Course outline:

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

Advanced Topics in Methods of Biofuel Production

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

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(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: www.yeastevolution.com

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. As an additional task, students will be guided, throughout the semester, to develop an extended abstract (up to 1000 words written in English) related to a paper presented during the course. This scientific writing is supervised by the instructors, who will guide students towards a final improved version. Students` final grading will be drawn from their presentations, participation in class discussions, and from the extended abstracts.

<u>Proposed course content per week:</u>

1) **<u>Week 1</u>: Course overview; basic concepts in experimental evolution, molecular genetics, and genomics.**
2) **<u>Week 2</u>: Fundaments of experimental evolution:**

   "Genome dynamics during experimental evolution" Jeffrey E. Barrick and Richard E. Lenski. Nat Rev Genet., 14(12): 827–839, 2013.

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

3) **Week 3: Genome projects of biotechnological relevant microorganisms 1:**

"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.

4) **Week 4: Evolutionary engineering applied to microbial metabolism:**

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

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

5) **Week 5: Genome projects of biotechnological relevant microorganisms 2:**

"Genome Sequence and Analysis of a Stress-Tolerant, Wild-Derived Strain of *Saccharomyces cerevisiae* Used in Biofuels Research". McIlwain SJ. Et al. G3 (Bethesda). Jun; 6(6): 1757–1766, 2016.

"Comparative genomics of xylose-fermenting fungi for enhanced biofuel production". Wohlbach ey al. PNAS, vol. 108 no. 32, 2011.

6) **Week 6: Yeast population genomics (pan-genomics):**

"The 100-genomes strains, an *S. cerevisiae* resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen". Strope PK et al. Genome Res., 25(5): 762–774, 2015.

"Comparative Genomics of *Saccharomyces cerevisiae* Natural Isolates for Bioenergy Production". Wohlbach et al. Genome Biol. Evol. 6(9):2557–2566, 2014.

7) **Week 7: 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.

8) **Week 8: 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.

9) **Week 9: Evolutionary engineering of stress tolerance to fermentation products 1:**

"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.

10) **Week 10: 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) **Week 11: 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) **Week 12: Proteomics applied to microbial biotechnology**

"Comparative Proteomics Analysis of Engineered *Saccharomyces cerevisiae* with Enhanced Biofuel Precursor Production."Tang et al. PLoS ONE 8(12): e84661, 2013.

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

13) **Week 13: 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.

14) **Week 14: Metabolomics applied to microbial biotechnology:**

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

"A metabolomics-based strategy for identification of gene targets for phenotype improvement and its application to 1-butanol tolerance in *Saccharomyces cerevisiae*." Teoh et al. Biotechnol Biofuels,8:144, 2015.

15) **Week 15: 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.

"Total synthesis of a functional designer eukaryotic chromosome". Annaluru N et al. Science, 344(6179):55-8, 2014;

Grading (evaluation):

Students´ grading will be based on their oral presentations, participations in paper discussions, and the writing of an extended abstract.

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).