II Scientific Abstract

BURROUGHS WELLCOME FUND

Scientific Abstract

Describe below the proposed work in a scientific abstract that is understandable to a multidisciplinary group of scientific reviewers (one page maximum).

Candidate Name:	**Vanessa Sperandio**
Institution:	**The University of Texas Southwestern medical Center at Dallas**
Title of Research Project:	**Interkingdom signaling in bacterial pathogenesis.**

Scientific Abstract:

Quorum sensing is a bacterial cell-to-cell signaling system in which communication occurs through hormone-like organic compounds referred to as autoinducers. These autoinducers then interact with bacterial transcription factors to drive gene expression in a coordinate manner. Quorum sensing has also recently been recognized as a cell-to-cell signaling system amongst prokarytes and eukaryotes. In particular, the AI-3 bacterial signaling system cross-signals with the human epinephrine/norepinephrine signaling system. The AI-3/epinephrine/norepinephrine signaling cascade is present in several bacterial pathogens such as enterohemorrhagic *E. coli* (EHEC) O157:H7, *Salmonella, Shigella, Yersinia, Francisella tularensis*, among others. We have extensivelly demonstrated that this signaling system is responsible for activating EHEC virulence genes. EHEC is responsible for major outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) throughout the world. One of the major problems in the control and prevention of EHEC outbreaks is the fact that it has a very low infectious dose. EHEC colonizes the large intestine where it causes attaching and effacing (AE) lesions, which are believed to be the first step towards infection of the host, and also produces Shiga toxins (Stx), which are responsible for the major symptoms of HUS. Treatment of EHEC infection with conventional antimicrobials is highly controversial, because these antimicrobials may activate the Shiga-toxin encoding bacteriophage to enter the lytic cycle, thereby producing and releasing Shiga toxin. The genes involved in the formation of these AE lesions are encoded within a chromosomal pathogenicity island named the Locus of Enterocyte Effacement (LEE). EHEC activates expression of its virulence genes via the AI-3/epinephrine/norepinephrine signaling system. The AI-3 signal is produced by the normal intestinal flora, and epinephrine/ norepinephrine are hormones produced by the host. By sampling both signals, EHEC recognizes that it is within the host, and refines the expression of its virulence genes. Given the widespread nature of this signaling system amongst several bacterial pathogens, and its defined role in EHEC pathogenesis, this proposal aims to characterize the AI-3 signal synthesized by the intestinal flora by determining its structure, and addressing to what extent it affects mammalian gene expression. Accordingly, in **Specific Aim 1** we will determine the chemical structure of AI-3, as well as design structural analogues of this signaling compound that can be used as antagonists. Inasmuch as the host hormones epinephrine and norepinephrine signal to EHEC, it would be expected that AI-3 would signal to eukaryotic cells. This hypothesis will be addressed in **Specific Aim 2** of this proposal, where we will assess the role of AI-3 signaling within mammalian cells.

III. Research progress Report

1) Aim 1: To determine the chemical structure of AI-3, and design potential antagonists to the AI-3 signal. This year we got a breakthrough in the identification of the chemical structure of AI-3. We started through an "in silico" approach surveying the genes surrounding the *qseBC* genes (QseC is the AI-3 receptor) searching for neighboring genes conserved in all bacterial species that harbor *qseBC*. Downstream of the *qseBC* operon, there is another operon composed of *mdaB* and *ygiN*. These genes act in concert in a quinone redox cycle. Bacteria synthesize quinones, biologically active molecules that perform important cellular functions. For example, *Escherichia coli* produces ubiquinone and menaquinone that are essential for electron transport; however, the high redox potential of quinones can result in the generation of reactive oxygen species that stress the cell. MdaB converts quinones to quinols, and YgiN quinols to quinone. Quinones lead to the synthesis of menaquinone, which is vitamin K. Vitamin K is abundant in the intestine, is synthesized by many members of the microbial intestinal flora, and is used by mammalian cells. The YgiN mutant, which does not produce menaquinone, had diminished transcription of the LEE genes, and was unable to form attaching and effacing (AE) lesions on epithelial cells. When we provided menaquinone to the *ygiN* mutant, it could now form AE lesions. Given that these phenotypes are AI-3 dependent, we reasoned that menaquinone could be the AI-3 signal. Hence, we performed QseC autophosphorylation studies in liposomes and indeed menaquinone increased QseC's autophosphorylation in a similar manner to QseC's other signals epinephrine and norepinephrine. We are close to conclude that AI-3 is menaquinone. This scenario makes sense given that menaquinone and quinones are linked to stress responses in bacterial and mammalian cells, and epinephrine and norepinephrine are mammalian stress signals.

