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## 0.1 Description of regulatory network

In figure 1, we present a simplified gene regulatory network that describes the interactions between the master regulator Spo0A, and the biofilm regulatory proteins SinI, SinR, and SlrR.

Surfactin is known to indirectly activate the expression of genes that produce matrix, although the precise molecular process is not fully understood. It has been suggested that surfactin creates pores in the membrane, leading to the leakage of potassium ions from the cytoplasm to the outside of the cell. This causes the activation of the membrane sensor kinase KinC when potassium levels are low, which then phosphorylates the master gene regulator Spo0A[20]. However, some studies contradict this exact mechanism.[21] Another mechanism responsible for indirectly activating the matrix-producing genes is nutrient depletion, by which kinases (such as KinA) phosphorylate Spo0A, which can then activate the transcription of SinI.

When Spo0A is activated (phosphorylated), it triggers the transcription of the SinI protein. The SinI protein binds to the SinR protein to form a SinI-SinR complex, which effectively titrates SinR, reducing the number of freely available SinR proteins. SinR is a protein that suppresses the transcription of genes related to matrix production. By disabling SinR through the formation of the SinI-SinR complex, the cell can activate the genes responsible for matrix production.[18]

Furthermore, SinR also inhibits the expression of another protein known as SlrR. If SlrR is expressed, it can also suppress SinR by interacting with it to form a SlrR-SinR complex.[18]

Spo0A has a self-regulatory mechanism that allows it to activate or repress its own transcription depending on the concentration of its phosphorylated form. When Spo0A~P is absent, the gene *spo0A* is transcribed at a basal low-rate during vegetative growth; this transcription is mediated by the housekeeping sigma factor  $\sigma^A$  and the promoter  $P_V$ . At non-zero low concentrations of Spo0A~P, the transcription of *spo0A* is further activated with the help of the  $P_S$  promoter and the  $\sigma^H$  factor. At these low concentrations, Spo0A~P activates biofilm formation and the expression of toxins involved in cannibalism. If the concentration of Spo0A~P increases further, it will reach high levels, which will lead to the repression of the *spo0A* gene and *sinI* gene transcription, while activating the transcription of genes responsible for sporulation.

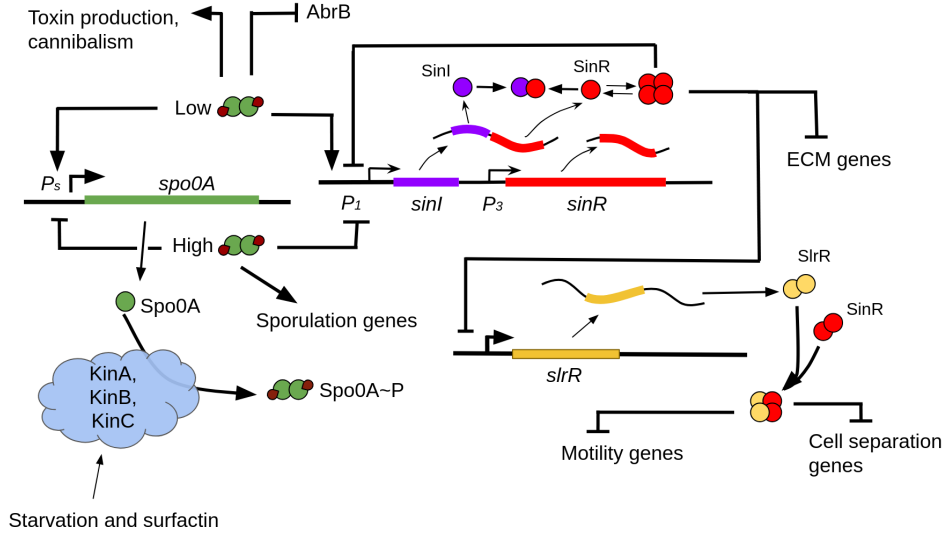


Figure 1: Gene regulatory network of genes involved in biofilm formation (*sinI*, *sinR*, *slrR*), sporulation (*spo0A*), and cannibalism.

