has been generated over decades by research on bacterial model organisms is extremely useful and usable as reference material for those bacterial species for which genetic material sporulation, an accessible starting point for further investigation research.

**SUPPLEMENTARY DATA**

Supplementary Data are available at NAR Online.

**ACKNOWLEDGEMENTS**

The authors are grateful for the time and dedication of Dr Adam Driks in thorough previewing of SporeWeb and they also thank many others in the microbiological and bacterial sporulation community who have come forward with useful suggestions and/or contributions.

**FUNDING**

TI Food and Nutrition, Wageningen, The Netherlands. Funding for open access charge: TI Food and Nutrition.

**Conflict of interest statement.** None declared.

**REFERENCES**

1. Eijlander, R.T., AbeC.T., and Kuipers, O.P. (2011) Bacterial spores in food: how phenotypic variability complicates prediction of spore properties and bacterial behavior. *Curr. Opin. Biotechnol.*, 22, 180–186.
2. Augustin, J.C. (2011) Challenges in risk assessment and predictive microbiology of foodborne spore-forming bacteria. *Food Microbiol.*, 28, 209–213.
3. Mock, M. and Fouet, A. (2001) Anthrax. *Annu. Rev. Microbiol.*, 55, 647–671.
4. Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R., and Dean, D.H. (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.*, 62, 775–806.
5. Amuguni, H. and Tzipori, S. (2012) *Bacillus subtilis*: a temperature resistant and needle free delivery system of immunogens. *Hum. Vaccin. Immunother.*, 8, 979–986.
6. Bader, J., Albin, A., and Stahl, U. (2012) Spore-forming bacteria and their utilisation as probiotics. *Benef. Microbes.*, 3, 67–75.
7. Permpoonpattana, P., Hong, H.A., Khaneja, R., and Cutting, S.M. (2012) Evaluation of *Bacillus subtilis* strains as probiotics and their potential as a food ingredient. *Benef. Microbes.*, 3, 127–135.
8. Gould, G.W. (2006) History of science–spores. *J. Appl. Microbiol.*, 101, 507–513.
9. Higgins, D. and Dworkin, J. (2012) Recent progress in *Bacillus subtilis* sporulation. *FEMS Microbiol. Rev.*, 36, 131–148.
10. Errington, J. (2010) From spores to antibiotics via the cell cycle. *Microbiology*, 156, 1–13.
11. Hilbert, D.W. and Piggot, P.J. (2004) Compartmentalization of gene expression during *Bacillus subtilis* spore formation. *Microbiol. Mol. Biol. Rev.*, 68, 234–262.
12. Moir, A. (2006) How do spores germinate? *J. Appl. Microbiol.*, 101, 526–530.
13. Liu, H., Bergman, N.H., Thomasson, B., Shallom, S., Hazen, A., Crossno, J., Rasko, D.A., Ravel, J., Read, T.D., Peterson, S.N. et al. (2004) Formation and composition of the *Bacillus anthracis* endospore. *J. Bacteriol.*, 186, 164–178.
14. Fisher, N. and Hanna, P. (2005) Characterization of Bacillus anthracis germinant receptors in vitro. J. Bacteriol., 187, 8055–8062.
15. van der Voort, M., Garcia, D., Moezelaar, R., and Abeel, T. (2010) Germinant receptor diversity and germination responses of four strains of the Bacillus cereus group. Int. J. Food Microbiol., 139, 108–115.
16. Vries de Y.P., Hornstra, L.M., de Vos, W.M., and Abeel, T. (2004) Growth and sporulation of Bacillus cereus ATCC 14579 under defined conditions: temporal expression of genes for key sigma factors. Appl. Environ. Microbiol., 70, 2514–2519.
17. Xiao, Y., Francke, C., Abeel, T. and Wells-Bennik, M.H. (2011) Clostridial spore germination versus bacilli: genome mining and current insights. Food Microbiol., 28, 266–274.
18. Paredes-Sabja, D., Setlow, P. and Sarker, M.R. (2011) Germination of spores of Bacillus and Clostridiales species: mechanisms and proteins involved. Trends Microbiol., 19, 85–94.
19. Steiner, E., Dago, A.E., Young, D.I., Heap, J.T., Minton, N.P., Hoch, J.A. and Young, M. (2011) Multiple orphan histidine kinases interact directly with Spo0A to control the initiation of endospore formation in Clostridium acetobutylicum. Mol. Microbiol., 80, 641–654.
20. Burns, D.A. and Minton, N.P. (2011) Sporulation studies in Clostridium difficile. J. Microbiol. Methods, 87, 133–138.
21. Rosenbusch, K.E., Bakker, D., Kuiper, E.J. and Smits, W.K. (2012) C. difficile 630Aerm Spo0A regulates sporulation, but does not contribute to toxin production, by direct high-affinity binding to target DNA. PLoS One, 7, e48608.
22. Labbé, R.G. and Dürr, P. (2005) Sporulation of clostridia. In: Dürr, P. (ed.), Handbook on Clostridia. CRC Press, Taylor & Francis Group, FL, USA, pp. 647–669.
23. de Hoon, M.J.L., Eichenberger, P. and Vitkup, D. (2010) Hierarchical evolution of the bacterial sporulation network.Curr. Biol., 20, R735–R745.
24. Galperin, M.Y., Mekhedov, S.L., Puigbo, P., Smirnov, S., Wolf, Y.J. and Rigden, D.J. (2012) Genomic determinants of sporulation in Bacilli and Rigden, D.J. (2012) Bacilli: Cytoscape plugins. Guide to Cytoscape plugins. CRC Press, Taylor & Francis Group, FL, USA, pp. 647–669.
25. Saito, R., Smoot, M.E., Ono, K., Ruscheinski, J., Wang, P.L., George, S., Aymerich, S., Pigeonneau, N., Bidnenko, E., Marchadier, E., Hoebeke, M., Aymerich, S. et al. (2012) Condition-dependent transcriptome reveals high-level regulatory architecture in Bacillus subtilis. Science, 335, 1103–1106.