PROJECT TYPE: EXPLORATORY RESEARCH PROJECTS - PN-II-ID-PCE-2011-3

FINANCING SOURCE: UEFISCDI

PROJECT CODE: 154/2011

PROJECT TITLE: Effects of *P. aeruginosa* quorum sensing molecules on *Drosophila* genome: a new tool to identify candidate genes involved in host-pathogen crosstalk

PROJECT SUMMARY:

Pseudomonas aeruginosa, an emerging multi-drug resistant organism, one of the major contributors to morbidity and mortality in opportunistic and nosocomial infections, communicates intra-species and with the eukaryotic hosts by cross-kingdom inter-cellular signaling mechanisms such as ***quorum sensing*** (QS), relying on low-molecular weight excreted molecules, to control the production of virulence factors. The *P. aeruginosa* QS signaling molecules (QSSMs) have *in vitro* pleiotropic effects on eukaryotic cells. In the present project we propose an original, integrative *in vivo* approach for the investigation of the way in which such signaling molecules modulate the *Drosophila melanogaster* genome, in order to identify the most sensitive eukaryotic genes targeted by the QSSMs and the specific orthologous genes in the *Homo sapiens* genome. In order to accomplish our purpose, we will evaluate the phenotypic effects of QSSMs and viable *P. aeruginosa* cells (virulent *versus* quorum sensing defective strains) on *D. melanogaster* wild type and mutant strains, highlight the genes expressing a significant up- or down-regulation, identify by bioinformatics the orthologous human genes, assess the effects of *P. aeruginosa* QSSMs and viable *P. aeruginosa* cells on the respective genes profiles in the human eukaryotic cells and finally formulate the clinical contribution of our findings to future development of novel therapeutics for a multitude of infectious and immunological diseases.

RESEARCH TEAM:

Project manager: Mariana Carmen Chifiriuc

Research team members:

Senior Researcher, Prof. Veronica Lazar

Senior Researcher, Lecturer Alexandru Ecovoiu

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PROJECT OBJECTIVES:

In the present project, we propose an original, integrative *in vivo* approach for the investigation of the way in which *P. aeruginosa* QSSMs modulate the genome of *D. melanogaster*, in order to further identify significantly up- or down-regulated genes. Our *in vivo* approach concerning gene expression modulation by QSSMs molecules is intended to reveal genes involved in the immune response, but also genes involved in other stress adaptive response pathways, such as apoptosis. This integrative model will allow us to identify specific orthologous

genes in the genome of *Homo sapiens* and to investigate how they are modulated by QSSMs . Since the concentration of the synthetic QS molecules is controllable, we will be able to simulate different scenarios of infections with various bacterial charges and to monitor accordingly the modulation of the transcription rate of GOIs (genes of interest). In order to accomplish our purpose, the following objectives will be followed:

Ob.1: Evaluation of the phenotypic effects of QSSM and viable *P. aeruginosa* cells (virulent *versus* quorum sensing defective strains) on *D. melanogaster* wild type and mutant strains.

Ob.2: Highlighting the gene expression profile of inoculated/infected fruit flies versus untreated organisms.

Ob. 3: Bioinformatic analysis of the functions of the genes expressing a significant up- or down-regulation and identifying orthologous human genes.

Ob. 4: Assessment of the effects of *P. aeruginosa* QSSMs and viable *P. aeruginosa* cells (virulent *versus* quorum sensing defective strains) on the identified orthologous human genes expression and subsequent phenotypic traits using eukaryotic cells in culture.

Ob.5: Formulation of the clinical significance of the findings, based on the evidenced bioactivities of the chemically defined QSSMs.

Ob.6: Project management, dissemination of results and human resource development

PRELIMINARY RESULTS

Drosophila melanogaster represents a genetically tractable model for studying the mechanisms used by the infectious microorganisms to colonize the healthy individuals. Taking into account that native microbiota plays an important role in the host resistance to colonization, in a first step we have assessed the diversity of the whole microbiota of *D. melanogaster* belonging to different lineages used in the project, the results demonstrating the microbial diversity is significantly varying among different genetic lineages, indicating the necessity of investigating the resident microbiota of a certain *D. melanogaster* line before using it as a potential candidate for the *in vivo* investigation of microbial infection and pathology. Further, we performed the microarray studies and selected 13 *D. melanogaster* genes with significant altered expression profiles during *P. aeruginosa* infection, out of which 6 have human orthologues. The evaluation of the expression of these genes in mammalian cell cultures will enable us to select the most responsive human genes as new valid model genes for the study of the eukaryotic host-bacterial pathogens interactions.

