

Cangene Gold Medal Award Lecture | Conférence Médaille d'or

Monday June 18 | Lundi 18 juin 13:00-14:00 DKN – 1C

Catherine Paradis-Bleau's undergraduate college degree in science (B.Sc.) was completed at the Université de Sherbrooke with a major in microbiology (1998-2001). As part of her education, she participated in three training periods in the field of microbiology; the first two at Agriculture and Agri-food Canada in the city of Lennoxville and the third one at Health Canada in the city of Longueuil. During these training periods, she worked to find a way to increase the antibiotic susceptibility of antibiotic resistant strains of *Staphylococcus aureus* causing mastitis infections in cows and also worked on a project to improve the microbiological quality of water and food. She then enrolled in the Université Laval in Quebec City for graduate studies in the microbiology-immunology program with Dr. Roger Levesque. As a masters-degree student, she principally worked on analyzing the bacterial cell division proteins FtsZ and FtsA from the perspective of identifying potent inhibitors as possible antibacterial agents (2002-2003). She then pursued her Ph.D. studies in the laboratory of Dr. Levesque and started a new project on phage-encoded lysis proteins. She concentrated her efforts on characterizing the antibacterial potential of the endolysin enzyme from the *Pseudomonas aeruginosa* phage ϕ KZ. Another project involved identifying inhibitors of essential bacterial cell wall biosynthesis enzymes (2003-2007). During her graduate studies, she received a NSERC Scholarship for her M.Sc. studies and a FRSQ Scholarship for her Ph.D. studies. Her Ph. D. work resulted in ten scientific papers on which she is the first author. She wrote seven primary scientific papers and three reviews. She also received outstanding evaluations from all four of her thesis committee members. At the moment, five of these scientific papers have been published in the scientific journals "FEMS Microbiology Letters", "Bioorganic & Medicinal Chemistry", "Peptides", "Protein Engineering, Design and Selection", and "Journal of Antimicrobial Chemotherapy". The other five papers will be soon published or submitted to the scientific journals "Proteins", "Journal of Antibacterial Chemotherapy", "Natural Products Report", "Current Opinion in Microbiology" and "Molecular Microbiology". She also has a co-authored paper published in the "Journal of Electronic Microscopy" and will have another co-authored paper published in the "Journal of Biological Chemistry". She has given three oral presentations as a guest speaker and 22 poster presentations. She received the Fondation Germain des Prés Student travel grant award in 2003 to present her work at the national Canadian Bacterial Disease Network meeting in Banff, the ACCEM Student travel grant award in 2005 to present her work at the General Meeting of the American Society of Microbiology in Atlanta. She was fortunate to have many opportunities to attend scientific meetings in the cities of Atlanta, Boston, Banff, Calgary, Laval and Quebec. She won an award for her poster presentation in 2003 from the Faculty of Medicine of the Université Laval, an award for her oral presentation at the Centre de Recherche sur la Fonction, la Structure et l'Ingénierie des Protéines (CREFSIP) symposium in 2005, an award for my oral presentation at the Faculty of Medicine of the Université Laval in 2005 and an award for my poster presentation at the CREFSIP symposium in 2006. She also participated in a public scientific educational event in 2005 and in voluntary work at Expo Sciences Bell as a judge for the regional finals in 2005, 2006 and 2007. Finally, she assisted two B.Sc. trainees and a Masters student. She has now moved to Boston to start her postdoctoral studies at Harvard Medical School under the supervision of Dr. Gerald B. Pier. She has received an FRSQ-funded postdoctoral fellowship for her work in the field of bacterial pathogenesis and immunity. Her main project is directed towards understanding how the Cystic Fibrosis Transmembrane Regulator participates in innate immunity to *P. aeruginosa* lung infection and how this impacts the course of cystic fibrosis.



Bacterial cell division and cell wall biosynthesis: the quest for essential proteins as new antibacterial targets

The incessant increase and spread of antibiotic resistance among emerging and re-emerging bacterial pathogens compromises the efficiency of available antibacterial therapies and increases the impact of bacterial infections on human morbidity and mortality. In order to develop novel classes of antibacterial agents, we focussed efforts to identify new drug targets using the opportunistic pathogen *Pseudomonas aeruginosa* as a model organism. We first analyzed the cell division proteins FtsZ and FtsA, and the bacterial cell wall biosynthesis amide ligase enzymes MurD, MurE and MurF as potential antibacterial targets. We characterized the enzymatic activities of the five purified proteins from *P. aeruginosa*. Using phage display approaches and a solid-phase parallel synthesis strategy to produce potentially inhibitory molecules, we selected specific inhibitors against the FtsZ, FtsA, MurD, MurE and MurF enzymes. Three peptides (FtsZp1, FtsZp2 and FtsZp3) inhibited the GTPase activity of FtsZ with IC_{50} values between 0.45 and 5 mM. A collection of GTP analogues was also synthesized. Nine compounds inhibited the GTPase activity of FtsZ with IC_{50} values between 450 μ M and 2.6 mM, and five compounds (10, 13, 14, 15 and 17) inhibited *Staphylococcus aureus* growth. Five peptides (FtsAp1, FtsAp3, FtsAp4, FtsAp5 and FtsAp6) showed specific inhibition of ATPase activity of FtsA with IC_{50} values between 0.7 and 35 mM. The C-7-C MurDp1 peptide specifically inhibited ATPase activity of MurD with an IC_{50} value of 4 μ M and the loop conformation of MurDp1 was shown to be important for the efficacy of the inhibitor. A dodecamer peptide, MurEp1, specifically inhibited MurE ATPase activity with an IC_{50} value of 500 μ M. MurEp1 blocked the amide bond formation between the amino acid and the nucleotide substrate of MurE, acting as a time-dependent mixed inhibitor and having K_i values of about 120 μ M. Another dodecamer peptide, MurFp1, specifically inhibited the MurF ATPase activity when MurF was pre-incubated with ATP or its nucleotide substrate. MurFp1 had an IC_{50} value of 250 μ M and a K_i value of 420 μ M with respect to the mixed type of inhibition against the amino acid substrate. These potent inhibitors, having novel modes of action against essential and unexploited enzymes, could be the basis for future development of new antibacterial agents targeting bacterial cell division and cell wall biosynthesis. In our ongoing efforts to identify potent antibacterial candidates active against essential bacterial constituents, we also studied the endolysin enzyme, gp144, from the *P. aeruginosa* phage ϕ KZ. This protein acted as a lytic transglycosylase and hydrolyzed the peptidoglycan of Gram-negative bacteria. gp144 passed through the bacterial membranes independently of the Sec secretion system. Circular dichroism spectroscopy and fluorescent calcein release experiments showed that gp144 specifically interacts with anionic lipids of bacterial membranes without altering zwitterionic lipids typical of eukaryotic membranes. Results obtained indicated that gp144 disorganizes bacterial membranes to access and hydrolyse the peptidoglycan layer to lyse Gram-negative bacteria. The enzyme structure was solved at a resolution of 1.7 Å. gp144 contains an N-terminal peptidoglycan-binding domain with a large carbohydrate binding channel which can accommodate at least six monosaccharide units, and a C-terminal domain responsible for the lytic transglycosylase activity with the catalytic residue Glu-115 present in the binding channel. The gp144 endolysin represents a strong candidate for evaluating the potential efficacy of this approach to address the critical problem of antibiotic resistance.



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