

## Research Project – Alessandro Cavazzani

Cultured meat is a rising technology that was conceived in order to tackle several emerging issues of traditional farming systems: first of all, as FAO projections (2011, 2017) prospect a world population reaching 11 billion people by the end of the century, along with increasing incomes in developing countries and expanding urban areas, the demand for meat might not be sustainable by conventional meat production processes only anymore; secondly, the last decades have witnessed a rising demand for alternative sources of protein out of an increased awareness of the moral, ethical and environmental issues underlying such processes. As of now, there are still many challenges hindering production scaling-up thus the insertion of cultured meat in the market. Most of these challenges are related to 1) the biological limit given by stem progenitor cells proliferative and differentiative potentials, to 2) the economical limit that's due, among many factors, to the lack of valid alternatives to fully synthetic culture media and to 3) the difficulty of yielding a product that replicates very closely the flavour, colour and texture of conventional meat (Fernandes et al., 2021).

Myoglobin is a heme protein that is highly abundant in muscle tissues. The presence of heme in meat is the main responsible for its red coloration, besides constituting an excellent iron supply. In 2019, Simsa et al. demonstrated that the implementation of myoglobin from equine skeletal muscle in cell culture media increased the proliferation of cultured bovine satellite cells and improved the overall coloration of the resulting 3D skeletal muscle tissue.

Since the possibility of myoglobin production in planta has already been investigated and successfully achieved (Carlsson et al., 2020), this project aims to set up a plant-based production system of myoglobin through the exploitation of biotechnological approaches on edible plants.

For this purpose, tomato plants will be stably transformed through an *Agrobacterium tumefaciens*-mediated approach, utilising binary vectors with the myoglobin gene inserted under either constitutive or fruit-specific promoters, in order to test both a whole-plant production and an organ-specific production approach.

The protein will be expressed in both its native and in a tagged form to facilitate its purification, and biochemical-functional analyses will be carried out in order to check the correct incorporation of heme thus the functionality of the protein.

Finally, the myoglobin produced will be implemented in the lab-scale production of cultural meat in order to verify its beneficial qualities.

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