POSTER COMMUNICATIONS

**Sustainable innovation to improve the quality of meat products in the era of the Green Deal**

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This document describes two activities that were completed as part of the PhD project. The antioxidant potential of 120 Lactic Acid Bacteria (LAB) strains belonging to *Lactiplantibacillus* and *Lacticaseibacillus* spp. was first investigated. Then, a screening to evaluate Nitric Oxide Synthetase (NOS) activity among 50 strains of *Staphylococcus* spp. and LAB strains was also carried out.

Innovazione sostenibile per migliorare la qualità dei prodotti carnei nell’era del *Green Deal*

Nel presente documento vengono descritte due delle attività svolte nell’abito del progetto di dottorato. In primo luogo, è stato investigato il potenziale antiossidante di 120 ceppi di batteri lattici appartenenti a *Lactiplantibacillus* e *Lacticaseibacillus* spp.. Successivamente è iniziato uno *screening* per indagare la capacità di ceppi di *Staphylococcus xylosus* e di batteri lattici di produrre Ossido Nitrico Sintetasi (NOS).

**Key words**: antioxidant activity, lactic acid bacteria, Nitric Oxide Synthase, *Staphylococcus xylosus*.

# **Introduction**

In recent years, there has been a significant increase in the demand for products with clean labels, that is, products with ingredients that are considered natural and healthy. Several authors have demonstrated that LAB have antioxidant capacity and thus could be used to replace synthetic antioxidants in meat products. Furthermore, some LAB and *Staphylococcus* spp. strains have been shown to produce NO from L-arginine via NOS. The activities presented in this work concern the screening of 120 LAB strains for antioxidant potential (A1) and the screening of LAB and *S. xylosus* strains for NOS activity (A2).

# **Materials and Methods**

* 1. Antioxidant activity (A1)

# 120 strains of LAB belonging to the species *Lacticaseibacullus casei, Lcb. paracasei, Lcb. rhamnosus* and *Lactiplantibacillus plantarum*, isolated from different reservoirs such as dairy products, wine and wine cellars, bread dough, faeces, human body, and coffee, were tested for their antioxidant potential after adaptation under both anaerobic condition and after activation of aerobic metabolism, as reported by Zotta *et al.* (2014). The cells were grown for 18 h at 30 °C, then they were centrifuged (6000 xg, 10 min), the pellet was resuspended in PBS (Sigma-Aldrich, Milan, Italy) and after standardization of their concentration, an inoculum of 106 CFU/mL was performed in MRS broth and supplemented M17 broth. This step was repeated twice and finally after centrigugation the supernatants and the washed cells were analysed using the DPPH method as described by Cao *et al.* (2019) and Yu *et al.* (2020), while two commercial kits, ABTS Assay Kit and Ferric Reducing Antioxidant Power (FRAP) Assay kit (Bioquochem, Asturias, Spain) were adopted following the manufacturer instructions for the ABTS and FRAP assay, respectively.

* 1. NOS enzymatic activity in *S. xylosus* and Lactic Acid Bacteria (A2)

Nitric Oxide Synthase activity of 50 strains of *S. xylosus* were investigated using a commercial kit Nitric Oxide Synthase (NOS) Activity Assay Kit (Colorimetric) (Sigma-Aldrich, Milan, Italy), following the protocol reported by the manufacturer.

Finally, 100 μL of each strain suspension was standardized at 107 CFU/mL and inoculated in MRS broth (LAB) and Luria Bertani (LB) broth (*S. xylosus*) (Oxoid, Milan, Italy) supplemented with 20 mg/mL myoglobin (Sigma-Aldrich, Milan, Italy) and 50 mM L-arginine (Sigma-Aldrich, Milan, Italy). After incubation for 18 h at 30 °C, the suspensions were centrifuged, the supernatant was collected and subjected to UV-Vis analysis with a Tecan Sunrise microplate reader (Tecan Italia Srl, Cernusco sul Naviglio, Italy) and the absorption spectrum between 450 and 700 nm was obtained (Ras *et al.*, 2018; Luo *et al.*, 2020; Xu *et al.*, 2023).

# **Results and Discussion**

* 1. Antioxidant activity

One of the main causes of meat quality depletion after microbial alteration is oxidation, which cause quality depletion, and the accumulation of toxic compounds, which can lead to the onset of non-transmissible chronic diseases (Carocho *et al.*, 2018). For these reasons, the antioxidant activity of the different strains was evaluated. From DPPH and ABTS analysis all the stains seemed to present antioxidant activity, however, FRAP analysis didn’t confirm these data. There was no correlation between antioxidant activity expressed by strains and their growth pattern, both under aerobic or anaerobic metabolism, but the antioxidant potential resulted to be strain-specific. The only exception was in the case of the cellular pellet grown under aerobic conditions, of which results showed lower average antioxidant activity than the other conditions. It was also discovered that the cells of aerobically grown strains had activity equal to, or lower than, the control consisting of ascorbic acid (1.5 ppm), whereas the other treatments have at least 20% of the strains with higher activity than the control. These findings are very promising, but they must be confirmed *in vivo* before to substitute the synthetic antioxidants in meat products with the use of this strains as bioprotective cultures.

* 1. NOS enzymatic activity

Nitrate and nitrite salts are commonly used to maintain the bright red colour of meat, but the residual content of nitrites reduces the ability of red blood cells to bind and transport oxygen through the body and contributes to the formation of nitrosamines (EFSA ANS Panel, 2017). Several studies have shown that bacteria such as *Staphylococcus* spp. and LAB can produce NO from L-arginine via the NOS enzyme (Yarullina *et al.*, 2011; Ras *et al.*, 2017; Xu *et al.*, 2023). The preliminary screening of 50 out of 100 strains belonging to the DI4A revealed that none of them showed NOS activity, as all of the tested strains had enzymatic activity levels lower than the detection limit of the used method (5 pmol/min/μg). As a result, additional research is being conducted to identify a producer strain by evaluating the bacteria's ability to develop colour in a broth culture supplemented with Met-Myoglobin and arginine. Same results were obtained for LAB, since none of the tested strains demonstrated the ability to produce NO from arginine. Indeed, no absorbance peaks were identified in the absorption spectra of the tested strains at 581 nm, the typical wavelength of NO-myoglobin. Thus, further research is required.

# **4. Conclusions**

A central theme in implementing the quality of meat products is the search for natural alternatives to synthetic antioxidants. To this purpose, a screening was carried out to assess the antioxidant activity of LAB strains in order to replace the compounds currently in use. It was discovered that the strains tested have good *in vitro* potential, though this needs to be confirmed *in vivo* as well. Furthermore, a screening was performed to determine whether strains of *S. xylosus* and LAB can produce NO from L-arginine via the NOS enzyme in order to reduce the concentration of nitrites in meat products. However, none of the tested strains demonstrated this ability, so additional research is being conducted.

# **4. References**

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