Transcriptomic response of phytopathogen Dickeya dadantii to an increase of DNA supercoiling by novel antibiotic seconeolitsin

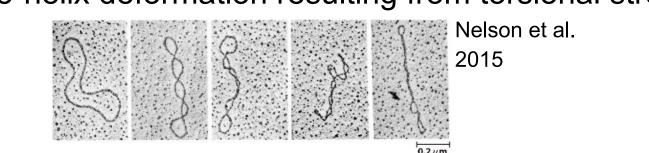
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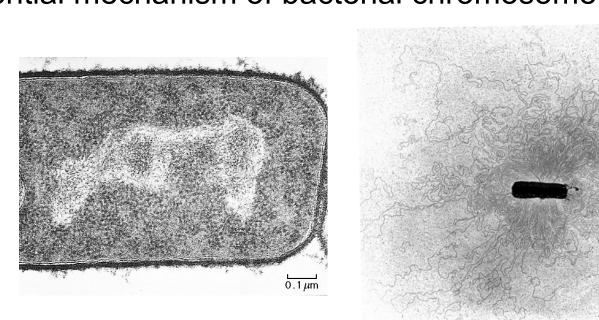


DNA supercoiling

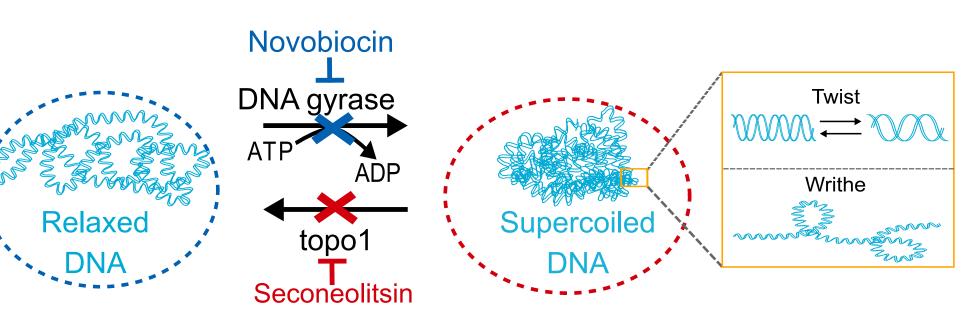
DNA double-helix deformation resulting from torsional stress



essential mechanism of bacterial chromosome compaction



regulated by topoisomerases: essential and conserved enzymes



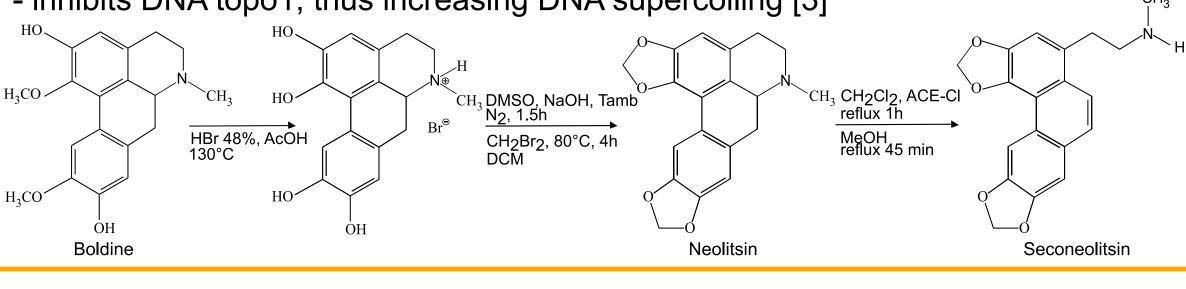
globally affects gene expression [1] but poorly studied mechanism

- → How does seconeolitsin affect transcription?
- → What are supercoiling -sensitive genes?



