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Growth and aroma production by *Staphylococcus xylosus*, *S. carnosus* and *S. equorum*—a comparative study in model systems

Anne K. Søndergaard, Louise H. Stahnke*

BioCentrum-DTU, Technical University of Denmark, Søltofts Plads Building 221, DK-2800 Kgs. Lyngby, Denmark

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Abstract

A laboratory medium inoculated with 20 different *Staphylococcus* strains was prepared in accordance with a full factorial experimental design investigating the effect of temperature, pH, NaCl and glucose on growth. The 12 strains most suited to growth in a fermented meat environment were inoculated in sausage minces together with *Pediococcus pentosaceus*, incubated at 25 °C for 1 week and the produced aroma compounds collected. The data were analysed by multiple linear regression and partial least squares regression analysis. The results showed that increasing pH and temperature from 4.6 to 6.0 and 10 to 26 °C, respectively, increased growth of all strains with strong synergy between temperature and pH. Increasing salt concentration from 5% to 15% w/v decreased growth of most strains, but the effect of pH and temperature was much stronger than the effect of salt. Strains of *S. carnosus* were more salt tolerant than strains of *S. equorum* and *S. xylosus*, especially at high pH and temperature. Addition of glucose up to 0.5% w/v had no significant influence on growth of any of the strains. With regard to aroma production, species characteristics were detected. *S. carnosus* and *S. xylosus* were quite different regarding the overall aroma profiles, whereas the profiles of *S. equorum* lied somewhere in-between. Contrary to *S. carnosus*, *S. xylosus* and *S. equorum* did not produce 2-methyl-1-butanol. On the other hand, in particular, *S. xylosus* produced more 3-methyl-1-butanol. Except for one of the strains of *S. equorum*, *S. xylosus* and *S. equorum* formed more diacetyl, 2-butanone and acetoin and also more of the methyl-branched ketones arising from degradation of leucine, isoleucine and valine. *S. carnosus* produced more methyl-branched aldehydes, acids and corresponding esters from leucine, isoleucine and valine—compounds that have been correlated with fermented sausage maturity in former studies. *S. equorum* produced the least of the methyl-branched aldehydes.

Keywords: Meat starter cultures; *Staphylococcus xylosus*; *S. carnosus*; *S. equorum*; Growth parameters; Aroma

1. Introduction

Staphylococcus species are used as starter cultures for fermented meat products such as sausages and ham. They are mainly used due to their nitrate reductase activity, which accelerates and stabilise colour, but also due to their ability to produce meat products with increased flavour (Montel et al., 1998; Stahnke et al., 2001). During fermented sausage production, the *Staphylococcus* cultures are exposed to processing conditions adverse to their survival, growth and metabolic activity. But except for the pathogens such as *S. aureus*,

* Corresponding author. Tel.: +45-4525-2605; fax: +45-4588-4922.

E-mail address: louise.stahnke@biocentrum.dtu.dk (L.H. Stahnke).

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only few investigations have been published on the influence of processing parameters on *Staphylococcus*.

Sørensen and Jakobsen (1996) and McMeekin et al. (1987) showed that two different strains of *S. xylosus* had higher growth rate at higher temperatures in yeast broth (growth investigated at, respectively, 10–30 and 5–30 °C). Sørensen and Jakobsen (1996) also found that growth decreased with decreasing pH and that the influence of pH was greatest at low concentrations of salt combined with high temperatures. Increasing the concentration of salt decreased growth, especially at high temperatures and high pH values. The authors concluded that the conditions under which sausage fermentation takes place, in general, are not favourable for growth of *S. xylosus* (Sørensen and Jakobsen, 1996). Other studies on various non-starter *Staphylococcus* spp. have shown that the temperature range for growth is 7–48 °C with an optimum of 35–40 °C for most species. For *S. aureus*, the pH growth range is 4–10 with an optimum at 6–7 (Baird-Parker, 1990).

McMeekin et al. (1987) found that growth rate decreased with increasing salt concentration in the range 3.5–23.4% w/v NaCl (a_w 0.976–0.848). The growth rate at a_w 0.996 was lower than at a_w 0.976, which indicated a growth optimum at relatively high salt concentration (pH = 7.0). This was not shown for the strain studied by Sørensen and Jakobsen (1996), but there seem to be strain differences with regards to salt tolerance and growth optima since studies of Hammes et al. (1995) showed that growth of one strain was reduced severely at 10% salt while another strain showed maximum growth at 10% salt. Likewise, Guo et al. (2000) found that one species of *S. xylosus* had maximum growth at 3% salt, whereas the investigated *Micrococcus varians* and *S. carnosus* species grew better at lower salt concentration.