## 0.2 Mathematical modelling

Several authors have formulated systems of differential equations to quantitatively model the biomolecular dynamics involved in the gene regulatory network that controls the expression of the extracellular matrix. The systems of equations describe the changes in the concentration of SinR, SinI, and SlrR proteins in the cytoplasm of individual *Bacillus subtilis* cells over time.[22][23][24][25][26][27]. Having considered the models proposed by these authors, we have decided to adopt a modified version of the model proposed by Dannenberg et al. [22] as the basis for our own model. The model is described by the following system of differential equations:

$$\frac{dR}{dt} = P_1 g(R) f(A) + P_3 - K_{on(RI)} RI - K_{on(RL)} RL - D_R R, \quad (1)$$

$$\frac{dI}{dt} = P_1 g(R) f(A) - K_{on(RI)} RI - D_I I, \quad (2)$$

$$\frac{dL}{dt} = P_L g(R) - K_{on(RL)} RL - D_L L, \quad (3)$$

$$\frac{dA}{dt} = P_V + P_S f(A) - D_A A, \quad (4)$$

where  $P_V$  and  $P_S$  represent the basal and activated production rates of Spo0A, respectively.  $P_3$ ,  $P_1$ , and  $P_L$  represent the production rates of SinR, SinI and SlrR due to the activation of their corresponding promoters.  $R$ ,  $I$ ,  $L$ , and  $A$  represent the protein concentrations of SinR, SinI, SlrR, and Spo0A~P within a single bacterial cell.  $K_{on(XY)}$  represents the rate constant for the formation of the  $(XY)$  complex, while  $D_Z$  encompasses both the dilution rate of component  $Z$  caused by cell growth and the degradation rate caused by protein breakdown. The phenomenological Haldane equation  $f(A)$  and the repression Hill function  $g(R)$  are defined as follows:

$$f(A) = \left( \frac{(A/K_A)^{n_A}}{1 + (A/K_A)^{n_A} + \frac{A^2}{K_A K_S}} \right)$$

$$g(R) = \left( \frac{1}{1 + (R/K_R)^{n_R}} \right)$$

where  $A$  represents the concentration of Spo0A~P in the cytoplasm.  $K_A$  and  $K_R$  represent the binding affinities of Spo0A and SinR, respectively, while  $n_A$  and  $n_R$  represent the cooperativities of the binding.  $K_S$  is a constant that accounts for the inhibition of Spo0A~P when it reaches high concentrations.

Equation (1) describes the dynamics of the SinR protein concentration in the cell. SinR is produced at a basal rate  $P_3$ , but its concentration is reduced by a combination of degradation and dilution at a rate  $D_R$  due to cell growth, or increased by the activation mediated by low levels of Spo0A~P. Additionally, the concentration of SinR is affected by its interaction with SinI and SlrR, which form complexes with SinR at rates  $K_{on(RI)}$  and  $K_{on(RL)}$ , respectively, leading to a reduction in free SinR molecules.

Equation (2) represents the changes in the concentration of SinI. SinI is produced according to its own maximum production rate  $P_1$ , which is modulated by the function  $g(R)$  that models SinR's repression, and the function  $f(A)$  which accounts for activation by low levels of Spo0A~P and repression by high levels of Spo0A~P. Just like SinR, the concentration of SinI is decreased both by degradation and dilution at a rate of  $D_I$ , and by its binding to SinR, forming the RI complex at a rate of  $K_{on(RI)}$ .

Equation (3) represents the dynamics of the SlrR protein concentration. The synthesis of SlrR is again dependant on a production rate ( $P_L$ ), modulated by the function  $g(R)$  representing the repressive effect of SinR on SlrR transcription. Similarly, it also considers SlrR's degradation and dilution through the rate  $D_L$  and its interaction with SinR, which sequesters SlrR into the RL complex at the rate  $K_{on(RL)}$ .

Lastly, (4) describes the rate of change of the concentration of Spo0A~P. Spo0A~P is produced at a basal rate  $P_V$  and at an activated rate  $P_S$  when the concentration of Spo0A~P is low. For simplicity, we assume that the phosphorylation of Spo0A is carried out at a constant rate, which allows us to ignore the concentration of the unphosphorylated form of Spo0A. However, this should be taken into account when considering the extracellular concentration gradients of nutrients and surfactin.

The Hill function and Haldane equation represent the regulatory effects of two proteins, Spo0A and SinR, in a phenomenological manner. The Haldane equation  $f(A)$  models the phenomenon in which Spo0A promotes gene transcription when its concentration is low, but inhibits the same transcription when its concentration is high. On the other hand, the repression Hill function  $g(R)$  describes how SinR inhibits the expression of genes that produce SlrR and SinI. As the concentration of SinR increases above a threshold ( $K_R$ ), the level of repression becomes significant. The Hill functions mathematically represent the biological processes of gene activation and repression in a sigmoidal, threshold-dependent manner.

