# **BLUE DISCOLORATION OF MEAT AND MEAT PRODUCTS**

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Abstract – This paper describes the phenotypic behavior of *Pseudomonas fluorescens* strains isolated from cases of blue discoloration of meat and meat products. Strains isolated from beef and belly cut were investigated in order to highlight the pigment production at different refrigeration temperatures (4, 6, 8, 10 °C). Some carbon sources (glucose, glycerol, and tryptophan) were also tested. In in-vitro tests, the color changes were inhibited at temperatures lower than 10 °C. Glucose and glycerol have stimulated the blue pigmentation. Meat strains showed peculiar dynamics of pigment production that suggests a microbial adaptation to different food niches.

Key Words - Pseudomonas fluorescens; Pigment, Meat Spoilage.

## I. INTRODUCTION

The meat color is largely considered as the most important attribute of product acceptability [1]. Usually, gray/brown and green discolorations are associated with the meat spoilage; these color changes on the meat surface are mainly related to oxidative processes and to the microbial growth [1]. Other abnormal discolorations could be a matter of concern for both, producers and consumers. Cases of food abnormal discoloration, such as the blue pigmentation, can induce alarmism to the public opinion [2]. The blue discoloration could be associated with several causes (chemical or microbiological agents); however, in the refrigerated products this peculiar spoilage was usually associated with the *Pseudomonas* contamination [3]. Two cases of blue discoloration were also described in meat and meat products [2]. Here, "blue strains" isolated from these finding were investigated in order to describe the dynamics of the blue pigment production.

## II. MATERIALS AND METHODS

Different cases of meat products blue discoloration were analyzed: i) a fresh belly cut showed blue discoloration on the fat surface (figure 1a), this anomalous pigmentation appeared after few days of refrigeration; ii) a dark-blue pigmentation was also reported in a dry-fermented salami (Figure 1b), only some part of the surface was affected during the ripening. Blue *Pseudomonas* were isolated on Potato dextrose agar, strains were characterized by phenotypic and genetic analyses [2]. A strain isolated from blue beef in United Kingdom was also considered. The blue pigment production was studied in several *in-vitro* tests. A standardized pre-inoculum of each strain (10<sup>7</sup> Colony Forming Units/ml) was inoculated in Minimal Bacterial Medium Agar (MBM Agar) on 6 cm Ø plates according to the procedures proposed by Fujikawa & Akimoto [4]. The pigment production was monitored by a chromameter (Minolta 500d Co., Osaka, Japan) and data were collected using Hunter-Lab (lightness, L; redness, a; yellowness, b) system. The magnitude of color change was expressed as  $\Delta E^*$  index [4]. Where color changes in the range of 2-3  $\Delta E^*$  were considered as distinguishable, while a  $\Delta E^* > 12$  indicated the genesis of different colors. Experiments were performed under different refrigeration temperatures (4, 6, 8, 10 °C), moreover a set of different carbon sources were also investigated (glucose, glycerol, and tryptophan).

## III. RESULTS AND DISCUSSION

Blue strains isolated from meat products (BB black belly cut; UK1 beef, SAL1 salami) were submitted to Multilocus sequence analysis (MLST) in a previous work [2]. All strains were ascribed in a specific genetic cluster (Blue branch) together with other strains collected during different cases of dairy product blue discoloration. The pigment production was studied in BB and UK1 strains in comparison with strains isolated from mozzarella cheese (Moz3) and butter (Bu1). Interestingly, the strains from meat products showed lower  $\Delta E^*$  values (Figure 2). After 10 days of observation, only the strain from black bacon grown at 10 °C gained the 12  $\Delta E^*$  threshold. All strains accounted the final concertation of 10<sup>9</sup> CFU/g, suggesting that temperature strongly influenced the pigment production but not the strains growth. Probably, thermal abuse over the 10 °C can increase the pigment production in meat strains, while in strains

from dairy products pigments were produced also at 4 °C. These bacteria can use glucose and glycerol as unique carbon sources to produce pigment. However, the highest  $\Delta E^*$  was registered at the highest glucose concentration (20 g/L); on the opposite the 10 g/L concentration of glycerol inhibited the blue production. Glycerol has been utilized by the *P. fluorecens* for pigment production and this can explain the association of the blue discoloration with the fat.



Figure 1. Blue discoloration of fresh belly cut (A) and dry-fermented salami (B). Photographs taken by authors.

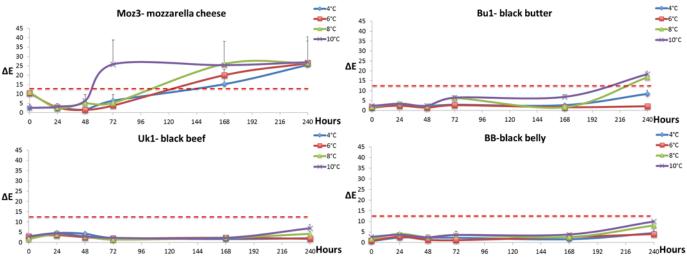


Figure 2.  $\Delta E^*$  values reported for strains selected from different food products grown at different temperatures. Dotted lines represented the 12  $\Delta E^*$  threshold.

## IV. CONCLUSION

Despite similar genetic profiles, strains from meat and meat products showed different dynamics of blue pigment production. This suggested a different adaptation of *P. fluorescens* to the environment niches; present data suggested that temperature less than 10  $^{\circ}$ C could reduce the blue discoloration phenomenon on meat matrices.

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#### REFERENCES

- Walker, H.W. (1980). Effects of microflora on fresh meat color. In: Proceedings 33rd reciprocal meat conference (pp. 33–36), 22–25 June 1980, West Lafayette, Indiana, USA.
- 2. Andreani, N.A., Martino, M.E., Fasolato, L., Carraro, L., Montemurro, F., Mioni, R., Bordin, P., Cardazzo, B., (2014). Tracking the blue: a MLST approach to characterise the *Pseudomonas fluorescens* group. Food Microbiology, 39, 116–126.
- Andreani N.A. & L. Fasolato, (2017). Chapter 2 *Pseudomonas* and Related Genera. In Woodhead Publishing Series in Food Science, Technology and Nutrition, Woodhead Publishing, (pp 25-59), The Microbiological Quality of Food, Elsevier Ltd. The Boulevard, Langford Lane, Kidlington, United Kingdom.
- 4. Fujikawa H. & Akimoto R. (2011). New blue pigment produced by *Pantoea agglomerans* and its production characteristics at various temperatures. Applied Environmental Microbiology. Jan;77(1):172-8.