Staphylococcus spp. catabolise glucose via the Embden–Meyerhof pathway and the hexose monophosphate pathway. Investigations have shown that addition of glucose to the substrate reduces the percentage of glucose being oxidised via the hexose monophosphate pathway and the subsequent oxidation via the citric acid cycle. In other words, glucose seems to suppress the citric acid cycle (Blumenthal, 1972). This is in accordance with the findings of Sørensen and Jakobsen (1996), who showed that addition of 1% w/v of glucose to the substrate did not have a positive effect on growth of *S. xylosus*, but on the contrary, gave a small reduction in growth rate at otherwise optimal conditions of temperature, pH and salt concentration. Stahnke (1999b) also found that the presence of glucose decreased the growth of *S. carnosus* in sausage minces to some extent.

The aroma of a fermented meat product is influenced by many different factors such as source and type of ingredients, temperature, processing time and choice of starter culture (Lücke, 1998). The biochemical formation of aroma is due to the activity of enzymes present in the meat, to enzymes from the microorganisms present and a combination of both. The starter culture used can be decisive for the aroma of the finished product. Studies of, for example, Berdague et al. (1993), Montel et al. (1996) and Stahnke (1999a) suggest that different species of *Staphylococcus* produce different aroma compounds in different amounts. However, it has never been studied as to whether there are characteristic species differences with regard to aroma production or with regard to optimal growth conditions for staphylococci used as starter cultures.

The objectives of the present study were as follows: (i) To investigate growth differences on species level for 20 *Staphylococcus* strains by studying the influence of temperature, pH, NaCl and glucose concentration on growth in a laboratory medium. (ii) To investigate species differences with regard to aroma production in sausage mince models.

2. Materials and methods

2.1. Experimental design of growth investigation

Twenty strains (S1–S20) of *Staphylococcus* were selected from the culture bank of Chr. Hansen (Hørsholm, Denmark) (Table 1). In two separate trials, all 20 strains were identified to species level by the API Staph system (bioMerieux, Marcy-l'Étoile, France) and 12 of those further verified by the 16s rRNA technique (Anon., 1999). The experimental design for each strain was set up as a full four-factorial design at two levels (2^4 structure) examining the influence of temperature, start pH, the amount of NaCl and glucose. Four centre points were included giving a total of 20 samples for each strain (Unscrambler version 7.5, Camo, Trondheim, Norway). The factor levels were chosen to cover a wide spectrum of production con-

Table 1
Identification of *Staphylococcus* strains to species level

Strain no.	Identification probability (%) according to API Staph test		Identification according to 16s rRNA	Conclusion
	Trial 1	Trial 2		
S1	xylosus>99	xylosus 83	xylosus	xylosus
S2	xylosus>99	xylosus>99	xylosus	xylosus
S3	xylosus>99	xylosus>99	equorum	equorum
S4	caprae 35, simulans 32	caprae 35, simulans 32	carnosus	carnosus
S5	carnosus>99	carnosus>99	carnosus	carnosus
S6	carnosus>99	carnosus>99	carnosus	carnosus
S7	lentus 80, xylosus 20	lentus 80, xylosus 20	–	lentus
S8	carnosus>99	simulans 85	carnosus	carnosus
S9	xylosus>99	xylosus 58, lentus 42	–	xylosus
S10	xylosus 99	xylosus 58, lentus 42	–	xylosus
S11	xylosus 83, lentus 17	xylosus 58, lentus 42	–	xylosus
S12	xylosus 99	xylosus 99	xylosus	xylosus
S13	xylosus>99	xylosus 83	xylosus	xylosus
S14	carnosus>99	carnosus>99	carnosus	carnosus
S15	xylosus>99	xylosus>99	–	xylosus
S16	xylosus 83, lentus 17	xylosus 98	–	xylosus
S17	xylosus>99	xylosus 99	–	xylosus
S18	xylosus>99	xylosus>99	equorum	equorum
S19	xylosus 99	xylosus 58, lentus 42	–	xylosus
S20	xylosus 99	xylosus 83, lentus 17	xylosus	xylosus

ditions for the bacteria in fermented meat products (Table 2). The whole 20-sample experiment with S1 was repeated three times in all, to check reproducibility from day to day ($S1^a$ versus $S1^b$ and $S1^c$) and within day ($S1^b$ versus $S1^c$).

2.2. Preparation of media and starter cultures

Before the experiments, the strains were grown overnight at 30 °C in a substrate consisting of 10 g/l of meat extract "Lab Lemco" (Oxoid L29, Hampshire, England), 8 g/l of yeast extract (Oxoid L21), 15 g/l of neutralized bacteriological peptone (Oxoid L34), and 5 g/l of NaCl dissolved in distilled water. pH in the substrate was 6.9 (PHM 92 pH meter, Radiometer, Denmark, electrode Mettler HA405-DXK-S8/120) before autoclaving. The same levels of meat extract, yeast extract and peptone were used as basic ingredients in the experimental substrates, but the salt, sugar concentration and pH varied according to Table 2. pH was adjusted with 1 N HCl.

