Adaptative dynamics of *S. cerevisiae* in fluctuating environnements

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Evolution under the rules of natural selection is usually considered through the prism of replicator dynamics. This framework assumes that the population is in constant exponential growth so that adaptive mutations, which increase the growth rate of individuals, can slowly take over the ancestral genotypes after many generations. However, in most natural habitats, microbes live in limited resources and remain only for short times (*i.e.* 5 to 10 generations depending on the initial dilution) in exponential phase. This means that most of the time microbial populations are at or near steady state without being diluted. In addition, a number of natural habitats exhibit daily and seasonal variations, which has forced microbes to cope with unsteady conditions. Thus, understanding how microbial populations near steady state imprint and respond to environmental variations is key for understanding the fundamentals of adaptive dynamics.

Traditionally, experimental evolution relies on serial dilutions or chemostats. Chemostats allow for continuous culture in exponential phase but do not allow for medium replacement. On the contrary, serial dilutions allow for medium replacement but impose strong selective bottlenecks at each passage. In order to experimentally investigate how a population near steady states behaves in fluctuating environments, we designed an automated bioreactor to impose environmental cycles, while retaining the whole population despite medium changes. We propose to use this device to measure the adaptive dynamics of the genetically well-characterised micro-organism, *Saccharomyces cerevisiae*, the yeast used in baking, brewing and winemaking.

In addition, rather than randomly fishing for adaptive traits, we propose to study the adaptation of a genetic switch in a well-defined set of environmental conditions. The central question we will address in the thesis project aims at determining how the switching rates between the two strategies adapt to the rate of environmental changes. To achieve this goal, we will control key parameters like the rate of environmental changes, the level of the selective pressure and the initial stoichiometry of the two strategies.

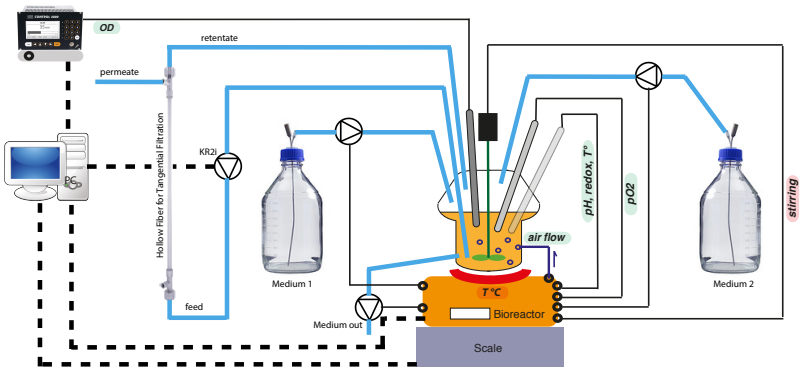

FIGURE 1 – Schematic of the bioreactor composed of a fermenter (Lambda) and a system for changing the culture medium (KR2i, Spectrum Labs). The filtration system uses a hollow fiber to perform a tangential filtration.

The bioreactor

The core of the bioreactor is composed of a vessel that holds a 400mL culture. The bioreactor is equipped with probes that measure environmental parameters like pH, RedOx potential and dissolved oxygen, as well as an OD (= optical density) probe that measures the population size. The vessel is connected to two 10L bottles that deliver the two media. Medium renewal is insured by peristaltic pumps. In order to retain cells while we change the environmental conditions, we will use tangential filtration. As a result, the entire population or part of it, as we decide, can be switched to a different environment in a couple of minutes. The whole setup is interfaced with LabView (National Instruments) in order to automatise measurements and the temporal sequence of environmental changes (Fig. 1).

A two-state strategy in fluctuating environments

We aim to study a situation for which we provide the initial population with means to switch between two phenotypes and measure how the switching frequency between the two phenotypes adapts to the rate of environmental changes. Therefore, we will use a genetic construct (collaboration with G Fischer, Sorbonne-Université) that allows for switching between two states. As shown in Fig. 2a, the *ura3* gene, which codes for an enzyme necessary for uracil synthesis, is split into two parts (light and dark blue) and flanked with recombination sites (pink). Thus a recombination event can either take the two parts apart or bring them together. So depending on recombination events, cells can activate or not the *ura3* gene, thus synthesise or not uracil.

We then will alternate between two distinct environments N and P (Fig. 2b), while keeping the size of the population despite media changes. We designed the two environment such that each phenotype is adapted to one condition but performs poorly in the other. Medium N contains Uracil but also a toxic chemical 5-FOA that kills cells producing Uracil. Medium N is thus favourable for the fraction of the population, that does not synthesise uracil. Medium P contains neither Uracil nor 5-FOA. Thus, cells that express *ura3* are favoured in medium P (positive), while they are killed in medium N (negative). All the experiment will be done under constant medium perfusion (chemostat mode).

