

5-Laboratory and large-scale culture

5-1-Laboratory culture:

Depending on the objective, fungi can be cultivated on solid media (in Petri dishes, test tubes or beakers) or on liquid media (in tubes or beakers).

Examples: If the goal is to study the microscopic appearance of mycelia and colonies, the culture is carried out on Petri dishes. If the goal is to preserve the fungi for a certain period, it is preferable to carry out a culture on slanted agar (in tubes).

*Cultivation media:

Numerous media are used in mycology, including Sabouraud and Potato Dextrose Agar. These media promote fungal growth and inhibit other microorganisms, particularly bacteria. They are referred to as "routine media" or "standard media."

To promote the growth of a particular group, family, or genus of fungi, "specific media" are used. These consist of a standard medium to which a growth inhibitor has been added. For example, Sabouraud medium with chloramphenicol and gentamicin.

* Environmental and nutritional factors

Although they are relatively undemanding, filamentous fungi require a number of nutritional and environmental factors such as aeration, pH, water availability, nutrients, and temperature for their growth.

* **The temperature** : The growth temperature of fungi varies between 25 and 35°C. In general, 25°C is an ideal temperature for the development of molds and 30 to 37°C is the appropriate range for the growth of yeasts (yeasts pathogenic to humans are preferably cultivated at 37°C).

* **pH** : Fungi grow in pH levels ranging from 4.5 to 8.0, but the majority of species prefer a slightly acidic pH, between 5.5 and 6.5.

* **oxygen** : Fungi are facultative aerobes, even though several

Some of them ferment carbohydrates. However, a few species are anaerobic and colonize specific habitats such as the rumen of animals.

* **The light** It is not essential for the vegetative growth of fungi. However, light can play a role in sporulation: it promotes sporogenesis in some species.

***Aw**: The amount of water available in the substrate and the surrounding environment are very important for mold growth.

***Nutrients**:

The most important are carbon and nitrogen, used in the form of organic compounds, and mineral ions (potassium, phosphorus, magnesium, calcium, etc.) in very small but essential quantities for stimulating and directing fungal growth. These nutrients are accessible to molds because they are available in nature.

5-2-Large-scale cultivation:

It is carried out in twin reactors or bio-fermenters, the types of which vary according to:

- The producing strain (yeast or mold, fast-growing or slow-growing, demanding or not, etc.)
- The medium used (liquid or solid, renewed or not, etc.)
- The product sought (constituent of cells or excreted into the environment, primary or secondary)
- The economic cost.

Depending on the setup, two types of cultivation can be carried out:

- **Batch farming or discontinuous farming:**

It requires bioreactors equipped with systems for measuring and adjusting culture parameters. Once the environment and physicochemical conditions become unfavorable (e.g., substrate depletion, acidity), the growth of microorganisms stops (Figure 10).

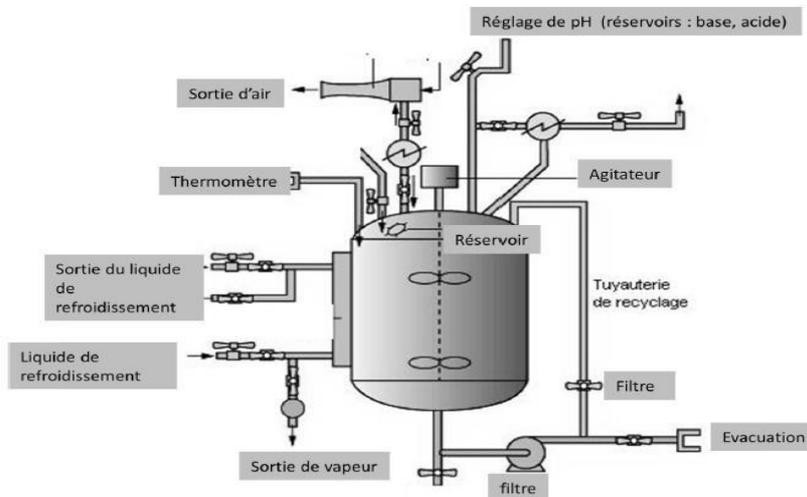


Figure 10:Example of a bioreactor for batch culture.

- "Culture in a renewed environment" or "continuous culture":

In addition to measuring and adjusting culture parameters, the bioreactor must allow for the renewal of the culture medium and strain if necessary.

6. Classification of fungi

The classification of fungi is based primarily on the type of thallus and the type of reproduction (sexual and asexual).

Five divisions are known among fungi: Chytridiomycetes, Oomycetes, Ascomycetes, Basidiomycetes and Zygomycetes. This classification encompasses unicellular thalli (yeasts), filamentous thalli (molds), and primitive thalli (plasmodia).

