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## Introduction

Fermented raw sausages as salami are often produced by using lactobacilli and koagulase-negative staphylococci in starter cultures. Their main functions are acidification by lactobacilli, nitrate reduction (via nitrite and NO, which binds to myoglobin) by staphylococci, and influence on aroma formation by both genera. For the development of starter cultures, a semiquantitative screening assay for nitrate reductase (NAR) activity in staphylococci strains is needed.

## Methods & Results

Staphylococci from different Swiss raw sausages, which had been spontaneously ripened (without starter cultures), were isolated and identified by 16S rRNA sequencing (Tab. 1). 40 different isolates, being negative for koagulase and hemolysis reaction, were screened for NAR activity. In this semiquantitative test, optical-density-adjusted cell suspensions from overnight grown cultures of staphylococci were incubated in small wells in nitrate-containing agar plates. After 3 hours of incubation, NAR activity was quantified by measuring the diameter of the red halo formed (nitrite detection after Griess; Fig. 1; method adapted from Mauriello et al., 2004).

Tab. 1. Identified staphylococci species in different raw sausages

No.	Sausage types	identified Staphylococci species (No. of isolates)
1	"Salami Nostrano"	<i>Staphylococcus xylosus</i> (3)
2	"Salami Superiore"	<i>Staphylococcus xylosus</i> (4)
3	"Bauernsalsiz"	<i>Staphylococcus xylosus</i> (4), <i>Staphylococcus carnosus</i> (2)
4	"Appenzeller Pantli"	<i>Staphylococcus xylosus</i> (3), <i>Staphylococcus equorum</i> (1)
5	"Hirschwurst"	<i>Staphylococcus xylosus</i> (3), <i>Staphylococcus carnosus</i> (3)
6	"Lauchwurst"	<i>Staphylococcus warneri</i> (1), <i>Staphylococcus carnosus</i> (1)
7	"Appenzeller Pantli"	<i>Staphylococcus equorum</i> (1)
8	"Salami"	<i>Staphylococcus carnosus</i> (2)
9	"Saucisse d'Ajoie"	<i>Staphylococcus saprophyticus</i> (1)
10	"Saucisson Vaudois"	<i>Staphylococcus saprophyticus</i> (1)
11	"Saucisson Vaudois"	<i>Staphylococcus equorum</i> (1), <i>Staphylococcus carnosus</i> (6), <i>Staphylococcus saprophyticus</i> (1)
12	"Mortadella Ticinese"	<i>Staphylococcus equorum</i> (1)
13	"Salametti Ticinesi"	<i>Staphylococcus equorum</i> (1)
14	starter cultures	<i>Staphylococcus xylosus</i> (2), <i>Staphylococcus carnosus</i> (2)

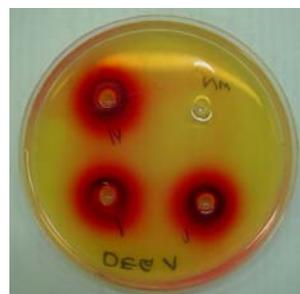


Fig. 1. Nitrate reductase activity of a *Staphylococcus xylosus* isolate. Determined as nitrite formation (red halo) in triplicates; top right: negative control (*E. faecalis*).

35% of the isolates were found to be NAR-negative (Fig. 2). Even some isolates of the same species isolated from an identical source differed in NAR activity. 11 of 14 *Staphylococcus carnosus*, 11 of 17 *Staphylococcus xylosus*, 3 of 5 *Staphylococcus equorum*, 1 of 3 *Staphylococcus saprophyticus*, and 0 of 1 *Staphylococcus warneri* isolates were found to be NAR-positive. Most of these strains showed an activity between 14 and 19 mm halo formation, comparable to values obtained from staphylococci of commercially available starters. In NAR-positive isolates, no halos below 11 mm were observed.

A first trial was performed to produce nitrate-cured salami sausages using two NAR-positive isolates of *Staphylococcus carnosus* (isolate 5.36; Halo  $\varnothing$ : 17 mm) or *Staphylococcus xylosus* (isolate 5.37; Halo  $\varnothing$ : 15 mm) in combination with *Lactobacillus sakei* or *Lactobacillus curvatus* (Fig. 3). The sensory quality of the resulting sausages was good (data not shown).

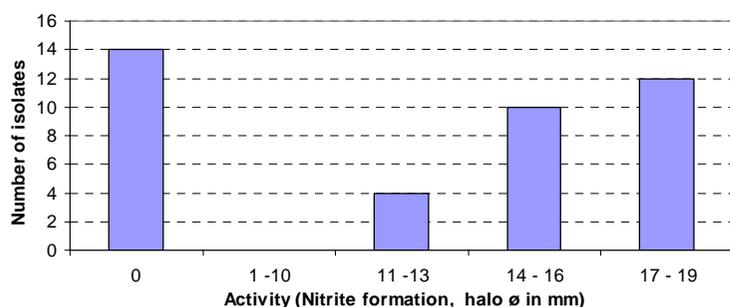


Fig. 2. Nitrate reductase activities of Staphylococci isolates



Fig. 3. Raw sausages of Salami type in a ripening cell

## Conclusions

The screening test for NAR activity was able to discriminate between different staphylococci isolates. It can be used as one criteria to select applicable starter culture strains for raw sausages.