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Nitric oxide synthase: What is its potential role in the physiology of staphylococci in meat products?

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ABSTRACT

Coagulase-negative staphylococci are frequently isolated from meat products and two species are used as starter cultures in dry fermented sausages. In these products, they face various environmental conditions such as variation of redox potential and oxygen levels that can lead to oxidative stress. Furthermore, when nitrate and nitrite are added as curing salts, staphylococci also experience nitrosative stress. A *nos* gene encoding a nitric oxide synthase (NOS) is present in the genome of all staphylococci. NOS produces nitric oxide (NO) and citrulline from arginine, but its activity is still poorly characterized, particularly in coagulase-negative staphylococci. NO is highly reactive with a broad spectrum of activity resulting from targeting metal centres (heme and non-heme) and protein thiols. At low concentration, NO acts as a signalling molecule, while at higher concentration it generates stress. Thus, it was initially suggested that staphylococcal NOS counteract oxidative stress in relation to PerR and Fur regulators. In the physiology of staphylococci, it has recently been highlighted that NO controls the rate of aerobic respiration and regulates the transition from aerobic to nitrate respiration and also helps maintain the membrane potential in relation to the two-component systems SrrAB and AirRS. As NO interacts with heme centres, it binds the heme iron atom of myoglobin to form nitrosomyglobin, which is the typical red pigment of cured meat. However, the contribution of NOS to this reaction in meat products has yet to be evaluated.

1. Introduction

The composition of the bacterial community of meat is influenced by processes such as curing, salting, fermentation and drying. Lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS) are the two major bacterial populations selected in those conditions (Leroy et al., 2006; Talon and Leroy, 2014). In naturally fermented meat products, *Staphylococcus equorum*, *Staphylococcus saprophyticus* and *Staphylococcus xylosus* are the three dominant species (Coton et al., 2010; Leroy et al., 2010). Some other species, such as *Staphylococcus carnosus*, *Staphylococcus succinus*, *Staphylococcus vitulinus* and *Staphylococcus warneri*, are present to a lesser extent (Blaiotta et al., 2004; Corbière Morot-Bizot et al., 2006; Iacumin et al., 2012; Marty et al., 2012; Ratsimba et al., 2017). Only *S. carnosus* and *S. xylosus* are currently used as starter cultures in meat products (Leroy et al., 2016; Talon and Leroy, 2014).

Curing salts such as nitrate and/or nitrite have been extensively used since ancient times to preserve meat (Talon and Leroy, 2014). Their ingoing concentrations and residual amounts are regulated: the European Parliament stipulates that 100 mg to 150 mg of nitrite per kg are allowed for thermally processed meats, while 150 mg of nitrite plus 150 mg of nitrate are allowed for non-thermally processed meats (Commission Regulation, 2011).

Most staphylococci reduce nitrate to nitrite *via* their nitrate reductase. In meat, subsequent chemical reduction of nitrite leads to nitric oxide (NO), which ensures microbial safety by inhibiting the growth of unwanted microorganisms such as *Clostridium botulinum*, and contributes to flavour and colour development (Pegg and Honikel, 2015; Sindelar and Milkowski, 2011).

NO can also be produced from arginine *via* nitric oxide synthase (NOS), which is widely distributed in staphylococci (Sapp et al., 2014). NOS activity is seen in *S. xylosus* and NO is produced in several species of CNS frequently found in meat (Ras et al., 2017; Ras et al., 2018). NOS-like activity is also seen in *Staphylococcus haemolyticus* (Sánchez Mainar et al., 2014). NO is highly reactive and acts as signalling molecule at low concentration and generates nitrosative stress at high concentration. Its interactions with numerous targets within the cell may modulate the physiology of bacteria.

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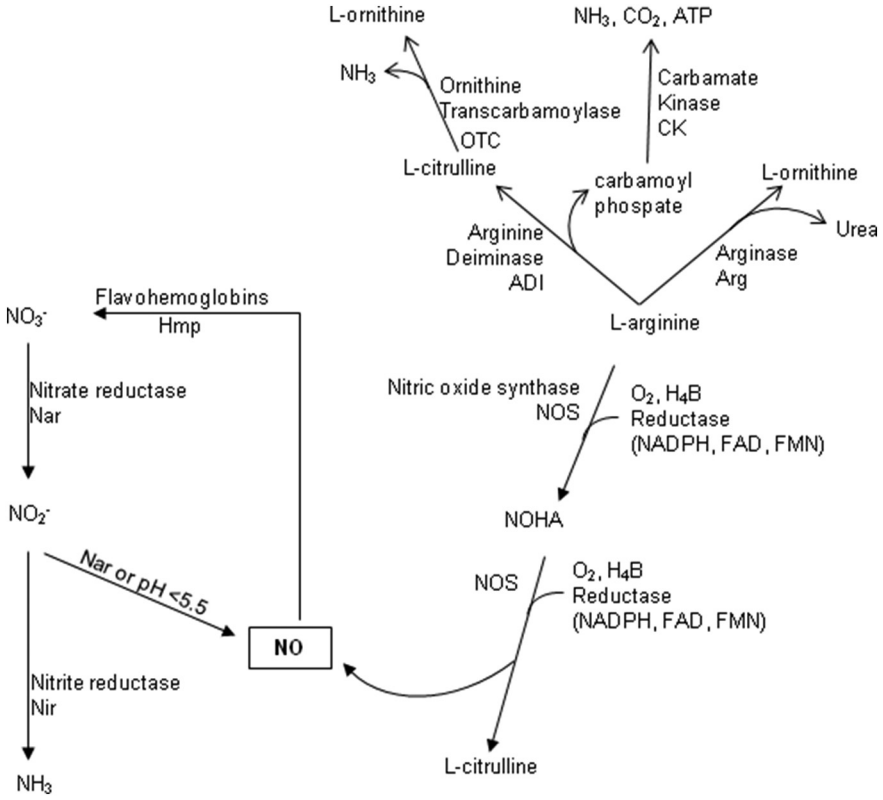