6.1. The yeasts: Yeasts are fungi with a single-celled thallus. The majority of them reproduce sexually by forming asci containing ascospores. They therefore belong to the Ascomycetes. Examples: *Saccharomyces*, *Candida*.

6.2. The Chytridiomycetes : These are perfect fungi: they are capable of sexual reproduction. These fungi are aquatic. They can live as parasites, saprophytes, or symbionts. They have a primitive (not well-defined) siphonous thallus.

The sexual reproduction of Chytridiomycetes occurs with monoflagellate sex cells called "zooids" or "sexual zoospores". The germination of these cells gives rise to thalli with "gametangia" or "sexual sporangia" which in turn produce zooids

Male or female. By fusing, these form diploid biflagellate zygotes (Figure 11). The transition to the asexual phase occurs through successive mitoses of the zygote, producing a multitude of asexual monoflagellate zoospores. The subsequent germination gives rise to new diploid thalli bearing asexual sporangia, which form the zoospores.

The return to the sexual phase occurs through mitosis of the nuclei of the sporangia, forming flagellated zooids, and the cycle begins again.



Figure 11: Monoflagellate zooids and biflagellate zygotes

6.3. The Oomycetes (Oomycota)

These are perfect fungi with a siphonous primitive thallus. They live in aquatic environments or on very moist soils. Their cell wall is distinguished from that of other fungi by the absence of chitin. They consist of creeping, filamentous thalli that attach to substrates via rhizoids. Oomycetes reproduce asexually with zoospores that lack a cell wall and are motile by means of two dissimilar flagella: one smooth, the other hairy.

"or "Hairy"; they are called "planospores".

Sexual reproduction occurs through the fusion of a male gametangium (spermatocyst) with a female gametangium (oogonium), forming an oospore. Fertilization takes place within the oospore without the release of gametes. The resulting spores are diploid and are released as biflagellate zoospores. After germination, each zoospore develops into a diploid siphonous thallus bearing asexual sporocysts. If conditions become unfavorable, the nuclei of these sporocysts undergo meiosis and regenerate into spermatocysts and oogonia; and the cycle begins again (Figure 12).

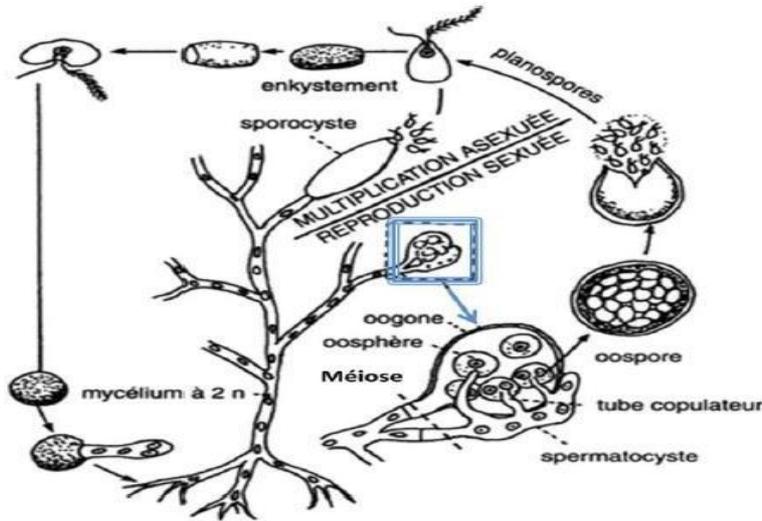


Figure 12:Oomycete reproductive cycle

6-4. Ascomycetes (Ascomycota)

Ascomycetes are perfect fungi. Unlike the first two divisions, the thallus here is well-defined: it is septate. These fungi reproduce sexually by forming asci containing ascospores in even numbers (2, 4, 8, 16, 32, etc.). The ascospores are released through the opening of the upper part of the asci. Therefore, they are described as "exogenous internal" spores (Figure 13).

