REQUIREMENT: Knowledge of molecular biology and genetics!

SELECTION CRITERIUM: Lattes curriculum analysis.

Course outline:

“Evolutionary and genomic approaches applied to microbial production of bio-based chemicals”

Proposed for the PhD Program in Bioenergy (UNESP, USP, and UNICAMP)

Offered by Dr. Jeferson Gross (jeferson.gross@gmail.com)

and Dra. Ana Paula Jacobus

Laboratory of Genomics and Experimental Evolution of Yeasts

(Institute for Research in Bioenergy, IPBEN, UNESP- Rio Claro)

Course length: 15 weeks.

Period of the year: To be held every second semester of the year.

Webpage: https://yeastevolution.wixsite.com/yeastevolution

Aimed audience: PhD students with a background in biological sciences and with knowledge of molecular biology and genetics.

Motivation:

The world is rapidly embracing the concept of “green chemistry”. Under this new paradigm, microorganisms (bacteria and single-cell eukaryotes) have important functions in biomass conversion into fuels and chemicals. For this reason, intense research is conducted worldwide to develop microbial strains suited for the new industry. More than ever before, revolutionary “omics” techniques (such as next generation sequencing, metabolomics or epigenomics) are combined to metabolic and evolutionary engineering approaches in vigorous research programs to shape microorganisms for new applications. On the top of that, breakthroughs in synthetic biology are even allowing the design of entire bacterial and yeast chromosomes to fulfill biotechnological tasks. The goal of this course to empower Ph.D. students to understand these new "omics" and synthetic biology technologies, and to learn how modern metabolic and evolutionary engineering are key approaches to develop microbial strains for the bio-based industry.

Course structure and focus:

The course will span a period of 15 weeks over one semester, with one class of four hours per week. The central focus will be the presentation and discussion of key up-to-date papers, covering the fields of structural and functional genomics, evolutionary and metabolic engineering, and systems/synthetic biology. All subjects are applied to microorganisms (bacteria and single-cell eukaryotes) relevant for production of bio-based chemicals and fuels. The topic of each class is proposed in advance, as listed below. The class will start with a theoretical exposure by the instructors, giving a general view of the field in focus. It follows a presentation and discussion (held by the students, invited speakers or the instructors themselves) of one or two relevant papers on the topic. After each oral presentation, students will be engaged in oriented discussions. Questions will be posed about the methods used or the scientific scope and prospects of each chosen paper. Students` final grading will be drawn from their presentations and participation in class discussions.

PROPOSED COURSE CONTENT PER WEEK (SUBJECTED TO ALTERATIONS IN 2021!):

1) **Course overview; basic concepts in experimental evolution, molecular genetics, and genomics.**

2) **<u>Week2</u>: Fundaments of experimental evolution:**

"Adaptive laboratory evolution – principles and applications for biotechnology". Dragosits and Mattanovich. Microbial Cell Factories, 12:64, 2013.

"Under pressure: evolutionary engineering of yeast strains for improved performance in fuels and chemicals production". Mans R, Daran JG, Pronk J. Curr Opin Biotechnol. 2018 Apr; 50:47-56.

"Selecting the Best: Evolutionary Engineering of Chemical Production in Microbes." Denis Shepelin ID , Anne Sofie Lærke Hansen, Rebecca Lennen, Hao Luo ID and Markus J. Herrgård. Genes (Basel). 2018 May 11;9(5).

3) **<u>Week3</u>: Metabolic and Evolutionary engineering for production of valuable products**

"Anaerobic and micro-aerobic 1, 3-propanediol production by engineered Escherichia coli with dha genes from Klebsiella pneumoniae GLC29." PMA Neto, et al., 2017. African Journal of Biotechnology 16 (35), 1800-1809.

"Increased production of L-serine in *Escherichia coli* through Adaptive Laboratory Evolution." Hemanshu Mundhada et al. Metabolic Engineering, 39, 141-150, 2017.

4) **<u>Week4</u>: Evolutionary engineering applied to microbial metabolism:**

"Evolutionary insight from whole-genome sequencing of experimentally evolved microbes." Dettman JR1, Rodrigue N, Melnyk AH, Wong A, Bailey SF, Kassen R. Mol Ecol. 2012 May;21(9):2058-77.

"Generation of an E. coli platform strain for improved sucrose utilization using adaptive laboratory evolution." Elsayed T. Mohamed, et al. Mohamed et al. Microb Cell Fact (2019) 18:116

"Evolutionary engineering of Escherichia coli for improved anaerobic growth in minimal medium accelerated lactate production." Baowei Wang et al. Applied Microbiology and Biotechnology (2019) 103:2155–2170.

5) **Week5: Genome projects of biotechnological relevant microorganisms:**

"Genome structure of a *Saccharomyces cerevisiae* strain widely used in bioethanol production" Argueso et al. Genome Res. Dec; 19(12): 2258–2270, 2009.

"Genomic analysis of thermophilic *Bacillus coagulans* strains: efficient producers for platform bio-chemicals". Fei Su & Ping Xu. Scientific Reports 4, 3926, 2014.