A novel antibiotic inhibiting topoisomerase I studied on *Dickeya dadantii*

- Seconeolitsin, a non-marketed antibiotic
- synthetized from boldine [2]
- previously studied on Streptococcus pneumoniae [3], never on Gram bacteria
- inhibits DNA topo1, thus increasing DNA supercoiling [3]



Novobiocin - inhibits DNA gyrase, thus inducing DNA relaxation

Dickeya dadantii, model organism

- Enterobacteriacea, Gram -
- causes the soft-rot disease in a wide range of plantspecies

Examples of *Dickeya* dadanti infected carrots and potato



High concentration: growth inhibition Seconeolitsin effect on *Dickeya dadantii* growth lag time increasing with concentration

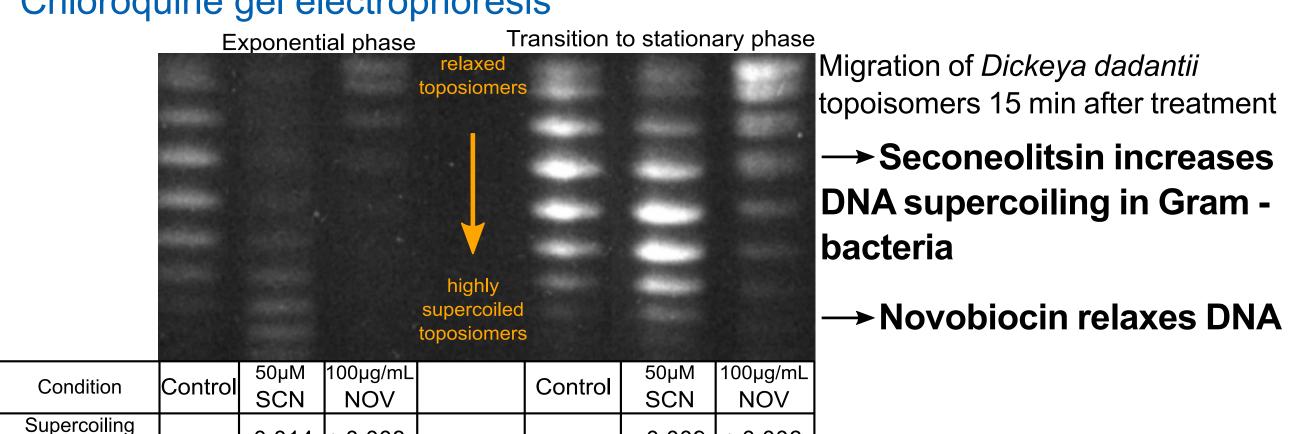
MIC ~ 100µM seconeolitsin

→ Chosen concentration to modify supercoiling with a sublethal dose : 50µM

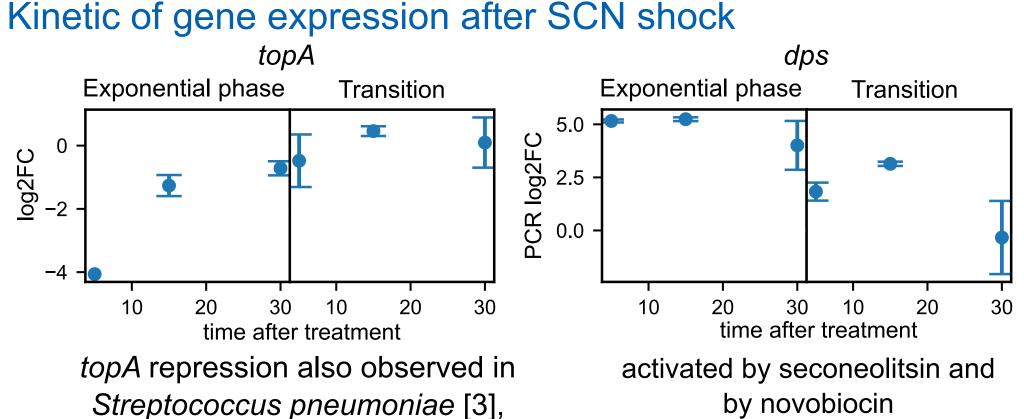
Supercoiling level modification

Chloroquine gel electrophoresis

-0.014 |>0.008



Transcriptomic response to seconeolistin shock



Exponential phase:

- large effect 5 min after shock,
- decreases but still present after 30 min

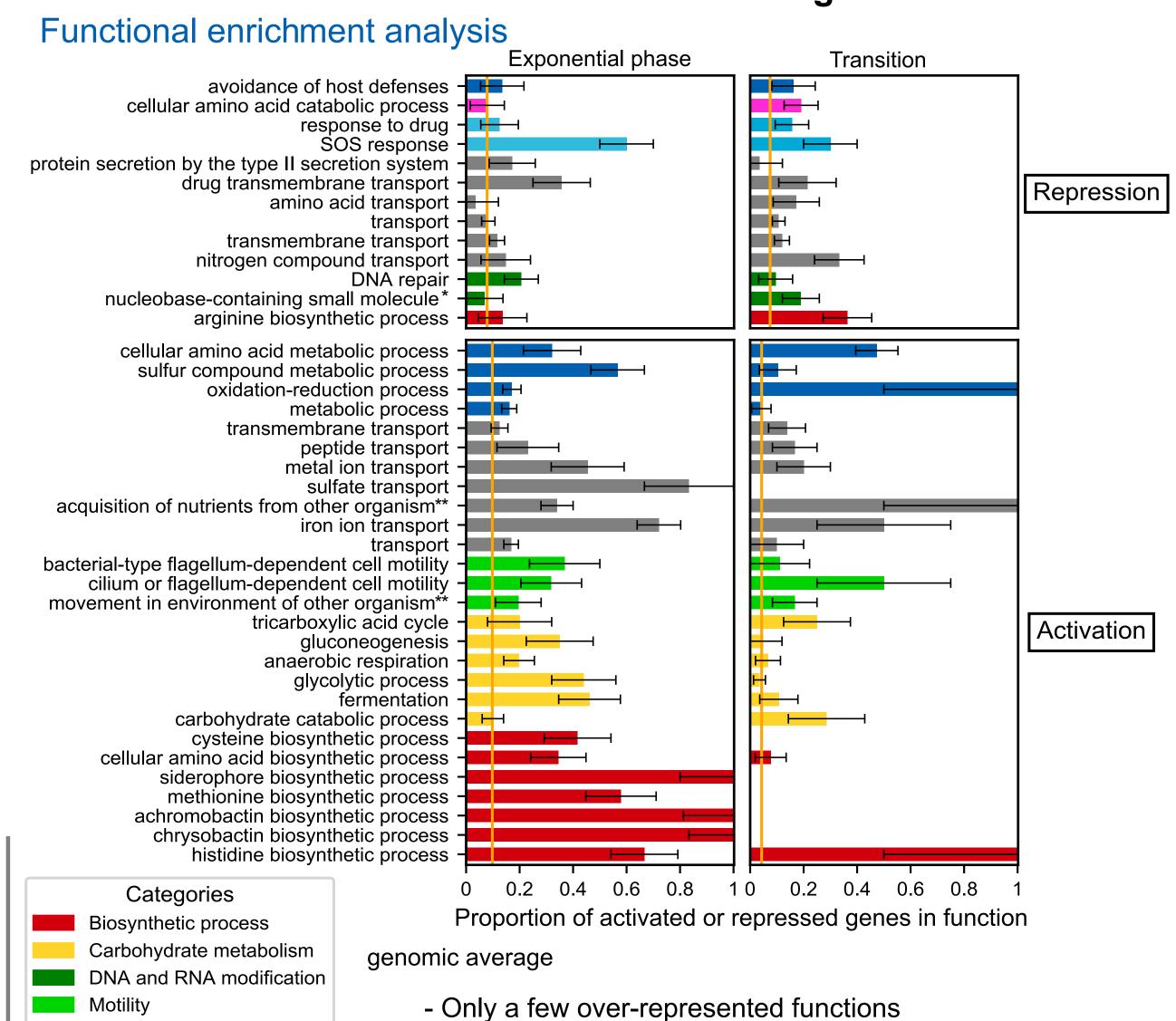
Transition to stationary phase:

- reduced effect in comparison to exponential phase

Seconeolitsin shock effect on biological functions

-0.009

>0.006



- Targeted genes functionally distant

Gene expression after seconeolitsin shock

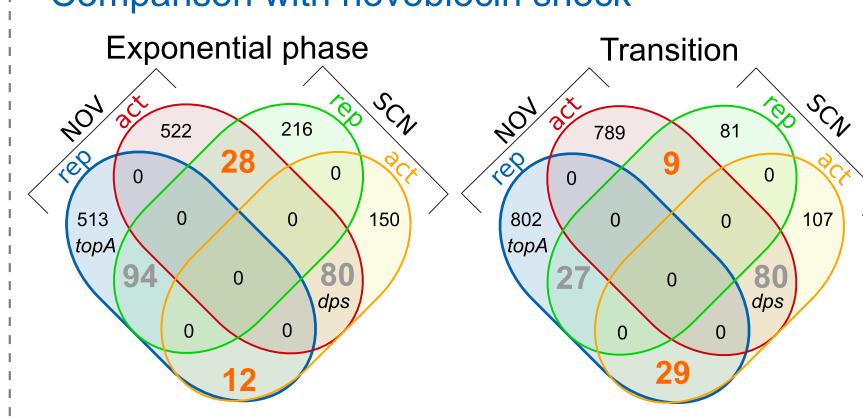
a b 400 ' 292 *** proportion of activated genes 0.6

More affected genes in expo, consistent with:

against supercoiling homeostasis

- the higher topo1 activity
- the stronger effect of seconeolitsin on supercoiling (chloroquine gel)

Comparison with novobiocin shock

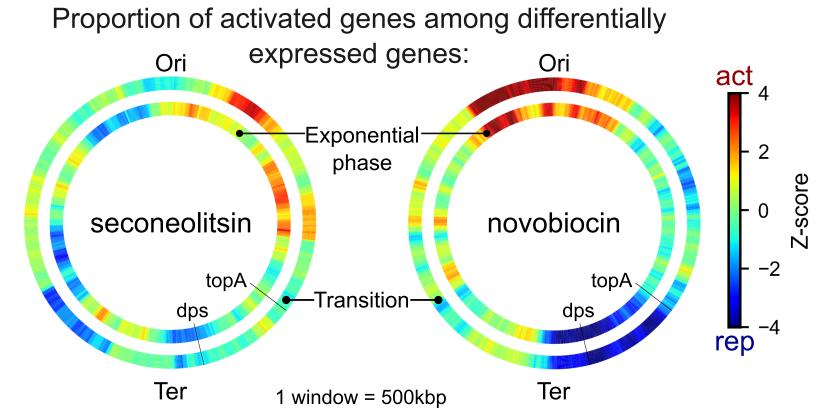


Supercoiling sensitive genes can:

→ Effective and sublethal treatment duration chosen for the study: 15 min

- respond to both antibiotics in same or opposite directions
- be only affected by relaxation or supercoiling increase
- → Transcriptomic response to DNA supercoiling variations strongly depends on conditions

