Journal of Applied Microbiology 2001, **90**, 365–371

Identification by 16S–23S rDNA intergenic region amplification, genotypic and phenotypic clustering of *Staphylococcus xylosus* strains from dry sausages

F. Rossi[1], R. Tofalo[2], S. Torriani[3] and G. Suzzi[2]

[1]Dipartimento Scientifico e Tecnologico, Facoltà di Scienze, Università degli Studi di Verona, [2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Potenza, and [3]Istituto di Industrie Agrarie – Università degli Studi di Catania, Italy

511/8/00: received 18 August 2000, revised 6 November 2000 and accepted 13 November 2000

F. ROSSI, R. TOFALO, S. TORRIANI AND G. SUZZI. 2001.

Aims: To ascertain the identification and typing of the Gram-positive, coagulase-negative cocci present in 'Salsiccia Sotto Sugna', an Italian artisanal sausage.

Methods and Results: Fifty-one strains were isolated and genotypically identified by amplification of the 16S–23S rDNA intergenic region with universal primers. Most isolates were identified as *Staphylococcus xylosus* and one strain as *Staph. condimenti*. Isolates were clustered by numerical analysis of both RAPD (Random Amplified Polymorphic DNA) PCR profiles and physiological characters. Genotypic clustering allowed the separation of strains showing nitrate reduction and amino acid decarboxylase activities. Phenotypic clustering distinguished strains isolated at diverse ripening stages.

Conclusions: The predominance of *Staph. xylosus* in Italian dry sausages was confirmed. Genotypic similarities related to the possession of single phenotypic traits.

Significance and Impact of the Study: In this study, a rapid method of *Staphylococcus* and *Kocuria* species distinction was proposed. The suitability of RAPD PCR to discriminate strains of *Staph. xylosus* with technologically relevant activities was reported.

INTRODUCTION

The characteristic flavour of fermented and ripened sausages is conferred by many different non-volatile and volatile compounds (Stahnke 1994), resulting from the activity of lactic acid bacteria, coagulase-negative staphylococci (CNS) and bacteria belonging to the genus *Kocuria* (Demeyer *et al.* 1974), and endogenous meat enzymes (Hierro *et al.* 1997, 1999). Berdagué *et al.* (1993) suggested that *Staphylococcus* spp., rather than lactic acid bacteria, could have a predominant effect on dry sausage aroma. The most important role of CNS and *Kocuria* spp. is the reduction of nitrate to nitrite to promote the desired red colour development and stability (Liepe 1983), peroxide decomposition and therefore, prevention of rancidity (Barrière *et al.* 1998), and nitrite permanence with consequent maintenance of hygienic safety. Furthermore, their lipolytic and proteolytic activity may contribute to the flavour of fermented meat products by formation of esters and other aromatic compounds from amino acids (Johansson *et al.* 1994). Mixed cultures of lactic acid bacteria and *Staph. xylosus*, *Staph. carnosus* and *Kocuria* spp. strains are increasingly being used to standardize the quality of fermented sausages.

Based on reports concerning identification of CNS and *Kocuria* spp. in cured and ripened meats, CNS are mainly isolated from dry cured meat products (Simonetti and Cantoni 1983; Comi *et al.* 1992; Rodríguez *et al.* 1994), whereas *Kocuria* spp. are dominant in freshly prepared sausages. Nevertheless, Fischer and Schleifer (1980) found 36% *K. varians* and 3% *K. kristinae* in dry sausages. Among the coagulase-negative staphylococcal species, *Staph. xylosus* is frequently involved in the fermentation of several types of sausages, but other species such as *Staph. carnosus*, *Staph. simulans*, *Staph. saprophyticus*, *Staph. epidermidis*, *Staph. haemolyticus*, *Staph. warneri* and *Staph. equorum* can be involved (Fischer and Schleifer 1980; Simonetti and Cantoni 1983; Seager *et al.* 1986; Pirone and Manganelli 1990;

Correspondence to: Dr Franca Rossi, Dipartimento Scientifico e Tecnologico, Facoltà di Scienze MM. FF. NN., Università degli Studi di Verona, Strada Le Grazie 15, I-37134 Verona, Italy (email: rossi@sci.univr.it).