The results obtained by microarray were further confirmed by real-time PCR, after experimental infections of young *D. melanogaster* males induced by feeding and by pricking, both in the whole body and at the intestine level. The relative expression level for some genes *of P. aeruginosa* involved in *quorum sensing* (QS) processes was also analyzed. We used fly males from the Oregon control strain and $gammaCop^{14a}/gammaCop^{14a}$ mutant males.

Four genes have been selected to be checked by qRT-PCR, namely *gammaCop*, *trpl*, *CG9466* and *CG9468*. Furthermore, we have analyzed the variation of expression levels for the reference genes *DptB* and *Dro*, known to be involved in the immune response. The qRT-PCR experiments have revealed the following aspects: **i)** when infected by pricking, the *DptB* and *Dro* genes are over-expressed in the whole body of infected *Oregon* males, as compared to the control. These data fit with the experimental results obtained on *D. melanogaster* larvae

(Vodovar *et al.*, 2005). *Dro* gene is also over-expressed in the body of *gammaCop*^{14a}/*gammaCop*^{14a} mutants, after infection by pricking. Nevertheless, *Dro* is 1.4 times under-expressed in the intestine of the mutants infected by feeding which suggests that their genetic background affects the expression of *Dro* gene during *P. aeruginosa* infection. The *gammaCop* gene is significantly over-expressed when infection with Gram-negative bacteria occurs. This gene might be involved in the cellular transport processes in hypoxia conditions induced by the development of *P. aeruginosa* at the pricking site (Legendre *et al*., 2012). The fact that, in mutant males, *gammaCop* gene is defective might influence its recruitment and usage by the immune system in the intestine, altering the expression of other genes which interacts with when forming an immune response. For *trpl* gene we were unable to identify a consistent pattern for the variations in its expression. Taking into account the biological processes in which *trpl* gene is involved (Agam et all., 2000; Xu et all., 2000; Zhang et all., 2013), it is possible that the expression level of *trpl* was influenced by events outside the experimental infection (for example, the light stimuli to which the males were inherently subjected during the infection protocol). The *CG9466* and *CG9468* genes are significantly under-expressed in case of infection by pricking, both for *Oregon* males and *gammaCop*^{14a}/*gammaCop*^{14a} mutant males. In the intestine, *CG9468* is over-expressed in both male categories, while *CG9466* is over-expressed only in the intestine of *Oregon* males, which might support an interaction between gene expression and genetic background of the mutants. In addition, it was shown that the expression level of *rhlI* gene, known to be part of QS modulation in *P. aeruginosa*, is influenced by the genetic background in which the infection occurs. The data from these experiments were obtained by using multiple biological replicates and support the hypothesis that an exchange of information occurs between *D. melanogaster* and *P. aeruginosa*, most probably mediated by QS molecules. A result of this host-parasite communication is the variation in the expression of target genes, as revealed both in the fly males and in *P. aeruginosa*, as it is the case of *rhLII* gene.

ALLOCATED BUDGET:

- **2011**- 125,000
- **2012**-500,000
- **2013**-225,745.43
- **2014**- 187,500
- **2015**-461,754.57

