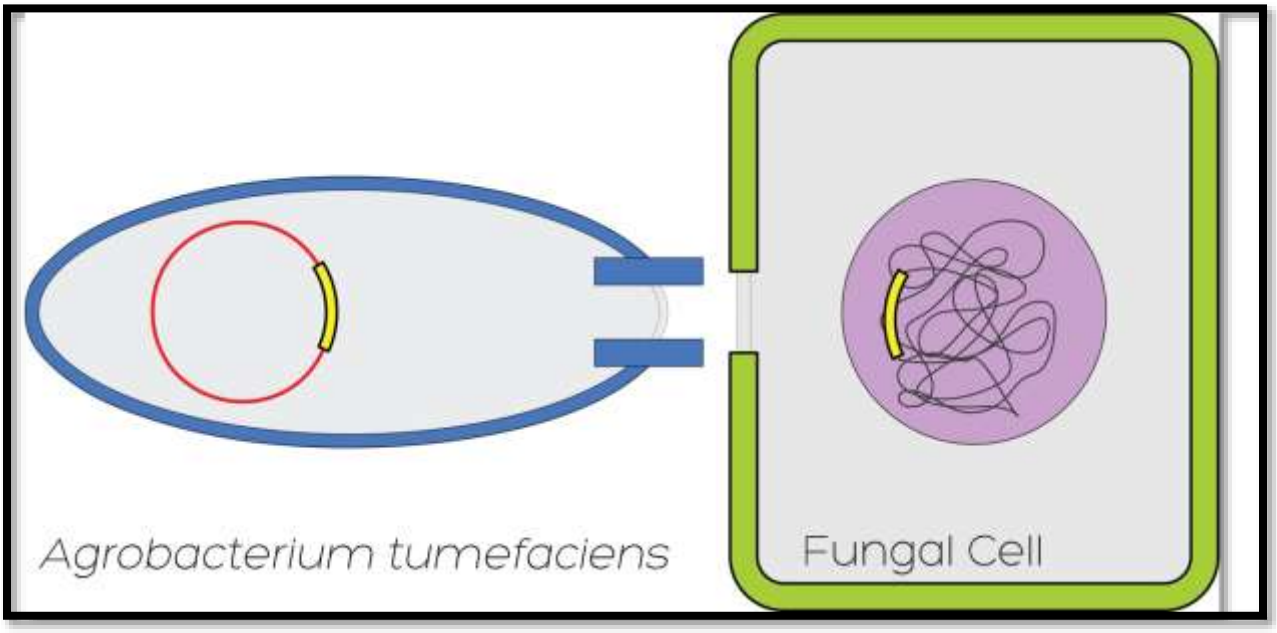
2- *Agrobacterium* -mediated transformation (AMT).

Agrobacterium is a Gram-negative bacterium commonly found in soil. *Agrobacterium tumefaciens* can infect injured plants. The tumor-inducing plasmid of > 200 kb, which is also referred to as the Ti plasmid.

Wide varieties of plant and fungal cells can be easily and efficiently transformed by agrobacterium-mediated transformation. In this process, plant or fungal tissue is co-cultured with *Agrobacterium tumefaciens*, an organism that is able to transfer part of its DNA to plants and fungi, known as T-DNA. Genetic constructs can be inserted into these T-DNA vectors and then transformed into *A. tumefaciens*, allowing it to insert this construct into a plant or fungal genome.

The AMT method opens up a new avenue for those fungi recalcitrant to transformation by conventional methods. The AMT method is especially suitable for generating knock-in mutations in fungi because T-DNA randomly inserts into the genome as a single copy. In addition, AMT can achieve high homologous recombination efficiency in various gene targeting experiments.



(AMT Method)

Major advantages of the AMT method include:

- firstly, diversified transformation recipients, including protoplasts, hyphae, and spores.
- secondly, the ability to integrate exogenous genes into the genome to form stable transformants.
- thirdly, high transformation efficiency resulting in a large number of transformants.

Factors that influence the AMT efficiency

Many factors affect the AMT efficiency, including: the type of starting fungal material (protoplast, spore, hypha, and fruit body tissue), concentration of the acetosyringone (phenolic natural product), ratio of fungus to Agrobacterium, and the condition for co-culturing.

