PRACTICAL CLASS IN MAURICE GENEVOIX HIGH SCHOOL

Notions we wanted to transmit

- **Yeasts:** Yeasts reproduce by budding or more rarely by scissiparity. Yeasts form conical colonies on a petri dish, unlike bacteria that tend to form colonies and fungi. Their size (5 to 15 µm) is greater than that of bacteria (1 to 3 µm), which makes them easy to observe at 400 magnification.
- **Saccharomyces cerevisae Ade2-:** It is a haploid unicellular eukaryote. It is the smallest known eukaryotic genome: 14,000 Kb and 6200 genes. The strain used carries a mutation that affects the ade2 gene involved in the adenine biosynthetic chain. The function of the ade2 gene is to transform an intermediate of this chain: Amino Imidazole Ribotide (AIR), which is oxidized to a red pigment in aerobic state. The Ade2- mutation has two effects:

As the strain is unable to synthesize adenine, it will not grow on minimal medium without adenine.

If you add adenine by supplementing the culture medium (or growing on rich medium), the mutated strain will use it to grow. Since the amount of adenine supplied to the strain is not very important, the strain does not have enough adenine to grow to the best of its ability. The strain will therefore operate the adenine biosynthetic chain. It will present a red phenotype because of the accumulation of the AIR and its oxidation.

- **Biosynthesis chain of adenine:** The red phenotype and the inability to grow on a poor environment allow us to have a "positive selection" visible to the naked eye and a selection system without the use of antibiotics. Strains which carry the Ade2- mutation after growth on a petri dish are thus easily differentiated. In fact, on a rich or adenine-enriched box, the yeasts without mutation will grow and have a white phenotype, whereas the Ade2- yeasts will grow but will have a red phenotype. On a low medium box without adenine, the unmutated yeasts grow and are white while the yeasts Ade2- do not grow.

- **Note:** Mutations are common in yeast Ade2- because the accumulating AIR pigment is toxic and increases the selection pressure. It is common for yeast to spontaneously mutate at the level of the respiratory chain so as to avoid oxidizing the adenine biosynthesis intermediate (AIR) to a toxic compound. These mutated colonies are white and larger than the red ones because they grow more easily (unhindered by the toxic pigment): it does not mean that they have recovered the ability to synthesize their own adenine.

- About mutagenic agents - UV concepts: The UVs used are type C and wavelength 254 nm. The DNA molecule can absorb UV light at 254 nm; UV causes mutations: uptake of UV energy by DNA results in the formation of adjacent thymine dimers, which causes breaks within the molecule due to distortions of the double helix. In the majority of cases, these breaks will be repaired by the enzymes involved in DNA replication (nucleases, polymerases, etc.). Only these enzymes can make mistakes and mutations can appear. The more one solicits these enzymes, the more the risk of appearance of mutation is important. These mutations can be lethal (if they affect a gene responsible for the synthesis of a vital protein) or not and, in this case, we have the appearance of a "mutant". The manipulation involves exposing our mutated strain to UV and observing the appearance of white colonies on medium supplemented with adenine.