PROJECT PUBLICATIONS

1. ISI published papers acknowledging the project Ideas 154/2011

- Holban Alina-Maria, Coralia Bleotu, Mariana Carmen Chifiriuc, Eugenia Bezirtzoglou, Veronica Lazar. Role of *Pseudomonas aeruginosa* quorum sensing (QS) molecules on the viability and cytokine profile of human mesenchymal stem cells. *Virulence* 2014, 5, 303-31. **IF 3.319**
- Curutiu Carmen, Balotescu-Chifiriuc Mariana Carmen, Iordache Florin, Bleotu Coralia, Lazar Veronica, Popescu Radu Cristian, Grigore Raluca, Bertesteanu Gabriel. *Fluorescence analysis of apoptosis induced by Pseudomonas aeruginosa in endothelial cells*. RJME 2014, 55(2):313-317 **IF 0.7**
- Chifiriuc M.C., Grumezescu A. M., Lazar V. Quorum Sensing Inhibitors from the Sea: Lessons from Marine Symbiotic Relationships. *Current Organic Chemistry*. 2014, 18, 823-839 **IF- 2.8**
- Gheorghe Irina, Ilda Czobor, Mariana Carmen Chifiriuc, Elvira Borcan, Camelia Ghiță, Otilia Banu, Veronica Lazăr, Grigore Mihăescu, Dan Florin Mihăilescu, Zong Zhiyong. Molecular screening of carbapenemase - producing Gram negative strains in Roumanian intensive care units during one year survey. *J Med Microbiol*. 2014, 63: 1303-1310. **IF 2.297**
- Gheorghe Irina, Mariana Carmen Chifiriuc, Ani Ioana Cotar, Veronica Lazar. Extended-spectrum Beta-lactamase Production in *Pseudomonas aeruginosa* and *Acinetobacter baumanii* Strains: Epidemiology, Molecular Characterization and Novel Proteomics-based Diagnostic Too ls. *Current Proteomics*. 2014, 11 (2): 108-115 **IF 0.44**
- Czobor Ilda, Irina Gheorghe, Otilia Banu, Alexandra Velican, Veronica Lazăr, Grigore Mihăescu, Mariana-Carmen Chifiriuc. ESBL genes in Multi Drug Resistant Gram negative strains isolated in a one year survey from an Intensive Care Unit in Bucharest, Romania. *Roumanian Biotechnological Letters*. 2014,19(4), 9553-9560. **IF 0.363**.
- Curutiu C., Chifiriuc M. C., Mitache M. *Pseudomonas aeruginosa* -Eukaryotic Cell Crosstalk: Mediators, Mechanisms and Implications for the Antimicrobial Therapy *Current Organic Chemistry*, 2013, 17 (2), 149-154. **IF- 2.8**
- Cotar A., Saviuc C., Nita A., R., Bezirtzoglou E., Lazar V., Chifiriuc, C. M.C. Anti-pathogenic Strategies for Fighting Pseudomonas aeruginosa Infections- probiotic

Soluble Compounds as Inhibitors of Quorum Sensing Genes Expression *Current Organic Chemistry*, 2013, 17 (2), 155-161. **IF- 2.8**

- Chifiriuc M.C. Special Issue on Quorum Sensing Inhibitors: Synthesis, Optimization, and Emerging Biomedical Applications. *Current Organic Chemistry*, 2013, 17 (2), 88-89. **IF- 2.8**
- Limban C., Grumezescu M.A., Chirea M., Matei L., Chifiriuc M.C. Antimicrobial Potential of Benzamides and Derived Nanosystems for Controlling in vitro Biofilm Development on Medical Devices *Current Organic Chemistry*, 2013, 17 (2), 162-175. **IF- 2.8**
- Ion Anghel, Carmen Limban, Alexandru M Grumezescu, Alina G Anghel, Coralia Bleotu and Mariana C Chifiriuc, *In vitro* evaluation of anti-pathogenic surface coating nanofluid, obtained by combining Fe3O4/C12 nanostructures and 2-((4-ethylphenoxy) methyl)-N-(substituted-phenylcarbamothioyl)-benzamides, *Nanoscale Research Letters* 2012, 7:513, **IF 2.481**
- Carmen Limban, Alexandru Grumezescu, Crina Saviuc, Georgeta Voicu, Carmen Chifiriuc, Optimized anti-pathogenic agents based on core/shell nanostructures and 2-((4-ethylphenoxy)methyl)-N-(substituted-phenylcarbamothioyl)-benzamides, *International Journal of Molecular Science*, *13*, 12584-12597, 2012, **IF 2.3**
- Chifiriuc Carmen Mariana, Alexandru Mihai Grumezescu, Crina Saviuc, Cristina Croitoru, Dan Eduard Mihaiescu, Veronica Lazar, Improved antibacterial activity of cephalosporins loaded in magnetic chitosan microspheres, *International Journal of Pharmaceutics*, *Volume 436, Issues 1–2, 15 October 2012, Pages 201-205;* doi: 10.1016/j.ijpharm.2012.06.031, 2012, **IF 3.7**
- Grumezescu Alexandru Mihai, Ecaterina Andronescu, Anton Ficai, Coralia Bleotu, Dan Eduard Mihaiescu, Mariana Carmen Chifiriuc, Synthesis, characterization and *in vitro* assessment of the magnetic chitosan-carboxymethylcellulose biocomposite interactions with the prokaryotic and eukaryotic cells, *International Journal of Pharmaceutics*, 2012, 436 (2012) 771– 777, **IF 3.7**
- Balaure Paul Catalin, Ecaterina Andronescu, Alexandru Mihai Grumezescu*, Anton Ficai, Keng-Shiang Huang, Chih-Hui Yang, Yung-Sheng Lin, Carmen Mariana Chifiriuc, Fabrication, characterization and *in vitro* profile based interaction with eukaryotic and prokaryotic cells of alginate-chitosan-silica biocomposite. *International Journal of Pharmaceutics*, 2012, **IF- 3.7**