The system of differential equations just presented is largely similar to the one proposed by Dannenberg et al. [22]. The main difference is that, unlike Dannenberg et al., this work will

consider the inhibitory effect of SinR on SinI transcription as well as the inhibitory effect of Spo0A~P at high concentrations.

# Bibliography

- [1] K. J. Niklas and S. A. Newman, “The many roads to and from multicellularity,” *Journal of Experimental Botany*, vol. 71, no. 11, R. Christine, Ed., pp. 3247–3253, Dec. 2019. DOI: 10.1093/jxb/erz547. [Online]. Available: <https://doi.org/10.1093/jxb/erz547>.
- [2] N. A. Lyons and R. Kolter, “On the evolution of bacterial multicellularity,” *Current Opinion in Microbiology*, vol. 24, pp. 21–28, Apr. 2015. DOI: 10.1016/j.mib.2014.12.007. [Online]. Available: <https://doi.org/10.1016/j.mib.2014.12.007>.
- [3] H. Vlamakis, C. Aguilar, R. Losick, and R. Kolter, “Control of cell fate by the formation of an architecturally complex bacterial community,” *Genes and Development*, vol. 22, no. 7, pp. 945–953, Apr. 2008. DOI: 10.1101/gad.1645008. [Online]. Available: <https://doi.org/10.1101/gad.1645008>.
- [4] S. Srinivasan, I. D. Vladescu, S. A. Koehler, X. Wang, M. Mani, and S. M. Rubinstein, “Matrix production and sporulation in bacillus subtilis biofilms localize to propagating wave fronts,” *Biophysical Journal*, vol. 114, no. 6, pp. 1490–1498, Mar. 2018. DOI: 10.1016/j.bpj.2018.02.002. [Online]. Available: <https://doi.org/10.1016/j.bpj.2018.02.002>.
- [5] X. Wang, F. Dong, J. Liu, Y. Tan, S. Hu, and H. Zhao, “The self-healing of bacillus subtilis biofilms,” *Archives of Microbiology*, vol. 203, no. 9, pp. 5635–5645, Aug. 2021, ISSN: 1432-072X. DOI: 10.1007/s00203-021-02542-w. [Online]. Available: <http://dx.doi.org/10.1007/s00203-021-02542-w>.
- [6] F. Dong, S. Liu, D. Zhang, J. Zhang, X. Wang, and H. Zhao, “Osmotic pressure induced by extracellular matrix drives bacillus subtilis biofilms’ self-healing,” *Computational Biology and Chemistry*, vol. 97, p. 107632, Apr. 2022, ISSN: 1476-9271. DOI: 10.1016/j.combiolchem.2022.107632. [Online]. Available: <http://dx.doi.org/10.1016/j.combiolchem.2022.107632>.
- [7] F. Dong, S. Liu, D. Zhang, J. Zhang, and X. Wang, “Matrix-producing cells formed ‘van gogh bundles’ facilitate bacillus subtilis biofilm self-healing,” *Environmental Microbiology Reports*, vol. 14, no. 5, pp. 822–827, Jun. 2022, ISSN: 1758-2229. DOI: 10.1111/1758-2229.13099. [Online]. Available: <http://dx.doi.org/10.1111/1758-2229.13099>.
- [8] D. Lopez, H. Vlamakis, R. Losick, and R. Kolter, “Paracrine signaling in a bacterium,” *Genes and Development*, vol. 23, no. 14, pp. 1631–1638, Jul. 2009. DOI: 10.1101/gad.1813709. [Online]. Available: <https://doi.org/10.1101/gad.1813709>.
- [9] A. Turing, “The chemical basis of morphogenesis,” *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, vol. 237, pp. 37–72, 641 1952. DOI: 10.1098/rstb.1952.0012.
- [10] A. N. Landge, B. M. Jordan, X. Diego, and P. Müller, “Pattern formation mechanisms of self-organizing reaction-diffusion systems,” *Developmental Biology*, vol. 460, no. 1, pp. 2–11, Apr. 2020. DOI: 10.1016/j.ydbio.2019.10.031. [Online]. Available: <https://doi.org/10.1016/j.ydbio.2019.10.031>.