1.1) Identification of the AI-3 synthase in *E. coli*. Because the synthetic pathway for menaquinone synthesis is known in *E. coli*, we are now generating mutants in all of the enzymatic steps for the synthesis of this molecule to further define the role of this pathway in EHEC pathogenesis.

1.2) LED209 inhibitor of QseC. The overall goal of this project was to develop small molecule inhibitors of the QseC sensor kinase (the receptor for AI-3/ epinephrine and norepinephrine) to prevent bacterial pathogenesis. Our Lead compound LED209 shows promise as an anti-virulence drug(*1*). The overall goal of this project is to transform LED209 into two versions for pre-clinical GLP-based development candidates: an absorbable version to target systemic bacterial infections, and a non-absorbable version that will be restricted to the gut to target enteric infections. We got funding in 2008 from NIH to further pursue these studies. A focused library of LED209 analogs was prepared to elucidate the pharmacophore, i.e., determine which parts contribute to inhibition of microorganism virulence, and also which components introduce any adverse side effects. This project continues to be productive, and we are figured the mechanism of action of LED209. LED209 is a pro-drug capable of delivering the active isothiocyanate (OM188) and avoid the metabolism and/or degradation experienced by OM188 en route to the target. Upon its release OMM188 react with free amines at the QseC periplasmic domain, causing an allosteric modification that prevents QseC from sensing its ligands (AI-3/ epinephrine and norepinephrine). OMM188 binding to QseC was achieved using clic-chemistry. This was exciting news, and we could potentially name LED209 as our lead. Thus far our preliminary toxicity studies suggest that LED209 is well tolerated *in vivo*, does not cross the blood brain barrier, does not affect the HERG channel, and does not cross-signal with mammalian adrenergic receptors. We are currently performing extensive pharmacology and toxicology of LED209, and if it passes these tests we are ready to call it our lead and initiate GMP.

None of the other non-absorbable compounds, other than OM110 had any effect *in vitro*, and OMM110 was toxic to mammalian cells and infant rabbits. Because our first two strategies for non-absorbable modification of LED209 (break Lipinsky rules, and conjugate to PEG) did not lead to the development of a suitable non-absorbable candidate, we will change our strategy to formulate LED209 with

an enteric coating that prevents release of the drug during passage through the stomach, only allowing release in the intestine.

Additionally, we have been developing small molecules based on and alternative scaffold as a backup. We moved away from the coumarin scaffold due to toxicity to mammalian cells. The promising scaffold that is being pursued is the 2-aminothiazole. The acylated 2-aminothiazole compounds CF308 and CF325 markedly inhibit virulence of *Salmonella*, but not EHEC; CF325 is more potent than CF308. CF326, CF329 and CF332 work to inhibit virulence of EHEC and *Salmonella* at 5pM. These compounds will progress to animal experiments.

1.3) Crystallization of apo QseC and QseC with ligand, biochemistry of QseC. We are still performing screens for crystals. We still have been able to generate bigger crystals of QseC's periplasmic domain, and we are working towards optimizing crystal formation so we can get better diffraction. To gain further insights into the biochemistry of QseC-ligand recognition we identified 8 conserved aminoacids on QseC's periplasmic loop, and have changed them to. All of these mutants were confirmed by sequencing, and express QseC at wild-type levels. These mutants were assayed in liposomes for authophosphorylation and for complementation of QseC-depended phenotypes. Four of these mutants had WT QseC phenotypes, but four of them seem to affect QseC function and were chosen for further characterization. Mutant D45A is less motile and has enhanced autophosphorylation and phosphotransfer to QseB, however it is normal for LEE and Shiga toxin (Stx) expression. Mutant V145A has normal motility, normal phosphorylation and phosphotransfer to QseB, Normal Stx expression, but diminished LEE expression. Mutants Q147A has increased autophosphorylation and phosphotransfer to QseB, increased LEE and Stx expression, and increased ability to form AE lesions. Mutant R152A Also has increased autophosphorylation and phosphotransfer to QseB, normal levels of LEE expression, but Stx expression is 100-fold higher than WT. QseC and QseB constitute a cognate two-component system, and the *qseBC* genes are co-transcribed in an operon that is also auto-regulated (*3*). QseC acts upstream of QseE (a second kinase that also senses Epinephrine, but not AI-3)(*4*), given that QseBC activates expression of the *qseEFG* operon (*5*) encoding the QseE sensor kinase, the QseF response regulator and the QseG outer membrane lipoprotein. Upon sensing AI-3/ epi and/or NE, QseC autophosphorylates and then transfers its phosphate to three response regulators (RR)s: its cognate RR QseB, QseF and KdpE(*6*). QseE only transfers its phosphate to its cognate RR QseF(*7*). The different phenotypes of the QseC point mutants suggest that the phosphotransfer signaling pathways in these mutants is altered, which is a very novel observation, given that this the first study suggesting that modifications in the periplasmic sensing domain of a sensor kinase alters its phosphotransfer abilities. We are continuing the characterization of these mutants.