2.3. Growth measurements

Growth was followed by optical density (OD) measurements at 600 nm (Spectronic 501, Milton Roy, USA). The experimental substrates were inoculated with overnight cultures diluted to OD ≈ 0.35 (preliminary unpublished investigations for staphylococci had shown 0.35 to be equivalent to approximately 5×10^7 cfu/ml) and incubated in closed glass tubes at 10, 18 or 26 °C according to Table 2. For all strains and substrates (totally 20 strains × 20 substrates), OD was measured after 24, 30 and 48 h and corrected for the values at 0 h.

2.4. Aroma investigation—experimental design

The 12 strains (S1–S6, S8, S12–S14, S18, S20) most suited to growth in fermented meat environments according to the growth experiments were investigated for aroma production in a sausage mince also inoculated with *Pediococcus pentosaceus*. The meat minces consisted of 31.5% w/w pork (15–20% w/w fat), 31.5% w/w beef (15–20% w/w fat) and 31.5% w/w back fat (80–90% w/w fat) that were stored until use at − 40 °C in sausage casings, wrapped in aluminium foil. The meat mince was thawed at 5 °C for 24 h and added 1.7% w/w NaCl blended with sodium nitrite (0.6% w/w $NaNO_2$), 1.0% w/w NaCl, 0.4% w/w glucose and 0.05% w/w sodium ascorbate according to a commercial sausage recipe. Afterwards, the minces were inocu-

Table 2
Full 2^4-factorial design with four centre points for investigating the effect of temperature, NaCl, pH and glucose on *Staphylococcus* growth in laboratory medium[a]

Substrate no.	Temperature (°C)	NaCl (% w/v)	pH	Glucose (% w/v)
1	10	5	4.6	0
2	26	5	4.6	0
3	10	15	4.6	0
4	26	15	4.6	0
5	10	5	6	0
6	26	5	6	0
7	10	15	6	0
8	26	15	6	0
9	10	5	4.6	0.5
10	26	5	4.6	0.5
11	10	15	4.6	0.5
12	26	15	4.6	0.5
13	10	5	6	0.5
14	26	5	6	0.5
15	10	15	6	0.5
16	26	15	6	0.5
17	18	10	5.3	0.25
18	18	10	5.3	0.25
19	18	10	5.3	0.25
20	18	10	5.3	0.25

[a] Each *Staphylococcus* strain was inoculated in the 20 substrates and OD_{600} measured after 24, 30 and 48 h.

lated with *Staphylococcus* (8×10^6 cfu/g) as prepared in Section 2.2 and 5×10^6 cfu/g *P. pentosaceus* (PC-1, freeze-dried sample from Chr. Hansen) and thoroughly mixed. All minces were made in duplicates and their pH (Hanna Instruments, Bedfordshire, England) and microbial count determined before and after fermentation—staphylococci by plate counting on S110 agar (Difco, Detroit, MI, USA) and lactic acid bacteria on MRS agar (Oxoid, Basingstoke, England). S110 was incubated for 2 days, aerobically at 30 °C and MRS for 3 days, anaerobically at 30 °C. The minces were put into closed 250 ml Erlenmeyer flasks (200 g in each), mounted with two short horizontal glass tubes. Tenax TA® tubes (200 mg, 60/80 mesh, Chrompack/Varian, Palo Alto, CA, USA) were fixed onto the glass tubes with Swagelok® unions/Teflon ferrules (Swagelok, Solon, OH, USA) and aroma compounds from the minces collected during growth by passive diffusion for 7 days at 25 °C. Tenax TA® tubes were conditioned before sampling by purging with nitrogen for 15 min at 340 °C with a flow rate of 75 ml/min (N_2 5.0, AGA, Ballerup, Denmark). Duplicate tubes were made for each mince.

2.5. Analysis of aroma compounds

The aroma compounds adsorbed onto the Tenax TA® were thermally desorbed in a two-step manner by an automatic thermal desorber with a Tenax TA® filled cold trap set at −30 °C (ATD400, Perkin-Elmer, Beaconsfield, England). The first desorption was carried out at 200 °C for 3 min, the second desorption at 250 °C for 1 min. The volatiles were automatically injected into a GC-MS through a transfer line set at 200 °C (Hewlett-Packard 5890 GC series II interfaced to a Hewlett-Packard MS 5972—both instruments from Agilent, Palo Alto, CA, USA). The separation was performed on a 30 m × 0.25 mm i.d. DB-1701 (1 μm film) fused silica capillary column (J&W Sci., Köln, Germany). Oven program was: 35 °C for 1 min, 4 °C/min until 175 °C, 10 °C/min until 260 °C and 5 min at 260 °C; carrier gas (He) velocity was 43 m/s at 35 °C. Ionisation energy was 70 eV, scan time 0.36 s, transfer line temperature 280 °C, scan range: 33–300 AMU. The desorption procedure was calibrated at every session by running three calibration tubes containing 5 μl of a 0.1 g/l octane in methanol solution. The calibration tubes were prepared according to Anon (1991). Peak areas from the experimental tubes were divided with the averaged area of the octane peaks from the three calibration tubes and multiplied by 10^{+6}. Identification was based on Kovats retention indices of authentic compounds and of MS spectra compared to the NBS75k database (National Bureau of Standards database in Hewlett-Packard Chemstation software, Agilent Palo Alto, California, USA).

