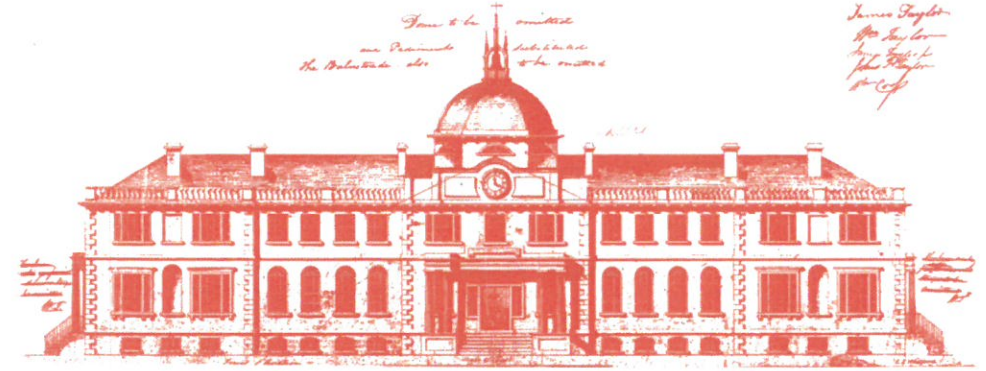


# The Aromatic Amino Acid Responsive TyrR Transcription Factor of *Enterobacter cloacae* UW5: Its Role in Regulation of Indole-3-Acetic Acid Biosynthesis and the Identification of an Expanded Regulon Using RNA-Sequencing

## Abstract

The control of transcription is an important process in all living cells. In the bacterial family *Enterobacteriaceae*, the transcription factor TyrR controls genes for aromatic amino acid uptake and biosynthesis. In this thesis, I explore the control of genes by TyrR in *Enterobacter cloacae* UW5, a soil bacterium commonly associated with plant roots that bestows beneficial effects on its host and is also an inhabitant of human intestinal microflora and an opportunistic pathogen. Chapter 1 provides a general introduction to bacterial activities in the plant rhizosphere and transcriptional regulation, especially by TyrR. In Chapter 2, I investigated the regulation of two divergently transcribed genes, *ipdC* and *akr*, by TyrR. The *ipdC* gene encodes indolepyruvate decarboxylase for the production of the plant growth hormone indole-3-acetic acid, which plays an important role in the plant beneficial behavior of *E. cloacae*. TyrR is required for activation of *ipdC* by binding a single DNA element upstream of the promoter. All three aromatic amino acids act as cofactors for TyrR to induce *ipdC* expression. Expression of *akr* was repressed by TyrR independently of aromatic amino acids and involved TyrR binding an atypical DNA site within the promoter. In Chapter 3, I assembled the *E. cloacae* UW5 genome sequence, which revealed genes and pathways that contribute to its plant-associated lifestyle and served as a reference for mapping RNA-sequencing data. In Chapter 4, I delineated the TyrR regulon by comparing transcription profiles in wild-type and *tyrR* mutant strains of *E. cloacae* generated through RNA-sequencing. Broad changes in gene expression were identified and several new TyrR members confirmed, including *dmpM*, a methyltransferase that is highly upregulated by tyrosine and phenylalanine, and *cpxP* and *cpxR*, components of the envelope stress response. Additionally, pathways for aromatic metabolism, anaerobic respiration, and motility were altered in the *tyrR* mutant. Chapter 5 summarizes this research that suggests that the *E. cloacae* TyrR regulon has expanded from that of *E. coli* to include genes for survival in the diverse environments that this bacterium inhabits and illustrates the expansion and plasticity of transcription factor regulons.



Home of the School of Graduate Studies, Sir Howard Douglas Hall was designed by J.E. Woolford in 1825 and is the oldest university building in Canada still in use.

The University of New Brunswick recognizes that the university sits on traditional Wolastoqey territory. The river that runs right by our university – the St. John River – is also known as Wolastoq, along which live the Wolastoqiyik -- the people of the beautiful and bountiful river.