1.4) Studies of QseC signaling in *Salmonella typhimurium* and *Citrobacter rodentium* utilizing epinephrine/norepinephrine knockout mice. In order to really study inter-kingdom communication, we needed a mouse model. Given that we have shown that *Salmonella* utilizes QseC to regulate virulence gene expression(*1*), we used *Salmonella typhimurium* so we could take advantage of DBH knockout mice (which do not produce either epinephrine or norepinephrine) to study how these host signals affect bacterial infection *in vivo*, as well as the contribution of QseC to this process. To have access to these DBH knockout mice we initiated a collaboration with David Weinshenker at Emory University. The *S. typhimurium qseC* mutant was attenuated for survival within J774 macrophages and invasion of epithelial HeLa cells. Invasion was restored to WT levels when it was complemented by *qseC* cloned in a plasmid. Survival within J774 macrophages was inhibited by both alpha and beta adrenergic antagonists. The *qseC* mutant was also attenuated for systemic infection in 129x1/SvJ mice. Transcription of *sopB, invF* and *sifA* was decreased in the *qseC* mutant compared to expression in WT in the spleens and livers of mice. Additionally, transgenic mice that do not express DBH (dopamine beta hydroxylase), i.e. unable to produce epinephrine/norepinephrine, had a different susceptibility to *Salmonella* infection compared to DBH heterozygous mice. Expression of virulence genes in the liver and spleens of DBH knockout mice was

decreased because of the lack of epinephrine and norepinephrine, and the sensing of these hormones *in vivo* was dependent on QseC. These data suggest that inter-kingdom signaling in *Salmonella enterica* serovar Typhimurium plays an important role in pathogenesis *in vivo*, and that the QseC sensor kinase regulates expression of virulence genes in *Salmonella(8)*. We extended these studies to infection of BALB-C mice by *Salmonella* and also knocked out the genes encoding the other adrenergic sensor (QseE) and generated a double sensor (*qseE, qseC*) mutant in *Salmonella*. These mutants were also tested in mice for pathogenesis and also showed diminished virulence.

We also performed transcriptome studies with the *Salmonella qseC* mutant in collaboration with Ferric Feng and Stephen Libby. Our microarray analysis revealed that *ygiW* was the most upregulated gene (65 fold) by QseC. The *ygiW* gene is transcribed in the opposite direction to the *qseBC* operon. YgiW has been described as member of the **B**inding **O**ligonucleotide/oligosaccharide **F**old (BOF) superfamily. Despite broadly found in different species from Enterobacteriaceae, YgiW has no function assigned, and it is predicted to be a periplasmic protein. A few potential functions have been reported, such as resistance to polymyxin B in *S.* Typhimurium, and some stress responses in *E.coli*. We showed that the *S.* Typhimurium *ygiW* mutant is highly attenuated for both oral and systemic murine infections. The *ygiW* mutant is also attenuated for survival within J774 macrophages, is impaired for motility, and also presented lower expression of SPI-1 and SPI-2 genes. The combination of these phenotypes explains its attenuation in vivo.

The YgiW from *E. coli* has been previously crystallized (PDB: 1NNX), we modeled the *Salmonella* YgiW structure on the *E. coli* structure, and only 3 amino acids differences were observed. The same 6 predicted active sites of BOF proteins are conserved. We have performed site direct mutagenesis on these 6 active sites, and the E110A YgiW point mutant was highly attenuated comparable to the *ygiW* knockout. YgiW in *S.* Typhimurium has an essential role in pathogenesis ant its potential function is currently under investigation. Preliminary data indicate YgiW to converge bacterial survival strategies and chemical signaling of host signals (epinephrine and norepinephrine) in *Salmonella* pathogenicity. In collaboration with Steven Trent we analyzed the LPS from the *ygiW* mutant, and found that although the O antigen is identical between the *ygiW* mutant and WT, the *ygiW* mutant has diminished lipid A modifications, which are important for intra-macrophage survival, suggesting that the main role of YgiW is to enhance lipid A modifications in *Salmonella*. We are now investigating with which of the lipid A modifying enzymes YgiW interacts. We also have preliminary data suggesting that YgiW binds to peptidoglycan, and we are further assessing whether this binding to the sugar portion of the peptidoglycan contributes to YgiW's function.