2.6. Statistical analyses

The relationships between OD and growth parameters were investigated separately for each strain (20 samples for each strain, see Table 2) by multiple linear regression–analysis of variance (ANOVA–MLR) and by partial least squares regression–analysis of variance (ANOVA–PLSR) in Unscrambler (version 7.5, Camo A/S, Trondheim, Norway). The **Y**-matrix consisted of samples versus OD values and the ***X***-matrix of the experimental design. The result matrix from the aroma experiment consisted of a total of 24 samples versus

relative peak areas of aroma compounds in the logarithmic$_{10}$ scale. The matrix was analysed by discriminant partial least squares regression (D-PLSR) in Unscrambler (version 7.5, Camo A/S, Trondheim, Norway). The ***Y***-matrix consisted of samples versus species in nominal numbers and the ***X***-matrix of samples versus aroma compounds. The PLSR models were validated by cross-validation.

3. Results and discussion

3.1. Strain identification

Table 1 shows the results from the identification trials using the 16s rRNA technique and/or the API Staph system. For the strains only identified by the API system, one should note that S9–S11, S15–S17 and S19 could be *S. equorum* even if stated as *S. xylosus* with a high probability—the API Staph test does not differentiate between *S. xylosus* and *S. equorum* (bioMerieux, Marcy-l'Étoile, France, January 2001). The two separate API system trials were, in general, in good agreement, though the identification probability in the two trials varied a lot for many of the strains. For the strains S4, S7, S9–S11 and S19, the identification probability was less than 80% in average, worst for S4 where the test could not give a straight answer. However, when comparing the API system with the 16s rRNA technique, only strains S4 and S8 were not correctly identified by the API test and those two cases would not have been misinterpreted at any rate due to the low identification probability for S4 and the different answers in trials 1 and 2 for S8.

Table 3
Parameters with a significant influence on growth of *Staphylococcus* according to multiple linear regression analysis (ANOVA-MLR)

Strain no.[a]	*Staphylococcus* species	Main effects[b,c]				Interaction effects					
		Temperature	NaCl	pH	Glucose	Temperature * NaCl	Temperature * pH	Temperature * Glucose	NaCl * pH	NaCl * Glucose	pH * Glucose
S1[a]	xylosus	+++	−−	+++	n.s.	−	+++	m	−	m	m
S1[b]	xylosus	+++	−−	+++	n.s.	−	+++	m	n.s.	m	m
S1[c]	xylosus	+++	−−	+++	n.s.	−−	+++	m	−	m	m
S2	xylosus	++	−−	+++	n.s.	−	+	m	−	m	m
S12	xylosus	+++	−−	+++	n.s.	−	+++	m	−	m	m
S13	xylosus	+++	−−	+++	n.s.	−	+++	m	−	m	m
S20	xylosus	+++	−−	+++	n.s.	−	+++	m	n.s.	m	m
S9	xylosus	+++	−−−	+++	n.s.	−−	+++	m	−−	m	m
S10	xylosus	+++	−−−	+++	n.s.	−	+++	m	−	m	m
S11	xylosus	+++	−−	+++	n.s.	−	+++	m	−	m	m
S15	xylosus	+++	−−−	+++	n.s.	−	+++	m	−−−	m	m
S16	xylosus	++	−−	+++	n.s.	−	++	m	−	m	m
S17	xylosus	+++	−−−	+++	n.s.	−−−	+++	m	−−	m	m
S19	xylosus	++	−−	+++	n.s.	−	++	m	−	m	m
S7	lentus	+++	−−−	+++	n.s.	−−	+++	m	−−	m	m
S3	equorum	+++	−−	+++	n.s.	m	+++	m	−	m	m
S18	equorum	+++	−−−	+++	n.s.	−−	+++	m	−−−	m	m
S4	carnosus	+++	n.s.	++	n.s.	m	+	m	m	m	m
S5	carnosus	+++	n.s.	+++	n.s.	m	+	m	m	m	m
S6	carnosus	+++	n.s.	+++	n.s.	m	++	m	m	m	m
S8	carnosus	+++	−	+++	n.s.	m	++	m	m	m	m
S14	carnosus	+++	n.s.	+++	n.s.	m	++	m	m	m	m

[a] S1[a], S1[b] and S1[c] refer to the same strain, but the experiment was repeated three times in all, please refer to text.