- Grumezescu A. M., A. Ficai, D. Ficai, G. Prdean, M. C. Chifiriuc, Polymeric magnetic silica microspheres as a drug loader for antimicrobial delivery substances, *Digest Journal of Nanomaterials and Biosctructures*, Vol. 7, No. 4, October-December 2012, p. 1891-1896.
- Mariana C Chifiriuc, Valentina Grumezescu, Alexandru M Grumezescu*, Crina M Saviuc, Veronica Lazar, Ecaterina Andronescu, Hybrid magnetite nanoparticles/Rosmarinus officinalis essential oil nanobiosystem with antibiofilm activity, *Nanoscale Research Letters*, 2012, 7:209 **IF 2.481**

2. International conferences

- Gheorghe, Â. Novais, F. Grosso, C. Rodrigues, C. Chifiriuc, V. Lazăr, L. Peixe. Identification of particular clonal complexes and mobile elements associated with the dissemination of *bla*$_{\text{OXA-23}}$-carrying *Acinetobacter baumannii* and *bla*$_{\text{VIM-2}}$-carrying *Pseudomonas aeruginosa* in Romania. Poster P0994. The 24rd European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain, May 2014.
- Pires J., Â. Novais, L. Silva, J. Campos, J. Bothelho, I. Czobor, I. Gheorghe, L. Peixe. Further validation of Blue-Carba, a recently described quick and reliable method for carbapenemase detection. Poster eP327. The 24rd European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spania, May 2014.
- Holban A.M., C. Bleotu, M.C. Chifiriuc, L.M. Diţu, L. Marutescu, C. Curutiu, V. Lazar. The impact of the Pseudomonas aeruginosa culture fractions on mesenchymal stem cells morphophysiology. ESCMID Conferences, Barcelona, Spain, May 2014
- Holban Alina M., Stephan Heeb, Mariana C. Chifiriuc, Paul Williams, Veronica Lazar. Host stress hormone noradrenaline interferes with Pseudomonas aeruginosa social behaviors in an iron dependent manner. FEBS/EMBO Conference, Aug-Sept Paris, France, 30th August-4th Sept 2014, oral presentation - travel grant fellowship
- Ecovoiu, A., Ratiu, A.C., Czobor, I., Chifiriuc, M. Whole genome expression profiles of *Drosophila melanogaster* consecutive to *Pseudomonas aeruginosa* infection allow selection of new model genes implicated in host-parasite relationship. 24th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain - P0130, May 2014
- Ecovoiu, A. Ratiu, A.T., Czobor, I., Chifiriuc, M.C. Qualitative and quantitative assessment of *Drosophila melanogaster* native microbiota. The 35th International

Congress of the Society for Microbial Ecology and Disease (SOMED). Valencia, Spania, May 15th - 17th, 2012.

3. IDB published papers acknowledging the project Idea 154/2011

- Cotar A.I., **Chifiriuc** M.C., Banu O., Lazar V.Molecular characterization of virulence patterns in *Pseudomonas aeruginosa* strains isolated from respiratory and wound samples. Bionterface Research in Applied Chemistry, 3(2), 551-558
- Holban A.M., Chifiriuc M.C., Lazăr V. Host cells response in *Pseudomonas aeruginosa* infections-role of quorum sensing African Journal of Microbiology Research, 2013, 7(21), 2420-2429.
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4. National conference with international contributions

- Irina Gheorghe, Ângela Novais, Filipa Grosso, Carla Rodrigues, Carmen Chifiriuc, Veronica Lazar, Luisa Peixe. Genetic characterization of *Pseudomonas aeruginosa* $bla_{\text{VIM-2}}$ and *Acinetobacter baumannii* $bla_{\text{OXA-23}}$ resistant to carbapenems in România Scientific Session of The Students of the Faculty of Biology. Anniversary Ed. Bucharest, 2014
- Ecovoiu, A. Ratiu,A.T., Czobor, I., Chifiriuc, M.C. *Drosophila melanogaster*, eukaryotic model for studying the host-parasite interactions in experimental infections with *Pseudomonas aeruginosa*. Diaspora Conference 2012. 21-24 Sept. 2012.