We also generated *qseC, qseB, qseBC, qseE, qseEC* mutants in *Citrobacter rodentium*, a mouse pathogen that contains the LEE genes. All of these mutants were attenuated for infection of mice. We intend to also use *Citrobacter* in the DBH mouse model of infection because it is an intestinal pathogens that forms AE lesions in mice.

2) Aim 2: To assess the role of AI-3 signaling in mammalian gene expression. Because we now know that AI-3 is menaquinone, vitamin K, it is clear that it modulates signaling functions in mammalian cells. Menaquinone is involved in the carboxylation of glutamate residues in proteins to form gamma-carboxyglutamate residues (abbreviated Gla-residues). mammalian proteins with Gla domains play key roles in the regulation of bllod coagulation, bone metabolism and vascular biology.

1. D. A. Rasko *et al., Science* **321**, 1078 (Aug 22, 2008).

IV. Mentoring/Institutional Environment

Current trainees in my laboratory:

1) Benjamin Habdas, Ph.D.. Post doctoral fellow (**Supported by Burroughs**)
Ben has graduated last February, and is currently doing a bridge post-doc in my laboratory in collaboration with Neal Alto. In this past year Ben has accumulated a lot of knowledge and data concerning AI-3 signaling to mammalian cells, and also worked in the signaling cascade describing a new member of this cascade, QseD. He is currently studying unidentified T3SS effectors in EHEC and C. rodentium, and is learning novel biochemistry techniques as well as gaining experience with mouse infections. He is currently looking for a position in industry.

2) Cristiano Moreira, Ph.D., Post-doctoral fellow (**Supported by Burroughs**)
Cris has conducted all of the Salmonella work delineated above in my laboratory. He is in the 5th year of his post-doc and has recently gotten his greencard. He is wrapping up two first author manuscripts, including a very exciting story with YgiW. With these two manuscripts he intends to go to the academic job market next year.

3) Christopher Parker, Ph.D., Post-doctoral fellow (**Supported by Burroughs**)
Christopher is at the his fourth year as a post-doctoral fellow. Chris has been working with the QseC receptor, attempting to monitor LED209 inhibition of this receptor, and crystallize it with AI-3 to model the antagonist. He has already generated a lot of the necessary tools to accomplish his goals, and obtained crystals of QseC. Chris aims to stay in academia, he favors undergraduate institutions, and my goal is to back him up for it.

4) Melissa Kendall, Ph.D., Post-doctoral fellow (**Supported by Burroughs**)
Melissa is a 6th year post doctoral fellow. She has mapped through transcriptomics the epinephrine and AI-3 signaling cascades in EHEC. She also mapped the QseA regulon. Recently she has been assessing the role of ethanolamine (the most abundant nitrogen source in the intestine) in regulating the expression of the LEE and Shiga toxin genes. She is also studying the role of Hfq in post transcriptional regulation in EHEC, and has found that this regulation is different in different strains. Melissa had her own NRSA fellowship for 2 years, having gotten this grant in her very first try. She recently applied for a K99 NIH grant that got scored and was reviewed favorably, and she is ready to submit the A1 version of this grant on July 15.

5) Alline Pacheco, M.S., Graduate Student

6) Regan Russell, M.S. Research Associate

7) Jacqueline Njoroge, B.S., Graduate Student

8) Darya Terekhova, Ph.D., Post-doctoral fellow (**Supported by Burroughs**)
Darya is a third year post doctoral fellow who has been working with Citrobacter rodentium. She is impressive! She has already generated and tested several mutants in the QseC signaling cascade in Citrobacter, and has already found that these mutants are very attenuated for infection of mice.

9) Charley Grueber, B.S. Graduate Student (**Supported by Burroughs**)
Charley is third year Ph.D. student and has just finished his qualifying exam. Charley has been working in the QseEFG system and how it integrates with QseBC to regulate virulence, by activating a small RNA.

Charley is already extremely independent. He is smart and driven, and a great troubleshooter. I intend to keep fostering his scientific maturation.

10) Maredith Mathis. Ph.D. Post-doctoral fellow

11) Y Nguyn. B.S. Graduate Student

Former trainees:
1) Marcie Clarke (former Ph.D. student): currently in Boston finishing law school and working as a patent specialist at a Law Firm.

2) Matthew Walters (former Ph.D. student): currently at the University of Michigan in Ann Arbor pursuing an academic post-doc.

3) Faith Sharp (former M.S. student): finished a Masters in teaching at the University of Illinois, and is currently teaching middle school

4) Nicola Reading (former Ph.D. student): currently at Brigham and Women's Hospital in Harvard pursuing an academic post-doc.