Chromosomal view of the transcriptomic response

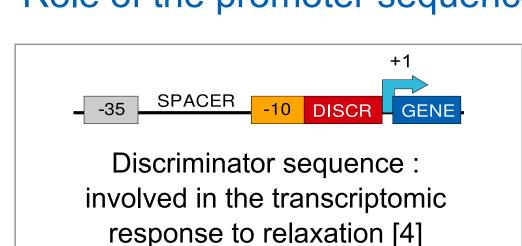


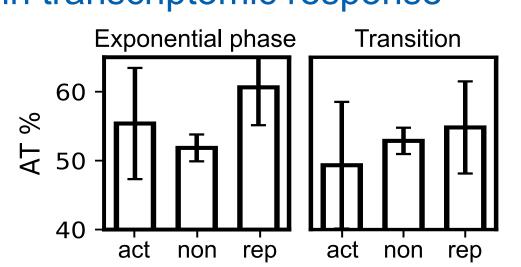
- → Genes affected by supercoiling increase are distributed along the chromosome
- → Complex and phase-dependent distribution

→ The transcriptomic response depends on the direction of supercoiling variations and on the conditions.

Selection mechanisms of differential expressed genes in response to seconeolitsin shock

Role of the promoter sequence in transcriptomic response

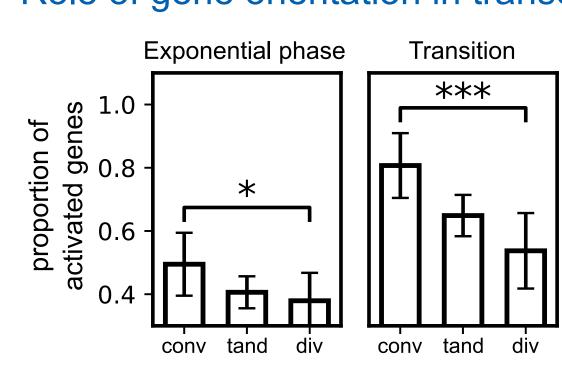




→ Seconeolitsin acts globally on gene expression

→ Discriminator AT-content not significantly involved in the transcriptomic response to supercoiling increase

Role of gene orientation in transcriptomic response



- Convergent genes (conv) Tandem genes (tand) Divergent genes (div)
- Convergent genes more activated than divergent ones
- Consistent with the need of topo1 between divergent genes to remove negative supercoils
- → Gene orientation involved in the transcriptomic response to supercoiling increase

Conclusion

Stress response

Other metabolic process

Other catabolic process

- → Topoisomerase I inhibition by seconeolitsin leads to supercoiling increase, contrary to novobiocin treatment that leads to relaxation.
- -> Conformational changes of the chromosome induce a global transcriptomic response, with little functional enrichment but a non-uniform spatial organization.
- → Supercoiling sensitivity is not an intrinsic property of a promoter. It depends on the physiological state of the cell and the direction of supercoiling variations.

I] S. Martis B., R. Forquet, S. Reverchon, W. Nasser, and S. Meyer, 'DNA Supercoiling: an Ancestral Regulator of Gene Expression in Pathogenic Bacteria?', Computational and Structural Biotechnology Journal, vol. 17, pp. 1047–1055, 2019, doi: 10.1016/j.csbj.2019.07.013. [2] A. González De La Campa, M. T. García Esteban, and M. A. Blázquez Ferrer, 'Use of Secone olitsine and N-Methyl-Secone olitsine for the Manufacture of Medicaments', Jun. 23, 2011. [3] M.-J. Ferrándiz, A. J. Martín-Galiano, C. Arnanz, I. Camacho-Soguero, J.-M. Tirado-Vélez, and A. G. de la Campa, 'An increase in negative supercoiling in bacteria reveals topology-reacting gene clusters and a homeostatic response mediated by the DNA topoisomerase I gene', Nucleic Acids Res, vol. 44, no. 15, pp. 7292–7303, Sep. 2016, doi: 10.1093/nar/gkw602. [4] R. Forquet, M. Pineau, W. Nasser, S. Reverchon, and S. Meyer, 'Bacterial promoter opening underpins ubiquitous transcriptional regulation by DNA supercoiling', bioRxiv, p.