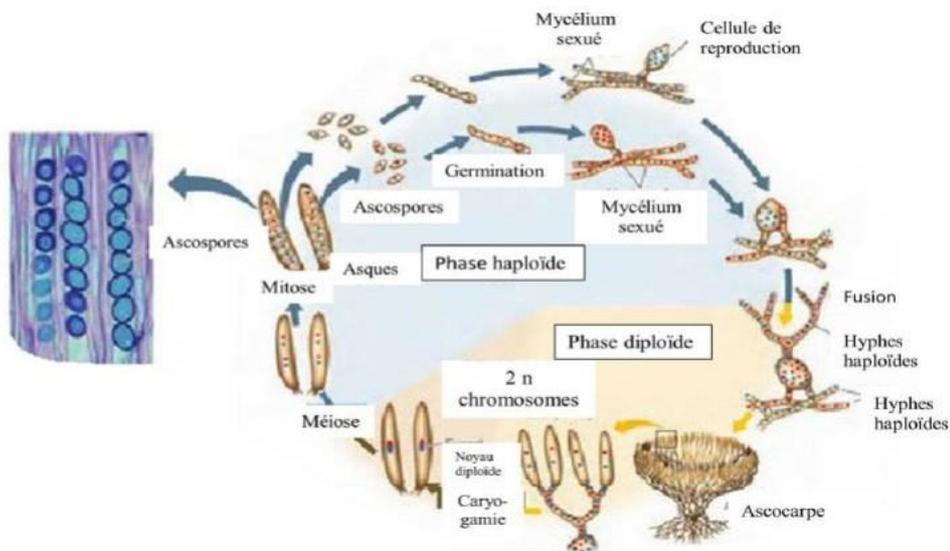


Figure 13:Reproductive cycle of Ascomycetes.

6-5 Basidiomycetes (Basidiomycota)

Basidiomycetes are perfect and higher fungi. They have a well-developed septate thallus and carry out sexual reproduction by forming basidia which produce basidiospores borne on sterigmata (small peduncles).

After meiosis in the basidium, sterigmata allow the haploid nuclei to move to the basidiospores formed directly outside. Once mature, these basidiospores are released by the rupture of the sterigmata. Unlike ascospores, basidiospores are called "external" spores. Figure 14 shows the reproductive cycle of Basidiomycetes.

The basidia are grouped together in a structure called the "basidiocarp" and commonly referred to as the "cap".

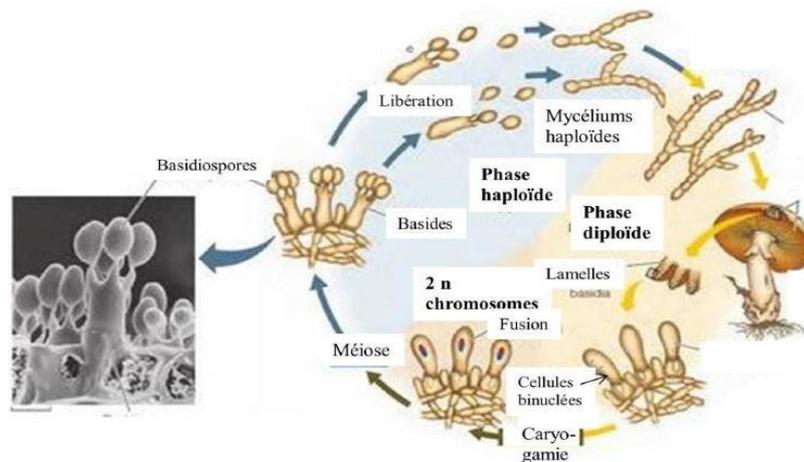


Figure 14:Reproductive cycle of Basidiomycetes.

6-6.The Zygomycetes (Zygomycota)

These are perfect fungi. They have a siphonous thallus and reproduce sexually by the formation of zygospores (Figure 15). Zygomycetes form a mycelium that is often invasive, attached to the substrate with rhizoids and topped with a large number of reproductive organs.

- Sporocysts or gametocysts: These represent the reproductive organs.

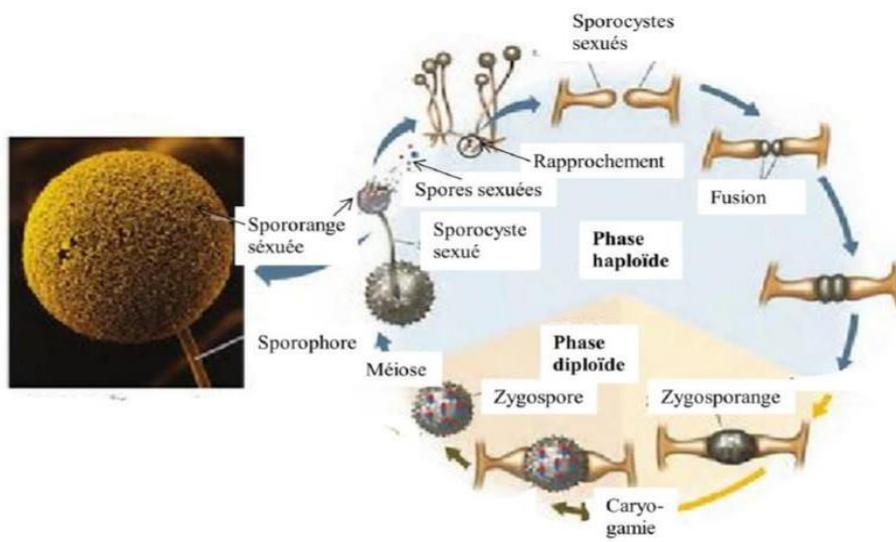


Figure 15:Sexual reproduction of Zygomycetes.