5) David Hughes (former Ph.D. student): currently in the University of Miami performing and academic post-doc.

6) Marcelo P. Sircili (former exchange Ph.D. student from Brazil): currently a faculty member at Instituto Butanta in Sao Paulo, Brazil

7) Juliana Falcao (former exchange Ph.D. student from Brazil): currently a faculty member in the University of Sao Paulo in Ribeirao Preto Brazil.

8) Sergio Rocha (exchange Ph.D. student from Brazil): defended his Ph.D. at Instituto Butanta in Sao Paulo, Brazil

9) Fernanda Pace (exchange Ph.D. student from Brazil): defended her Ph.D. at State University of Campinas in Campinas, Brazil

10) Carlos Esteban Nieto (former post-doctoral fellow): currently pursuing a second post-doc at Imperial College in London, UK

11) Scott Waterman (former post-doctoral fellow): Lost contact and I do not know his current position.

12) Shane Flickinger (former post-doctoral fellow): currently pursuing a second post-doc at the University of Wisconsin in Madison

13) David Rasko (former research Assistant Professor): currently Assistant Professor Tenure track at the University of Maryland School of Medicine in Baltimore.

V. Carrier Impact/Comments/Concerns

Impact: The Investigator in Pathogenesis of Infectious Diseases award from Burroughs Wellcome Fund had a very positive impact on my career. It allowed me to network with several scientists that I did not know before, and broaden my scientific horizon. The award also positively impacted my tenure and promotion review, as well as my recent recommendation by the P&T committee to be promoted to Full Professor as of September 1st 2011. This award was essential to financially allow me to take more risks in my research, and learn novel expertise that will make my scientific research more rounded.

Comments/ Concerns: I have been pleased with my Institution. I have been granted tenure promoted to Associate Professor on September 1st 2007. I also received a secondary appointment in the Biochemistry department on July 1st 2008. I also was recently recommended by the P&T committee to be promoted to Full Professor as of September 1st 2011. I have no concerns with my Institution and/or BWF administration of the grant.

Most important accomplishment that occurred in the past year: We have published a manuscript in SdiA in PNAS, discovering that AHLs in the rumen of cattle prime EHEC to establish a commensal relationship with its natural reservoir.

VI . Other Funding Sources

2 RO1 AI 053067 (Sperandio) 1/1/2008 – 12/31/2012 25% effort
NIHH/NIAID $300,000 (yearly direct costs)
Quorum sensing regulation of EHEC virulence genes
The major goals of this project are generate deeper understanding of the quorum sensing signaling cascade in EHEC.

Burroughs Wellcome Fund. Investigators in Pathogenesis of Infectious Diseases 10% effort
07/01/06 to 07/01/11
Requested funds $80,000 (yearly direct costs)
Interkingdom signaling in bacterial pathogenesis
The goal of this proposal is to determine the chemical structure of the bacterial autoinducer-3 involved in interkingdom signaling, and design potential antagonists to this signal. The second aim of this study is to assess the role of AI-3 signaling in mammalian gene expression

UO1 AI 077853 Sperandio PI 06/01/08 – 05/31/20013 25% effort
NIH/ NIAID $1,015,199 (total yearly direct costs)
Sperandio yearly direct costs portion $ 574,914
AI-3 inhibitors as treatment for bacterial infections
We will develop AI-3 antagonists to hinder EHEC, *Salmonella* and *F. tularensis* infections

1RO1 AI 077613 Sperandio PI 2/1/2009 – 1/31/2014 25% effort
NIH/NIAID $250,000 (yearly direct costs)
SdiA regulation of EHEC virulence.
In this grant we are studying the role of SdiA-AHL regulation in the expression of the LEE and gad (acid resistance system) in EHEC bovine colonization.

VII. Curriculum Vitae

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Vanessa Sperandio	POSITION TITLE Associate Professor
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY/ MENTOR
State University of Campinas, Brazil	B.S.	1991	Biological Sciences
State University of Campinas, Brazil	M.S.	1993	Molecular Genetics
State University of Campinas, Brazil	Ph.D.	1995	Molecular Genetics

A. Positions and Honors.

-Research and Professional Experience:

- Recommended for promotion to Full Professor by the P&T committee January 2011, to be effective 09/2011

-UT Southwestern Medical Center Associate Professor with tenure 09/2007 to present
-UT Southwestern Medical Center Assistant Professor of Microbiology 07/2001 to 08/2007
-University of Maryland School of Medicine Postdoctoral Fellow 08/1997 to 06/2001
-State University of Sao Paulo Postdoctoral Fellow 07/1995 to 07/1997
-State University of Campinas Graduate student 12/1994 to 07/1995
-University of Maryland School of Medicine Exchange Graduate student 05/1993 to 11/1994
-State University of Campinas Graduate student 03/1992 to 03/1993