[b] Significance levels: +++/−−− $p \le 0.1\%$, ++/−− $p \le 1\%$, +/− $p \le 5\%$, where the signs + and − indicate a positive and a negative influence, respectively.

[c] m = parameter excluded from regression model since it was non-significant in the first analysis step ($p > 5\%$), n.s. = not significant in the final model ($p > 5\%$).

3.2. Growth experiments

Table 3 shows the results from the ANOVA–MLR analysis on the OD measurements after 24 h of growth at defined conditions where the strains were still in the exponential growth phase. The results after 30 and 48 h of growth were similar (data not shown). The table lists both the significant main effects and the interaction effects between the parameters. The results for the three experiments with the strain S1 ($S1^a$, $S1^b$ and $S1^c$) show that the reproducibility of the growth experiments within day and also from day to day was good. The same main effects and interaction effects were significant with the same degree of significance except for one case.

Table 3 shows that increasing temperature and pH had a positive and synergistic influence on growth of all strains due to the significant interaction between temperature and pH, whereas increasing salt concentration had a significantly negative influence on all strains, except for four of the *S. carnosus* cultures. Salt and the interactions involving salt, in general, seemed to have no significant effect on the growth of *S. carnosus*. However, the linear regression analysis indicated that the growth results for the centre batches of the experimental design (samples 17–20 in Table 2) were off-line, so there may have been a growth optimum around the centre points (10% w/v NaCl) as shown by Hammes et al. (1995) for several *S. carnosus* strains. The experimental design in the present investigation was linear and not quadratic, and therefore, such an optimum was not possible to see directly from the regression results.

Glucose did not have a significant influence on growth of any of the strains, but the regression analyses showed a tendency ($p < 10\%$) towards a negative influence on growth of some. As mentioned in the Section 1, the authors Sørensen and Jakobsen (1996) and Stahnke (1999b) also found that glucose had a negative effect on growth of two different *Staphylococcus* strains. However, one should notice that the growth medium was not a buffered system in any of the studies, that is, adding glucose most probably resulted in a pH lowering—the lowering depending on the efficiency of oxygen transfer through the media (Arkoudelos and Nychas, 1995)—and this would have affected growth negatively. It is assumed that the expected positive effect of glucose was levelled out by the negative effect of a pH lowering. Unfortunately, the final pH of the growth medium was not measured, so this assumption cannot be checked.

The interaction effect between salt and temperature was negative for all *S. xylosus* strains, the *S. lentus*

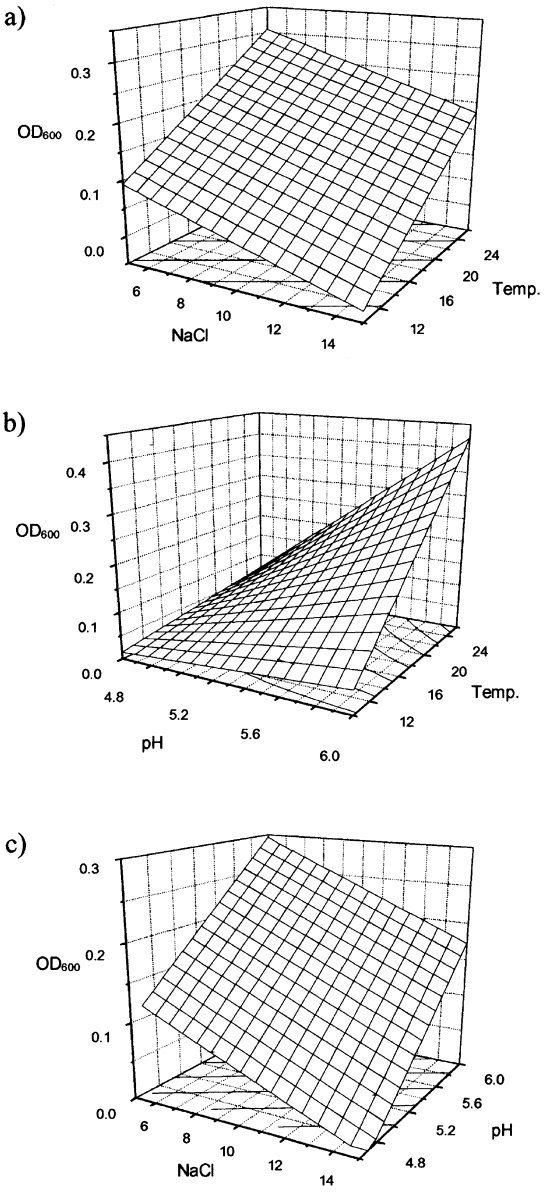

Fig. 1. Response surface plots from ANOVA–PLSR on growth (OD_{600}) versus NaCl, temperature and pH for *S. xylosus* S12 growing in laboratory medium at glucose content 0.25% w/w. (a) pH = 5.3, (b) NaCl = 10% w/w, (c) temperature = 18 °C.