- [11] K. Nagarajan, C. Ni, and T. Lu, "Agent-based modeling of microbial communities," *ACS Synthetic Biology*, vol. 11, no. 11, pp. 3564–3574, Oct. 2022. DOI: 10.1021/acssynbio.2c00411. [Online]. Available: <https://doi.org/10.1021/acssynbio.2c00411>.
- [12] L. Chai, H. Vlamakis, and R. Kolter, "Extracellular signal regulation of cell differentiation in biofilms," *MRS Bulletin*, vol. 36, no. 5, pp. 374–379, 2011. DOI: 10.1557/mrs.2011.68.
- [13] J. S. Webb, M. Givskov, and S. Kjelleberg, "Bacterial biofilms: Prokaryotic adventures in multicellularity," *Current Opinion in Microbiology*, vol. 6, no. 6, pp. 578–585, Dec. 2003. DOI: 10.1016/j.mib.2003.10.014. [Online]. Available: <https://doi.org/10.1016/j.mib.2003.10.014>.
- [14] N. Ido, A. Lybman, S. Hayet, *et al.*, "Bacillus subtilis biofilms characterized as hydrogels. insights on water uptake and water binding in biofilms," *Soft Matter*, vol. 16, no. 26, pp. 6180–6190, 2020. DOI: 10.1039/d0sm00581a. [Online]. Available: <https://doi.org/10.1039/d0sm00581a>.
- [15] T.-F. Mah, "Biofilm-specific antibiotic resistance," *Future Microbiology*, vol. 7, no. 9, pp. 1061–1072, Sep. 2012. DOI: 10.2217/fmb.12.76. [Online]. Available: <https://doi.org/10.2217/fmb.12.76>.
- [16] H. Vlamakis, Y. Chai, P. Beauregard, R. Losick, and R. Kolter, "Sticking together: Building a biofilm the bacillus subtilis way," *Nature Reviews Microbiology*, vol. 11, no. 3, pp. 157–168, Jan. 2013. DOI: 10.1038/nrmicro2960. [Online]. Available: <https://doi.org/10.1038/nrmicro2960>.
- [17] S. Arnaouteli, N. C. Bamford, N. R. Stanley-Wall, and Á. T. Kovács, "Bacillus subtilis biofilm formation and social interactions," *Nature Reviews Microbiology*, vol. 19, no. 9, pp. 600–614, Apr. 2021. DOI: 10.1038/s41579-021-00540-9. [Online]. Available: <https://doi.org/10.1038/s41579-021-00540-9>.
- [18] L. Chai, H. Vlamakis, and R. Kolter, "Extracellular signal regulation of cell differentiation in biofilms," *MRS Bulletin*, vol. 36, no. 5, pp. 374–379, May 2011. DOI: 10.1557/mrs.2011.68. [Online]. Available: <https://doi.org/10.1557/mrs.2011.68>.
- [19] F. B. Rahman, B. Sarkar, R. Moni, and M. S. Rahman, "Molecular genetics of surfactin and its effects on different sub-populations of bacillus subtilis," *Biotechnology Reports*, vol. 32, e00686, Dec. 2021. DOI: 10.1016/j.btre.2021.e00686. [Online]. Available: <https://doi.org/10.1016/j.btre.2021.e00686>.
- [20] D. Lopez, M. A. Fischbach, F. Chu, R. Losick, and R. Kolter, "Structurally diverse natural products that cause potassium leakage trigger multicellularity in bacillus subtilis," *Proceedings of the National Academy of Sciences*, vol. 106, no. 1, pp. 280–285, Jan. 2009. DOI: 10.1073/pnas.0810940106. [Online]. Available: <https://doi.org/10.1073/pnas.0810940106>.
- [21] S. N. Devi, M. Vishnoi, B. Kiehler, L. Haggett, and M. Fujita, "In vivo functional characterization of the transmembrane histidine kinase KinC in bacillus subtilis," *Microbiology*, vol. 161, no. 5, pp. 1092–1104, May 2015. DOI: 10.1099/mic.0.000054. [Online]. Available: <https://doi.org/10.1099/mic.0.000054>.
- [22] S. Dannenberg, J. Penning, A. Simm, and S. Klumpp, *The motility-matrix production switch in bacillus subtilis – a modeling perspective*, 2023. DOI: 10.48550/ARXIV.2308.12780. [Online]. Available: <https://arxiv.org/abs/2308.12780>.
- [23] C. A. Voigt, D. M. Wolf, and A. P. Arkin, "The bacillus subtilis sin operon," *Genetics*, vol. 169, no. 3, pp. 1187–1202, Mar. 2005, ISSN: 1943-2631. DOI: 10.1534/genetics.104.031955. [Online]. Available: <http://dx.doi.org/10.1534/genetics.104.031955>.
- [24] J. A. Newman, C. Rodrigues, and R. J. Lewis, "Molecular basis of the activity of sinR protein, the master regulator of biofilm formation in bacillus subtilis," *Journal of Bio-*