-Awards:

1) Kavli Fellow, National Academy of Sciences, since 2007
2) Burroughs Welcome Fund Investigator in Pathogenesis of Infectious Diseases. (07/2006-07/2011)
3) Ellison Medical Foundation New Scholar Award in Global Infectious Diseases. (08/2004-08/2008).
4) Dam Charitable foundation travel grant. (10/2000 to 11/2000)
5) PEW fellow in biomedical sciences. (08/1997-08/1999)
6) Post-doctorate fellowship award from Brazilian council of National research (CNPq). (07/1995-07/1997).
7) Training grant from Brazilian federal research council (CAPES) (05/1997-06/1997).
8) Doctorate fellowship award for training abroad from CAPES. (05/1993-05/1994).
9) Doctorate fellowship award for training abroad from CNPq. (06/1994-10/1994).
10) Masters fellowship award from (Scientific research foundation of Sao Paulo State) FAPESP (03/1992-03/1993)
11) Undergraduate Scientific training fellowship award from FAPESP. (03/1991-02/1992).

B. Peer reviewed publications:

1) Sperandio, V. and Silveira, W. D. 1993. Comparison between enterotoxigenic *Escherichia coli* strains expressing "F42", F41 and K99 Colonization Factors. *Microbiol. Immunol***., 37**(11), 869-875.
2) Sperandio, V., Girón, J. A., Silveira, W. D., and Kaper, J. B. 1995. The OmpU Outer Membrane Protein: a Potential Adherence Factor of *Vibrio cholerae*. *Infect.Immun.*, **63**(11), 4433-4438.
3) Girón, J.A., Viboud, G.I., **Sperandio, V**., Gomez-Duarte, O.G., Maneval, D.R., Albert, M.J., Levine, M.M., and Kaper, J.B.1995. Prevalence and association of Longus Pilus structural gene (*lgnA*) with colonization factor antigens, enterotoxin types, and serotypes of enterotoxigenic *Escherichia coli*. *Infect. Immun.,* **63** (10):4195-4198.
4) Sperandio, V., Bailley, C., Girón, J.A., DiRita, V.J., Silveira, W.D., Vettore, AL. and Kaper. 1996. Cloning and characterization of the gene encoding the OmpU outer membrane protein of *Vibrio cholerae*. *Infect. Immun*, **64**:5406-5409.
5) Gonçalves, AG., Campos, L.C., Gomes, T.AT., Rodrigues, J. **Sperandio, V.,** Whittam, T.A, and Trabulsi, L.R. 1997. Virulence properties and clonal structure of strains of *Escherichia coli* O119 serotypes. *Infect. Immun.* **65**:2034-2040.
6) Sperandio, V., Kaper, J.B., Bortolini, M.R., Neves, B.C., Keller, R., and Trabulsi, L.R. 1998. Characterization of the locus of enterocyte effacement (LEE) in different enteropathogenic *Escherichia coli* (EPEC) and Shiga-toxin producing *Escherichia coli* (STEC) serotypes. *FEMS Microbiol Lett.* **164**:133-139.
7) Neves, B.C., Knutton, S., Trabulsi, L.R., **Sperandio, V.,** Kaper, J.B., Dougan, G., and Frankel G. 1998. Molecular and ultrastructural characterisation of EspA from different enteropathogenic *Escherichia coli* serotypes. *FEMS Microbiol. Lett*. **169**:73-80.
8) Bortolini, M.R., Trabulsi, L.R., Keller, R., Frankel, G. and **Sperandio, V**. 1999. Lack of expression of bundle-forming pili in some clinical isolates of enteropathogenic *Escherichia coli* (EPEC) is due to a conserved large deletion in the *bfp* operon. *FEMS Microbiol. Lett.* **179**: 169-174.
9) Pelayo, J.S., Scaletsky, I.C.A., Pedrozo, M.Z., **Sperandio, V**., Giron, J.A., Frankel, G., and Trabulsi, L.R. 1999. Virulence properties of atypical EPEC strains *J. Med. Microbiol*. **48**: 41-9.
10) Sperandio, V., Mellies, J.L., Nguyen, W., Shin, S. and Kaper, J.B. 1999. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc. Natl. Acad. Sci*. *USA* **96**: 15196-15201.
11) Mellies, J.L., Elliott, S.J., **Sperandio, V**., Donnenberg, M.S. and Kaper, J.B. 1999. The Per regulon of enteropathogenic *Escherichia coli*: identification of a regulatory cascade and a novel transcriptional activator, the locus of enterocyte effacement (LEE)-encoded regulator (Ler). *Mol. Microbiol*. **33**: 296-306.
12) Sperandio, V., Mellies, J.L., Delahay, R.M., Frankel, G., Crawford, J.A., Nguyen, W. and Kaper, J.B. 2000. Activation of enteropathogenic *E. coli* (EPEC) *LEE2* and *LEE3* operons by Ler. *Mol. Microbiol*. **38**: 781-793.
13) Sperandio, V. 2000. The elusive type III secretion signal. *Trends in Microbiol*. 8(9):395.
14) Sperandio, V. 2000. How the bacterial flora and the epithelial cell get along. *Trends in Microbiol*. 8(12):544.
15) Elliott, S.J., **Sperandio, V**., Girón, J.A., Shin, S., Mellies, J.L., Wainwright, L.A., Hutcheson, S.W., McDaniel, T.K. and Kaper, J.B. 2000. The Locus of enterocyte effacement (LEE)-encoded regulator (Ler) controls expression of both LEE and non-LEE encoded virulence factors in enteropathogenic and enterohemorrhagic *Escherichia coli. Infect. Immun*. **68**: 6115-6126.
16) Sperandio, V., Torres, A.G., Girón, J.A. and Kaper, J.B. 2001. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* (EHEC) O157:H7. *J. Bacteriol.* **183**: 5187-97.
17) Sperandio V. 2001. Genome sequence of *E. coli* O157:H7. *Trends in Microbiol*. 9(4): 159.
18) Sperandio, V., Li, C.C. and Kaper, J.B. 2002. Quorum-sensing *Escherichia coli* regulator: a regulator of the LysR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic *E. coli*. *Infect. Immun.* **70**: 3085-3093.