1.The type of starting fungal material

The AMT method can use the protoplasts, spores, hyphae, and fruit body tissue of fungi as the recipient. Appropriate starting materials should be selected for different strains. For instance, the AMT method only works for the protoplasts of *Rhizopus oryzae* and *Mucor circinelloides*, while spores or germinal spores would not produce transformants.

2.The concentration of acetosyringone (AS)

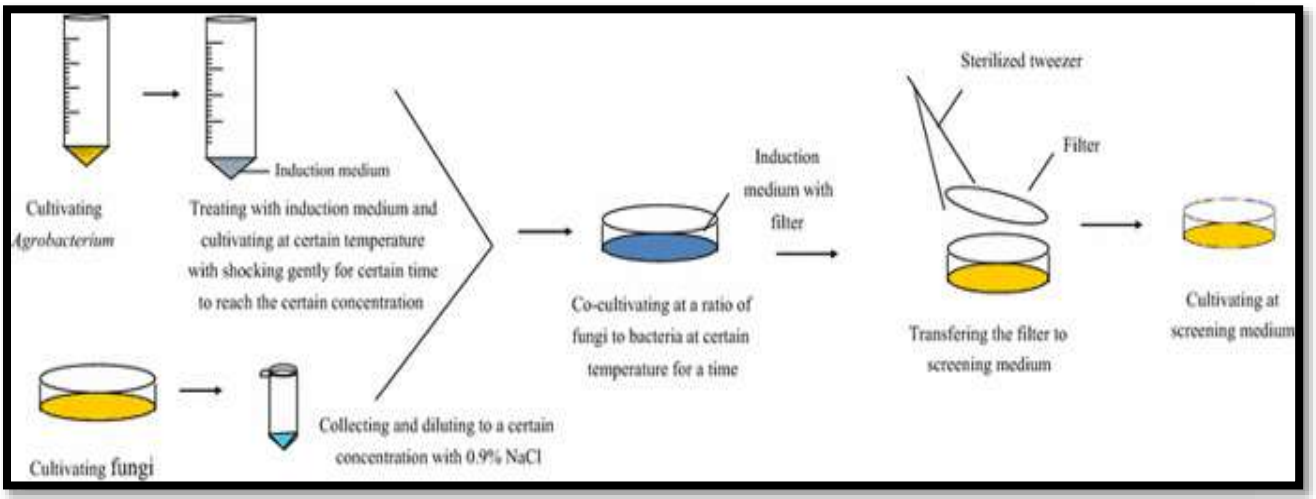
AS acts on two stages during the AMT process. One is the induction process, and the other is the transformation process. AS is generally used to induce the expression of the Vir domain of T-DNA, and the gene in the Vir domain activate the transfer of T-DNA.

3.The ratio of fungi to Agrobacterium Within certain limit

the transformation efficiency will reach the maximum level with the increase of amount of fungus or Agrobacterium. An optimal ratio for the AMT for different fungi must be empirically determined.

4.The condition for co-culturing

An important factor in the AMT method. This includes culture time, temperature, pH, and the selection of filter. The temperature and time for co-culturing are the key factors among the AMT steps. In the fungus-Agrobacterium transformation, an appropriate condition to start is a temperature of 20–28 °C and a co-culturing time of 16–96 h. A lower temperature (20–25 °C) is usually beneficial for the AMT method. The filter, which is hydrophilic and serves as support for fungus-Agrobacterium co-culturing, facilitates the transfer of single colonies to the screening plate. A nitrocellulose membrane, nylon membrane, filter paper, cellophane and polyvinylidene fluoride (PVDF) membrane can be used as the filter.



The basic steps of the *Agrobacterium*–mediated transformation

3- Electroporation transformation:

A simple, rapid, and efficient transformation method for filamentous fungi. electric charges are stored in a capacitor to build a high voltage, the sample is struck by the impulse voltage, and the exogenous nucleic acid can be transferred instantly into cells. Usually, square waves are used in the transformation of fungi

4- Biolistic transformation:

also known as particle bombardment. Its principle is that foreign DNA is adsorbed on the surface of tungsten or gold particles. Under the push of high pressure, the particles are injected into host cells. Particle bombardment can realize both stable and transient transformation.