## UNIVERSITY OF NEW BRUNSWICK SCHOOL OF GRADUATE STUDIES

### ORAL EXAMINATION

**Thomas J.D. Coulson**

**IN PARTIAL FULFILMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF**

**DOCTOR OF PHILOSOPHY**

Ph.D. Candidate

**Thomas Joseph Dana Coulson**

Graduate Academic Unit

**Biology**

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**July 31, 2019**

**1:00 p.m.**

**Forestry/Geology Bldg.  
Room 202**  
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**Examining Board:**

Dr. René Malenfant (Biology)

Dr. Shawn MacLellan (Biology)

Dr. Ghislain Deslongchamps (Chemistry)

Dr. Cheryl Patten (Biology)

Supervisor

**External Examiner:**

Dr. Ji Yang

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**The Oral Examination will be chaired by:**

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**Publications:**

Published

**Coulson, T.J.D.** and Patten, C.L. (2015) Complete genome sequence of *Enterobacter cloacae* UW5, a rhizobacterium capable of high levels of indole-3-acetic acid production. *Genome Announcements*, 3(4): e00843-15; doi10.1128/genomeA00843-15

**Coulson, T.J.D.** and Patten, C.L. (2015) The TyrR transcription factor regulated the divergent *akr-ipdC* operons of *Enterobacter cloacae* UW5. *PLoS One*, 10(3): e0121241; doi10.1371/journal.pone.0121241

Patten, C.L., Blakney, A.J. and **Coulson, T.J.D.** (2013) Activity, distribution and function of indole-3-acetic acid biosynthetic pathways in bacteria. *Critical Reviews in Microbiology*, 39(4): 395-415.

English, M.M., **Coulson, T.J.D.**, Horsman, S.R. and Patten, C.L. (2010) Over expression of *hns* in the plant growth promoting bacterium *Enterobacter cloacae* UW5 increases root colonization. *Journal of Applied Microbiology*, 108(6): 2180-2190.

Mesanza, N., Crawford, B.D., **Coulson, T.J.D.**, Iturrutxa, E., and Patten, C.L. 2019. Colonization of *Pinus radiata* D. Don seedling roots by biocontrol bacteria *Erwinia billingiae* and *Bacillus simplex*. *Forests*, 10:552.

In Preparation

**Coulson, T.J.D.**, Malenfant, R., Patten, C.L. 2018. Characterization of the TyrR regulon in the rhizosphere bacterium *Enterobacter cloacae* UW5 reveals overlap with the CpxR envelope stress response. *Submitted, Journal of Bacteriology*.

**Conference Presentations:**

**Coulson, T.J.D.** and Patten, C.L. (2018) Characterization of the aromatic amino acid responsive TyrR transcription factor in *Enterobacter cloacae* UW5 reveals overlap with the CpxR envelope stress response. UNB Graduate Student Research Conference. Fredericton, Canada.

**Coulson, T.J.D.** and Patten, C.L. (2012) Regulation of overlapping, divergent promoters by the global transcription factor TyrR. American Society of Microbiology 112<sup>th</sup> General meeting. San Francisco, USA.

**Coulson, T.J.D.** and Patten, C.L. (2011) Regulation of indole-3-acetic acid biosynthesis in *Enterobacter cloacae* UW5 by the transcription factor TyrR. Rhizosphere 3 International Conference. Perth, Australia.

**Coulson, T.J.D.** and Patten C.L. (2010) Characterization of the *Enterobacter cloacae* UW5 *akr* gene and its implications in indole-3-acetic acid production. Canadian Society of Microbiology 60<sup>th</sup> Annual Conference. Hamilton, Ontario.

**Coulson, T.J.D.** English, M.M., Horsman, S.R. and Patten C.L. (2009) Quantification of *hnsA* gene expression in the rhizosphere in a hyper-colonizing *Enterobacter cloacae* UW5 mutant. Canadian Society of Microbiology 59<sup>th</sup> Annual Conference. Montreal, Quebec.