19) Sperandio, V., Torres, A.G., and Kaper, J.B. 2002. Quorum sensing *Escherichia coli* regulators B and C (Qse BC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. *Mol. Microbiol*. **43**:809-821.
20) Sperandio V. 2002. Quorum sensing in *Pseudomonas aeruginosa*: yet another player. *Trends in Microbiol*. 10(3): 118.
21) Sperandio V. 2002. Flagella: multipurpose structures in EPEC. *Trends in Microbiol*. 10(6): 262.
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54) Njoroge, J., and Sperandio, V. 2009. Jamming Bacterial Communication: New Approaches for the Treatment of Infectious Diseases. EMBO Molecular Medicine (Invited publication) 1:201-210.
55) Sperandio, V. 2009. Deciphering bacterial language. Nature Chemical Biology. 5(12) 870
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68) Sperandio, V. 2010. SdiA sensing of acyl-homoserine lactones by enterohemorrhagic *E. coli* (EHEC) serotype O157:H7 in the bovine rumen.Gut Microbes. 1: 432-435 (invited publication)
69) Curtiss, M.M., and **Sperandio V.** 2011. A Complex Relationship: the Interaction among Symbiotic Microbes, Invading Pathogens, and their Mammalian Host. *Mucosal Immunity*. 4: 133-138 (invited publication)
70) Pace, F, Paive, J.B., Nakazato, G., Lancellotti, M., Sircili, M.P., Silveira, W.D.*, **Sperandio, V.***. 2011. Mutation of the gene *icmF* gene of the type VI secretion system alters pathogenicity in an avian pathogenic *Escherichia coli* (APEC) strain. Microbiology in revision. *co-corresponding authorship.
71) Rocha, S.P.D., Abe, C.M., **Sperandio, V**., Bando, S.Y., Elias, W.P. 2011. Analysis of the Locus of Enterocyte Effacement (LEE) of Atypical Enteropathogenic *Escherichia coli* Displaying Different Patterns of Interaction with Cultured Epithelial Cells. *Infect. Immun*. 79: 1833-1841.
72) Njoroge, J.W., Nguyen, Y., Gruber, C, and **Sperandio V.** 2011. Virulence meets metabolism and stress: Cra and KdpE gene regulation in enterohemorrhagic Escherichia coli (EHEC). Submitted.

VIII. BWF Supported Publications.

1) Parker, C.T., and **Sperandio, V.** 2009. Cell-to-cell signaling during pathogenesis. Cellular Microbiology. (Invited publication) 11(3):363-9.

2) Pacheco, A.R., and **Sperandio, V.** 2009. Inter-kingdom signaling: chemical language between bacterial and host. (Invited publication). Current Opinion in Microbiology (2):192-8

3) Rasko, D.A., and **Sperandio, V.** 2010. Anti-virulence strategies to combat bacteria-mediated disease. Nature Drug Review (Invited publication). 9:117-128.