- logical Chemistry*, vol. 288, no. 15, pp. 10 766–10 778, Apr. 2013, ISSN: 0021-9258. DOI: 10.1074/jbc.m113.455592. [Online]. Available: <http://dx.doi.org/10.1074/jbc.m113.455592>.
- [25] Z. Chen, B. Zarazúa-Osorio, P. Srivastava, M. Fujita, and O. A. Igoshin, “The slowdown of growth rate controls the single-cell distribution of biofilm matrix production via an sini-sinr-slrr network,” *mSystems*, vol. 8, no. 2, Á. T. Kovács, Ed., Apr. 2023, ISSN: 2379-5077. DOI: 10.1128/msystems.00622-22. [Online]. Available: <http://dx.doi.org/10.1128/msystems.00622-22>.
- [26] T. Pedreira, C. Elfmann, and J. Stülke, “The current state of subtiwiki, the database for the model organism bacillus subtilis,” *Nucleic Acids Research*, vol. 50, no. D1, pp. D875–D882, Oct. 2021, ISSN: 1362-4962. DOI: 10.1093/nar/gkab943. [Online]. Available: <http://dx.doi.org/10.1093/nar/gkab943>.
- [27] J. S. Hallinan, G. Misirli, and A. Wipat, “Evolutionary computation for the design of a stochastic switch for synthetic genetic circuits,” in *2010 Annual International Conference of the IEEE Engineering in Medicine and Biology*, IEEE, Aug. 2010. DOI: 10.1109/iembs.2010.5626353. [Online]. Available: <http://dx.doi.org/10.1109/IEMBS.2010.5626353>.
- [28] Y. Alharbi, “Mathematical modelling of gene regulation network governing biofilm formation in bacillus subtilis,” en, 2022. DOI: 10.15132/20000260. [Online]. Available: <https://discovery.dundee.ac.uk/en/studentTheses/mathematical-modelling-of-gene-regulation-network-governing-biofi>.
- [29] E. Catto, *Box2d.ts*, Available at <https://github.com/flyover/box2d.ts.git>, commit 7b387c368244fddde9cff9cbd643df3993aa698d, 2019. [Online]. Available: <https://github.com/flyover/box2d.ts.git>.
- [30] T. Ursell, *Rpdata.caltech.edu*, <http://rpdata.caltech.edu/courses/aph162/2006/Protocols/diffusion.pdf>, [Accessed 07-01-2024], 2005.
- [31] G. Nervadof, *Solving 2D Heat Equation Numerically using Python — levelup.gitconnected.com*, <https://levelup.gitconnected.com/solving-2d-heat-equation-numerically-using-python-3334004aa01a>, [Accessed 07-01-2024], 2020.
- [32] *Uni-muenster.de*, [https://www.uni-muenster.de/imperia/md/content/physik\\_tp/lectures/ws2016-2017/num\\_methods\\_i/heat.pdf](https://www.uni-muenster.de/imperia/md/content/physik_tp/lectures/ws2016-2017/num_methods_i/heat.pdf), [Accessed 07-01-2024], 2016.
- [33] A. N. Bashkatov, E. A. Genina, Y. P. Sinichkin, V. I. Kochubey, N. A. Lakodina, and V. V. Tuchin, “Glucose and mannitol diffusion in human dura mater,” *Biophysical Journal*, vol. 85, no. 5, pp. 3310–3318, Nov. 2003, ISSN: 0006-3495. DOI: 10.1016/S0006-3495(03)74750-X. [Online]. Available: [http://dx.doi.org/10.1016/S0006-3495\(03\)74750-X](http://dx.doi.org/10.1016/S0006-3495(03)74750-X).
- [34] J. van Gestel, T. Bareia, B. Tenennbaum, *et al.*, “Short-range quorum sensing controls horizontal gene transfer at micron scale in bacterial communities,” *Nature Communications*, vol. 12, no. 1, Apr. 2021, ISSN: 2041-1723. DOI: 10.1038/s41467-021-22649-4. [Online]. Available: <http://dx.doi.org/10.1038/s41467-021-22649-4>.