and one of the *S. equorum* strains (Table 3). It means that raised concentrations of salt had a greater negative impact at high temperatures than at low temperatures. The same phenomenon was seen for the interaction between pH and salt. At high pH values, salt had a negative impact, as opposed to low pH values, where salt did not have an impact. Those interaction effects were also shown in a study of one strain of *S. xylosus* by Sørensen and Jakobsen (1996). Likewise, McMeekin et al. (1987) noticed that the minimum growth temperature of the investigated *Staphylococcus* strains increased as the water activity decreased. The impact of the interaction effects is illustrated in Fig. 1a–c, which show growth as a function of salt, temperature and pH for the *S. xylosus* S12. The strain S12 was selected for Fig. 1 since it represents most of the strains regarding the influence of the three parameters and their degree of significance (Table 3). It is clearly seen in Fig. 1 that there is strong synergy between pH and temperature, but not between salt and pH and salt and temperature.

To detect species differences with respect to growth parameters, the data were analysed by ANOVA–PLSR. Fig. 2 illustrates the relationship between the investigated parameters, their interactions and all of the 20 strains at once. The non-significant parameters ($p>5\%$) have been removed from the model. The PLSR plot shows that especially temperature and pH have an influence on growth (PC1 explains 85%), whereas the effect of salt is much smaller in the investigated salt span (PC2 explains only 4%). Both high pH and high temperature increase growth, but temperature more for the *S. carnosus* strains. Salt and its interactions with pH and temperature are placed opposite the strains indicating that salt has a negative effect on the growth of all strains, but mostly on the group of strains positioned in the lower right-hand corner. This shows that the strains of *S. carnosus* are the most salt-tolerant species, and especially at high pH and temperature. *S. carnosus* strains are positioned in the top group and *S. xylosus*, *S. lentus* and *S. equorum* in the bottom group. DNA–DNA hybridisation studies have also shown that *S. xylosus* and *S. equorum* are more alike than *S. carnosus* (Schleifer and Kroppenstedt, 1990).

3.3. Aroma profile

Based on the regression analysis, the growth potential was evaluated for all 20 strains at the conditions persisting during fermented sausage production, that is, at conditions with high salt concentration, low pH

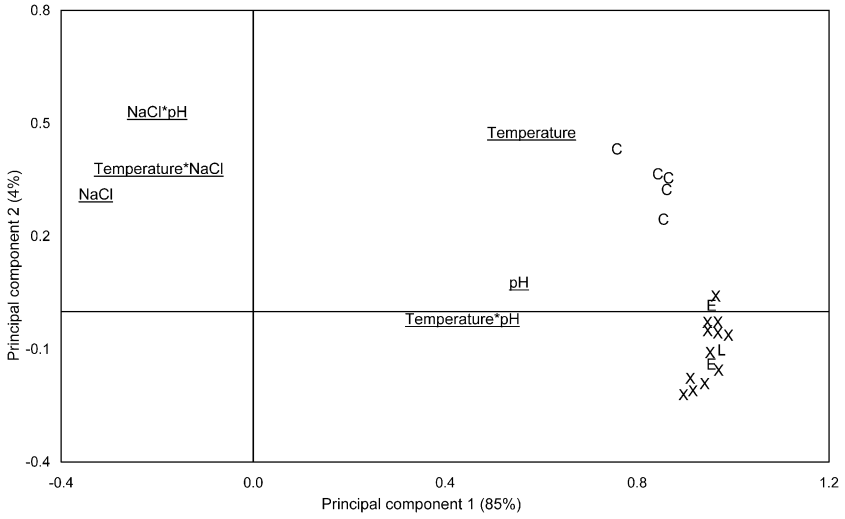

Fig. 2. Plot of principal components 1 and 2 from the ANOVA–PLSR analysis on *Staphylococcus* growth in laboratory medium versus temperature, pH, NaCl and glucose concentration. Glucose and its interaction effects were not significant for the regression model and have been excluded from the plot. Growth was measured as OD_{600}. Validated number of principal components was two. C = *S. carnosus*, X = *S. xylosus*, E = *S. equorum*, L = *S. lentus*.

and with either a high or low temperature at different times during the process (regression models not shown). The 12 strains that seemed most capable of growth according to the regression models were investigated for aroma production. The 12 selected strains were 5 strains of *S. xylosus* and *S. carnosus* and 2 strains of *S. equorum*.

Table 4 lists those 44 aroma compounds that were detected in the headspace above the minces and which earlier studies have suggested to be directly or indirectly important for sausage aroma (Stahnke, 1994, 1995). Most of the compounds were present in all samples in different amounts, though some compounds were specific for some strains.