4) Njoroge, J., and Sperandio, V. 2009. Jamming Bacterial Communication: New Approaches for the Treatment of Infectious Diseases. EMBO Molecular Medicine (Invited publication) 1:201-210.

5) Reading, N.C., Rasko, R.A., Torres, A.T., and **Sperandio, V.** 2009. The two-component system QseEF and the membrane protein QseG link adrenergic and stress sensing to bacterial pathogenesis. *PNAS* 106(14):5889-94.

6) Hughes, D.T., Terekhova, D.A., Liou, L., Hovde, C.J., Sahl, J. , Patankar, A.V., Gonzalez, J.E., Edrington, T.S., Rasko, D.A., and **Sperandio, V.** 2010. Chemical sensing in mammalian host-bacterial commensal associations. *PNAS* 2107:9831-6

7) Hughes, D.T., Clarke, M.B., Yamamoto, K., Rasko, D.A., and **Sperandio, V**. 2009. The QseC adrenergic signaling cascade in enterohemorrhagic *E. coli* (EHEC). *PLoS Pathogens* 5:e1000553

8) Moreira, C.G., Weinshenker, D. and **Sperandio, V.** 2010. QseC mediates *Salmonella enterica* serovar Typhimurium virulence *in vitro* and *in vivo*. *Infect. Immun*. 78:914-26

9) Kendall, M.M., Rasko, D.A., and **Sperandio, V.** 2010. The LysR-Type Regulator QseA Plays an Extensive Role in Enterohemorrhagic *Escherichia coli* Virulence Gene Regulation. *Mol. Microbiol*. 76(5):1306-21.

10) Habdas, B.J., Smart, J. Kaper, J.B, and **Sperandio, V.**. 2010. The LysR-type Transcriptional Regulator QseD Alters Type Three Secretion in Enterohemorrhagic *Escherichia coli* and Motility in K-12 *Escherichia coli*. *J. Bacteriol.* 192:3699-712

11) Sperandio, V. 2010. SdiA sensing of acyl-homoserine lactones by enterohemorrhagic *E. coli* (EHEC) serotype O157:H7 in the bovine rumen.Gut Microbes. 1: 432-435 (invited publication)

12) Curtiss, M.M., and **Sperandio V.** 2011. A Complex Relationship: the Interaction among Symbiotic Microbes, Invading Pathogens, and their Mammalian Host. *Mucosal Immunity*. 4: 133-138 (invited publication)

13) Njoroge, J.W., Nguyen, Y., Gruber, C, and **Sperandio V.** 2011. Virulence meets metabolism and stress: Cra and KdpE gene regulation in enterohemorrhagic Escherichia coli (EHEC). Submitted.

IX. SUPPORT FOR SEMINAR SPEAKER IN THE MOLECULAR MICROBIOLOGY SEMINAR SERIES

The Invited speaker was Arturo Casadevall, Professor and Chair of Biological Sciences at University of Notre Dame , he was a member of the advisory board of the Pathogenesis of Infectious Diseases. Bellow is the itinerary of his visit.

Itinerary Arturo Casadevall
November 3rd and 4th , 2008

04/25/11

Arrive DFW flight AA0753
Please take a taxi from the airport to the hotel

Renaissance Hotel: 2222 Stemmons Freeway, Dallas TX 75207

6:20 pm Vanessa will pick you up at the hotel

6:30 Dinner:
Shinsei Restaurant

7713 Inwood Road
Dallas, TX 75209
(214) 352-0005
Guests: Vanessa Sperandio, Julie Pfeiffer and Nicholas Conrad

April 26th

8:00am Breakfast Post-docs: Melissa Kendall, Meredith Curtis and Cristiano Moreira

9:00am	Lora Hooper	NB9.106	etx. 8-7306
9:30am	Neal Alto	NL3.108	ext. 3-1373
10:00am	Vanessa Sperandio	NL4.140A	ext. 3-1378
10:45am	prepare for seminar		
11:00-12:00 p.m.	Seminar		

12:00pm Lunch A.W. Harris Faculty Club with graduate students and post docs: Alline Pacheco, Jacqueline Njoroge, Y Nguyen, Charley Gruber, Christopher Parker, Darya Terekhova, and Benjamin Habdas

1:00pm	Julie Pfeiffer	NL4.140B	ext 3-1377

1:30pm	Michael Norgard	NL4.114B	ext. 3-0015
2:00pm	Gregory Robertson	NL4.120A	ext. 3-1392
2:30pm	David Hendrixson	NL4.138A	ext. 3-1385

3:00pm Leave to the airport (Vanessa will take you)

Flight Dallas To NY AA0744