Project type: EXPLORATORY RESEARCH PROJECTS - PN-II-ID-PCE-2011-3

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.

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

Project publications

1. **International conferences**

   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. Al. Ecovoiu, Attila Cristian Ratiu, Ilda Czobor, Mariana Carmen Chifiriuc

2. **National conference with international contributions**

   Drosophila melanogaster, eukaryotic model for studying the host-parasite interactions in experimental infections with *Pseudomonas aeruginosa*. Diaspora Conference 2012. 21-24 Sept. 2012. Alexandru Al. Ecovoiu, Attila Cristian Ratiu, Ilda Czobor, Mariana Carmen Chifiriuc

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

   3.1. 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

   3.2. Holban A.M., Chifiriuc M.C., Lazăr V. Host cel ls response in *Pseudomonas aeruginosa* infections-role of quorum sensing African Journal of Microbiology Research, 2013, 7(21), 2420-2429. **SRI 0.49**

4. **ISI published papers acknowledging the project Ideas 154/2011**

1. 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. **SRI 2.13**
2. 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. **SRI 2.13**

3. Chifiriuc M.C. Special Issue on Quorum Sensing Inhibitors: Synthesis, Optimization, and Emerging Biomedical Applications. *Current Organic Chemistry*, 2013, 17 (2), 88-89. **SRI 2.13**
4. 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. **SRI 2.13**
5. 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, **SRI 1,64**
6. 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, **SRI- 1,6**
7. 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, **SRI- 1,31**
8. 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, **SRI 1,31**
9. 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, **SRI- 1,31**

10. 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.
11. 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 doi:10.1186/1556-276X-7-209, **SR-1,64**