To identify species differences between *S. carnosus*, *S. xylosus* and *S. equorum* regarding aroma production, D-PLSR was performed on the aroma data. The most optimal model separating the three species consisted of three principal components totally, overall explaining 59% and 80% of the variance in the **X**- and ***Y***-matrix, respectively. Fig. 3a and b displays the scores and loading weights plots of the first two principal components.

The scores plot (Fig. 3a) shows that the reproducibility of the duplicate mince samples was good. The replicates A and B are placed close to each other except for the strains S1, S2 and S14 which are scattered due to discrepancies for a few compounds between the duplicates. The scores plot also shows that minces inoculated with the three species were different with regard to aroma pattern. The strains of *S. carnosus*, *S. xylosus* and *S. equorum* lie in two or perhaps three separate groups based on their aroma production. It is uncertain whether the *S. equorum* strains S3 and S18 rather belong to the *S. carnosus* and *S. xylosus* clusters, respectively, than to a separate cluster.

The loading weights plot (Fig. 3b) shows the aroma compounds that were formed in higher or lower

Table 4
Aroma compounds collected from sausage minces added *P. pentosaceus* and *Staphylococcus* and incubated for 7 days at 25 °C

	Identification[a]		Identification[a]
ALDEHYDES		ESTERS	
2-methylpropanal	MS/RI	ethylacetate	MS/RI
3-methylbutanal	MS/RI	1-methyl-ethylacetate	MS/RI
2-methylbutanal	MS/RI	ethylpropanoate	MS/RI
acetaldehyde	MS/RI	methyl-3-methylbutanoate	MS
butanal	MS/RI	ethylbutanoate	MS/RI
pentanal	MS/RI	ethyl-2-methylbutanoate	MS/RI
hexanal	MS/RI	ethyl-3-methylbutanoate	MS/RI
octanal	MS/RI	3-methyl-1-butylacetate	MS/RI
nonanal	MS/RI	2-methyl-1-butylacetate	MS
benzaldehyde	MS/RI	3-methylbutyl-3-methylbutanoate	MS
KETONES		ACIDS	
acetone	MS/RI	acetic acid	MS/RI
3-methyl-2-butanone	MS/RI	2-methyl-propanoic acid	MS/RI
4-methyl-2-pentanone	MS/RI	2-methyl-butanoic acid	MS/RI
3-methyl-2-pentanone	MS/RI	3-methyl-butanoic acid	MS/RI
2-butanone	MS/RI	ALCOHOLS	
2,3-butandione	MS/RI	ethanol	MS/RI
2-pentanone	MS/RI	2-propanol	MS/RI
3-pentanone	MS/RI	1-propanol	MS/RI
2,3-pentandione	MS/RI	2-methyl-1-propanol	MS/RI
3-hydroxy-2-butanone	MS/RI	3-methyl-1-butanol	MS/RI
2-heptanone	MS/RI	2-methyl-1-butanol	MS/RI
acetophenone	MS/RI	SULFIDES	
		dimethyldisulfide	MS/RI
		dimethyltrisulfide	MS

[a] MS = compound has been identified by its mass spectrum compared to the NBS75k database (Agilent, Palo Alto, CA, USA), RI = compound has been identified by Kovats retention index compared to the authentic compound.