6) **Week6: RNA-seq applied to microbial biotechnology**

"Transcriptional profiling reveals molecular basis and novel genetic targets for improved resistance to multiple fermentation inhibitors in *Saccharomyces cerevisiae*". Chen et al. Biotechnol Biofuels, 9:9, 2016.

"Comparative transcriptomics elucidates adaptive phenol tolerance and utilization in lipid-accumulating *Rhodococcus opacus* PD630". Yoneda et al. Nucleic Acids Research, Vol. 44, No. 5, 2016.

"Unravelling evolutionary strategies of yeast for improving galactose utilization through integrated systems level analysis". Hong K-K et al. vol. 108 no. 29, 2011.

7) **Week7: Modern Genomics applied to environmentally relevant microorganisms:**

Paper yet to be assigned

8) **Week8: Evolutionary engineering of stress tolerance to fermentation products 1:**

"Experimental evolution: its principles and applications in developing stress-tolerant yeasts." Krishna B. S. Swamy & Nerve Zhou. Applied Microbiology and Biotechnology (2019) 103:2067–2077

"Adaptation to High Ethanol Reveals Complex Evolutionary Pathways." Voordeckers et al. PLoS Genet 11(11): e1005635, 2015.

"Improvement of isopropanol tolerance of *Escherichia coli* using adaptive laboratory evolution and omics technologies." Horinouchia et al. Journal of Biotechnology, 255, 47–56, 2017.

9) **Week9: Evolutionary engineering of the xylose metabolism**

"Unraveling the genetic basis of xylose consumption in engineered Saccharomyces cerevisiae strains" Santos LV et al. Scientific Reports 6, 38676, 2016.

"Development of a D-xylose fermenting and inhibitor tolerant industrial *Saccharomyces cerevisiae* strain with high performance in lignocellulose hydrolysates using metabolic and evolutionary engineering". Demeke et al. Biotechnology for Biofuels, 6:89, 2013.

10) **Week10: Evolutionary engineering of stress tolerance to fermentation products 2:**

"Metabolic engineering and adaptive evolution for efficient production of D-lactic acid in *Saccharomyces cerevisiae*." Baek SH et al. Appl Microbiol Biotechnol, 100(6):2737-48, 2016.

"GSF2 deletion increases lactic acid production by alleviating glucose repression in *Saccharomyces cerevisiae*" Baek SH et al. Scientific Reports 6, Article number: 34812, 2016."

11) **Week11: Evolutionary engineering of stress tolerance to environmental factors:**

"Altered sterol composition renders yeast thermotolerant". Caspeta et al. Science, 346(6205):75-8, 2014.

“Thermotolerant yeasts selected by adaptive evolution express heat stress response at 30°C.” Caspeta et al. Scientific Reports 6, 27003, 2016.

12) Week12: Evolutionary engineering of stress tolerance to fermentation inhibitors:

“A new laboratory evolution approach to select for constitutive acetic acid tolerance in *Saccharomyces cerevisiae* and identification of causal mutations”. Daniel González-Ramos et al. Biotechnol Biofuels, 9:173, 2016.

“Evolutionary Engineering of *Saccharomyces cerevisiae* for Enhanced Tolerance to Hydrolysates of Lignocellulosic Biomass.” Almario et al. Biotechnol Bioeng., 110(10):2616-23, 2013.

13) Week13: Proteomics and metabolomics applied to microbial biotechnology

“Quantitative proteomic analysis of the influence of lignin on biofuel production by *Clostridium acetobutylicum* ATCC 824.” Raut et al. Biotechnol Biofuels, 9:113, 2016.

“Recent applications of metabolomics to advance microbial biofuel production.” Julia I Martien and Daniel Amador-Noguez. Current Opinion in Biotechnology, 43:118–126, 2017.

“Metabolic constraints on the evolution of antibiotic resistance”. Mattia Zampieri et al. Mol Syst Biol. (2017) 13: 917.

14) Week14: Experimental evolution with synthetic microorganisms

“Adaptive evolution of genomically recoded Escherichia coli.” Wannier et al. PNAS | March 20, 2018 | vol. 115 | no. 12

“SCRaMbLE generates evolved yeasts with increased alkali tolerance”. Ma et al. Microb Cell Fact (2019) 18:52

15) <u>Week15</u>: **Synthetic genomes of microorganisms for biotechnological purposes.**

"Creation of a bacterial cell controlled by a chemically synthesized genome." Gibson DB et al., Science, 329(5987):52-6, 2010.

Documentário "Vida sintética"

Grading (evaluation):

Students´ grading will be based on their oral presentations, participations in paper discussions.

Bibliography:

Barrick, J. E., Lenski, R. E. Genome dynamics during experimental evolution. *Nat Rev Genet* **14**, 827-39 (2013).

Lenski, R.E. Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. *ISME J* (2017).

Dragosits, M. & Mattanovich, D. Adaptive laboratory evolution -- principles and applications for biotechnology. *Microb Cell Fact* **12**, 64 (2013).

Winkler, J.D. & Kao, K.C. Recent advances in the evolutionary engineering of industrial biocatalysts. *Genomics* **104**, 406-11 (2014).