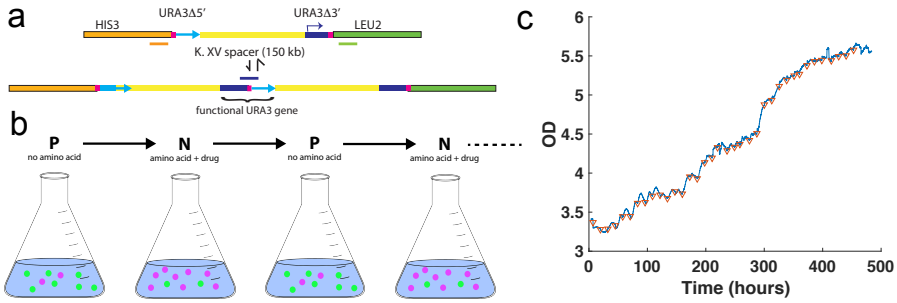

FIGURE 2 – **a)** The *ura3* gene, which is necessary for Uracil synthesis, is split in two pieces (light and dark blue regions) and flanked by a sequence that allows homologous recombination (pink region). **b)** Medium P positively selects for Uracil production, as it does not contain Uracil. Medium N, negatively selects for Uracil production, as it contains a drug (5-FOA), which inhibits growth if yeast synthesize Uracil. **c)** Dynamics of the population size under environmental changes. Medium changes are labelled with open orange triangles.

Adaptive dynamics

Important dimensionless parameters are $\alpha = \frac{\nu_{change}}{\nu_{growth}}$, where ν_{change} is the frequency of change between N and P media, and ν_{growth} is the yeast growth rate. In the case of $\alpha \ll 1$, environmental

variations are slower than generation time and the evolution of the system is expected to be essentially genetic. In the case of $\alpha \gg 1$, environmental variations are faster than generation time and the evolution of the system is expected to be essentially non-genetic (*i.e.* at the metabolic level).

In our system, it is easy to control these two parameters independently. We can control ν_{growth} by adjusting the renewal rate of the medium (*i.e.* the perfusion rate), and we impose ν_{change}. Typically, we will be able to scan a range of ν_{growth} from $1h^{-1}$ to 0.1^{-1} (corresponding to division rates from 1h to 10h) and a range of ν_{change} from $1h^{-1}$ to 0.05^{-1} (corresponding to change frequencies from 1h to 1j).

We started a collaboration with David Lacoste (Gulliver, ESPCI) to model this system in the absence of adaptation, *i.e.* where switching rates does not depend on the history of the culture. The model is based on a set of linear coupled ODEs that describe the temporal dynamics of the two subpopulations plus periodic changes in the medium composition (with or without uracil). With this model we can already capture the linear increase of the total population size (Fig. 2c). In order to test model predictions, we will regularly sample and sequence (by quantitative PCR) the population in the bioreactor in order the measure relative frequency of the two phenotypes.

Finally, we also plan to make environmental changes unpredictable to measure how it impacts the switching frequency.

A considerable amount of theoretical work has been devoted to understand optimal evolutionary strategies under fluctuating environment, but it has still seldom be confronted to experimental validation. In addition, as mentioned above, experimental strategies of environment switching have relied almost exclusively on serial dilution of the population, imposing strong selective pressure on the genetic making of the population. Our approach will allow to test evolutionary strategies in a more general context and will therefore provide a strong test for evolutionary models. In addition, since we have full control on the number of generation across the experiment, our study will likely provide an important test on the role of non-genetic mechanisms on the adaptive route followed by a population under fluctuating constraints, which is an important question emerging in the field of experimental ecology.

PhD program and candidate profile

The work of the PhD candidate will first consists in improving the design and the automation of the bioreactor. A considerable part of the work will then be dedicated to data analysis (fluctuations, correlations, etc...). The project will start by measuring the population dynamics for constant switching rates. We will then vary the environmental pressure in the two media by adding small amounts of Uracil in the medium P and lowering the concentration of 5FOA in the medium N to change either the growth rate or the carrying capacity of the environment. We will also vary the initial fraction of the two subpopulations in order to test the sensitivity to initial conditions.

The candidate should have a good expertise in instrumentation and data processing to be able to face the development of a dedicated bioreactor that would allow to dynamically control the external environment while monitoring the population dynamics. Notions in molecular biology would be a plus, but are not mandatory.

We believe that the complementarity competences between our two groups (ND, JBB), together with regular discussions with the group of David Lacoste, will provide a highly favourable basis for the success or our project, and a motivating and interdisciplinary environment allowing the fulfilment of a multi-skilled young scientist.

References

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