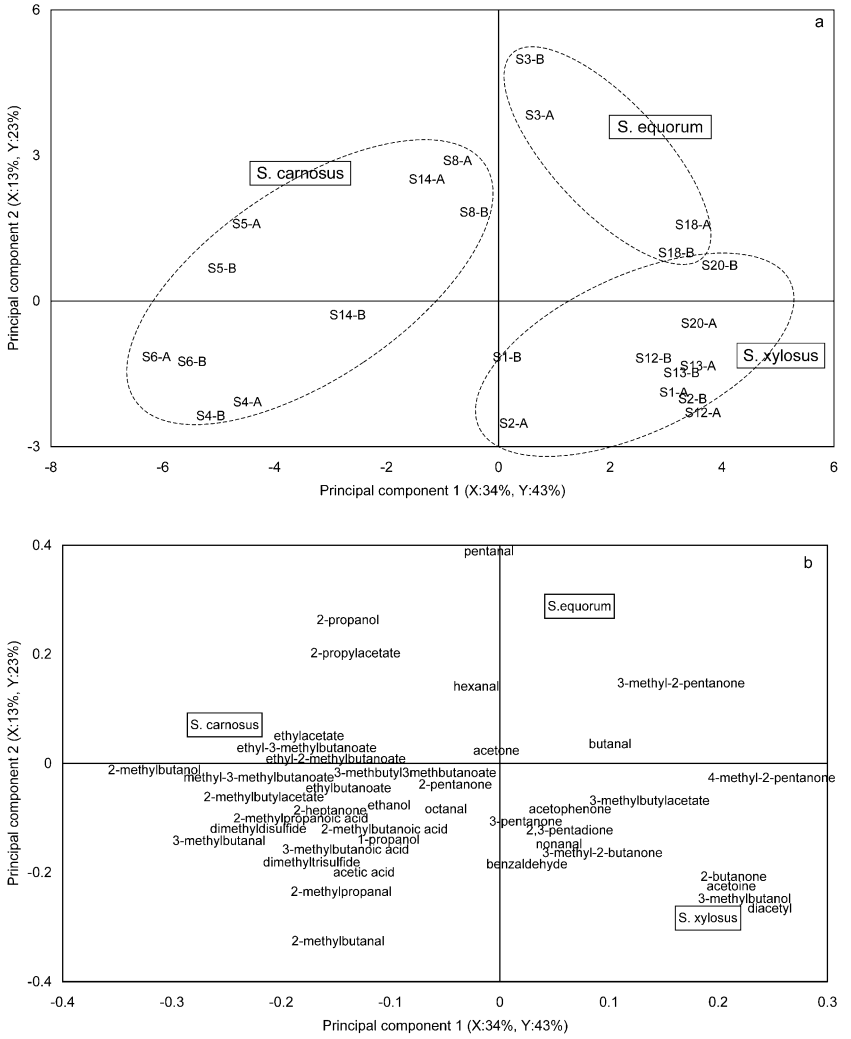

Fig. 3. Plots of principal components 1 and 2 from the discriminant partial least squares regression (D-PLSR) analysis on *Staphylococcus* species versus aroma compounds. Sausage minces were inoculated with *P. pentosaceus* and *S. xylosus* (S1, S2, S12, S13, S20), *S. carnosus* (S4, S5, S6, S8, S14) or *S. equorum* (S3, S18) and the produced aroma compounds collected during 7 days of incubation at 25 °C. (a) Scores plot. A and B symbolise duplicate minces. (b) Loading weights plot.

amounts by the different species. Most predominately, it is seen that *S. xylosus* strains produced more diacetyl, 2-butanone and acetoin than *S. carnosus* strains. Similar results were obtained in an earlier study on two commercial strains of *S. xylosus* and *S. carnosus*, but that study showed that *S. carnosus* did not produce diacetyl and the corresponding acetoin at all (Stahnke, 1999a). This difference may be due to differences in the analysis conditions (GC-FID versus GC-MS) since most of the strains of *S. carnosus* in the present investigation did produce both compounds, though in much lower amounts than *S. xylosus*. However, diacetyl and acetoin may also have arisen from the added *P. pentosaceus*, which produces both diacetyl and acetoin (data not shown). In the former study, *S. carnosus* was grown as a pure culture (Stahnke, 1999a).

A former study by Stahnke (1999a) for just one strain of *S. carnosus* and *S. xylosus* showed that *S.*

xylosus did not produce 2-methyl-1-butanol. This was also the case in the present study—only *S. carnosus* strains produced 2-methyl-1-butanol. This feature could possibly be used as a means of separating the species upon identification. On the other hand, *S. xylosus* strains produced more 3-methyl-1-butanol and the corresponding 3-methylbutyl acetate. In the former study, *S. xylosus* also produced more 3-methyl-1-butanol than *S. carnosus* (Stahnke, 1999a). The same pattern is true for the branched ketones, 3-methyl-2-butanone, 3-methyl-2-pentanone and 4-methyl-2-pentanone arising from valine, isoleucine and leucine, respectively. Those compounds were formed in higher amounts by *S. xylosus* strains in this study as in the former study.

The methyl-branched aldehydes, acids and their esters (2-methylpropanal, 2- and 3-methylbutanal, 2-methylpropanoic acid, 2- and 3-methylbutanoic acid and the corresponding ethyl and methyl esters) were produced in highest amounts by the *S. carnosus* strains. This was also shown by Stahnke (1999a) for one commercial strain of *S. carnosus*. All of those compounds are degradation products of the amino acids leucine, isoleucine and valine. The *S. carnosus* strains also seemed to form higher amounts of methyl ketones from fatty acid β-oxidation (2-pentanone and 2-heptanone), whereas degradation products of the aromatic amino acids (benzaldehyde and acetophenone) were produced in similar amounts by all species. Also the straight-chain aldehydes (pentanal, hexanal, octanal and nonanal) are placed in the centre of the plot, indicating that they were formed in similar amounts in all minces. This is not surprising since those aldehydes are believed to arise from chemical lipid oxidation not involving microbial action (Grosch, 1982).

A very recent study showed that sausages fermented with a strain of *S. carnosus* instead of *M. varians* matured faster, at the same time containing higher amounts of the above mentioned degradation products of leucine, isoleucine and valine, in addition to several methyl ketones (Stahnke et al., 2001). Together with the present results, this indicates that *S. carnosus* strains produce more of the aroma compounds, which are important for fermented sausage maturity and that *S. carnosus* may be a better choice than *S. xylosus* and *S. equorum* when looking at aroma-producing capacity. However, this needs to be investigated further in more details.

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