Fig. 1. Pathways for nitric oxide (NO) production and detoxification in staphylococci.

2. Production of nitric oxide: at the interface between nitrate/nitrite and arginine catabolism

The reactions involved in the production of NO are schematized Fig. 1.

2.1. Curing salts

2.1.1. Nitrate reduction

In meat, nitrate added as curing salt is initially reduced to nitrite by the nitrate reductase activity of staphylococci. This property is widespread among staphylococci, albeit with some heterogeneity. Considering the two species used as starter cultures, all strains of *S. carnosus* have nitrate reductase activity, which is mostly high, whereas such activity ranges from zero to high in some *S. xylosus* strains (Mauriello et al., 2004; Sánchez Mainar and Leroy, 2015). In a cured meat model, *S. xylosus* reduced more than 80% of nitrate after 24-h incubation (Vermassen et al., 2014). Among other species frequently isolated, nitrate reductase activity has been found always in *S. equorum* and less frequently in *S. saprophyticus* (Gøtterup et al., 2007; Gøtterup et al., 2008; Mauriello et al., 2004; Sánchez Mainar and Leroy, 2015).

The nitrate reductases of *S. carnosus* and *S. xylosus* are membrane enzymes encoded by the *nar* operon composed of four genes (*narGHJI*) and downstream the *narT* gene is involved in the transport of nitrate and the *nreABC* operon is involved in regulation (Leroy et al., 2017; Rosenstein et al., 2009).

Nitrate can also arise from the detoxification of NO by flavohaemoglobins (Hmp), which function as NO scavengers. In the presence of O_2, Hmp oxidize NO to nitrate, while in anaerobiosis Hmp reduce NO to N_2O_2, but with a much lower activity (Forrester and Foster, 2012; Gonçalves et al., 2006). In *S. aureus*, microaerobiosis or nitrosative stress induced the expression of an *hmp* gene encoding a homologue of a flavohaemoprotein (Gonçalves et al., 2006; Richardson et al., 2006; Schlag et al., 2007). *S. aureus* achieves *hmp* regulation by placing it under the control of the regulator SrrAB, which senses reduced electron flow through the respiratory chain (Kinkel et al., 2013; Richardson et al., 2006). In *S. xylosus* C2a, two genes encoding flavohaemoglobins have been identified (LN554884), but their role remains to be characterized.

2.1.2. Nitrite reduction

2.1.2.1. Nitrite reductase. S. carnosus and *S. xylosus* have a nitrite reductase. For *S. carnosus*, this activity is regulated by nitrate, so when the nitrate concentration becomes limiting, nitrite is imported and reduced to ammonia by its nitrite reductase (Neubauer and Götz, 1996). In both species, nitrite reductase is encoded by the *nir* operon composed of five genes (*nirR, sirA, nirB, nirD, sirB*) located upstream of the *nar* operon (Leroy et al., 2017; Rosenstein et al., 2009).

The *nreABC* operon regulates both the *nar* and *nir* operons under anaerobiosis and in the presence of nitrate in *S. aureus* (Schlag et al., 2008). These three operons are upregulated in *S. xylosus* grown in a meat model without added nitrite or nitrate, probably due to anaerobic conditions (Vermassen et al., 2016).

2.1.2.2. Nitrate reductase. Nitrate reductase is often described as being involved in the reduction of nitrate to nitrite, but the reduction of nitrite that leads to the production of NO, independently of respiration, can be due to a molybdenum enzyme such as nitrate reductase (Maia and Moura, 2015). It has been suggested that the synthesis of NO in *Salmonella enterica* serovar Typhimurium and *Escherichia coli* is ensured mainly by the membrane NaR nitrate reductase (Gilberthorpe et al., 2008; Vine et al., 2011). The conditions required to observe NO synthesis by nitrate reductase result from anaerobic conditions associated with a decrease in nitrate concentration combined with the accumulation of nitrite in the medium (Maia and Moura, 2015).

2.1.2.3. Chemical reactions in meat. In fermented meat products, pH decreases to 5.5, at which nitrite is dissolved in the water contained in