Torriani *et al.* 1994; Coppola *et al.* 1996). Several CNS isolates from dry sausages have not been identifiable to recognized species by physiological identification keys (Pirone and Manganelli 1990; Simonetti and Cantoni 1983; Torriani *et al.* 1994). Moreover, very few genetic fingerprinting techniques have been applied to differentiate some of these bacteria (Moschetti *et al.* 1997; Irlinger and Bergère 1999).

This study was aimed at confirming the identification at the species level of Gram-positive, coagulase-negative cocci isolated from dry sausages. Therefore, a rapid PCR method for discriminating between bacteria, i.e., amplification of the 16S–23S rDNA intergenic region with universal primers (Jensen *et al.* 1993), was applied to differentiate reference strains belonging to CNS and *Kocuria* species and isolates from 'Salsiccia Sotto Sugna', a traditional sausage from southern Italy (Amato *et al.* 1999). Results are presented in relation to physiological identification data.

In addition, an intraspecies genotypic and phenotypic characterization of isolates belonging to the species *Staph. xylosus*, the most widely represented CNS species in dry sausages (Pirone and Manganelli 1990; Comi *et al.* 1992; García-Varona *et al.* 2000), was carried out by numerical analysis of RAPD PCR and phenotypic profiles, respectively. The purpose of this investigation was to gain an insight into the potential of a rapid response characterization technique, such as RAPD PCR, in the selection of strains to be used as starters in typical productions.

MATERIALS AND METHODS

Strains and culture conditions

The following reference strains were used: *K. varians* LMG 14231^T, *K. kristinae* LMG 14215^T, *K. roseus* LMG 14224^T, *Staph. carnosus* subsp. *carnosus* DSM 20501^T, *Staph. carnosus* subsp. *utilis* DSM 11676^T, *Staph. condimenti* DSM 11674^T, *Staph. xylosus* DSM 20266^T and *Staph. simulans* DSM 20322^T.

Mannitol Salt Agar (MSA; Unipath) was used for isolation of 51 strains at different times during ripening of six batches of 'Salsiccia Sotto Sugna' from two producers. The samples analysed comprised all zones of the product section. Isolates were checked by Gram staining and catalase activity. Gram-positive, catalase-positive and coagulase-negative cocci grouped in pairs, tetrads or irregular clusters were further identified according to Kloos and Schleifer (1986) and Kloos *et al.* (1992). Each isolate was tested for: oxidation/fermentation of glucose in Hugh/Leifson medium; acid production from D-fructose, D-galactose, glycerol, D-mannose, lactose, D-mannitol, maltose, raffinose, sucrose, trehalose, D-xylose, L-arabinose, cellobiose, D-melezitose and D-ribose in Baird-Parker medium (Unipath); citrate utilization; starch and Tween 80 hydrolysis; growth in the presence of 10, 15 and 20% NaCl at 10 and 30°C; growth at 4, 10, 15, 37 and 45°C; growth on Simmons citrate agar; urease, ornithine decarboxylase, nitrate reduction, esculin hydrolysis, Voges-Proskauer reaction, oxidase and β-haemolysis. Susceptibility to novobiocin (5 mg l^{-1}), lysostaphin (200 mg l^{-1}) and furazolidone (200 mg l^{-1}) were tested by the agar plate method described by Schleifer and Kloos (1975). Isolates were tested by qualitative assays of proteolysis on FSDA (Huggins and Sandine 1984) and gelatine (Difco), and lipolysis on tributyrin agar (Fluka) and on fat, according to the Berry method (Tiecco 1992). In all cases, the plates were incubated at 30°C for 4 days. Decarboxylase activity towards L-histidine, L-tyrosine, L-ornithine, L-lysine and L-phenylalanine (Fluka) was checked in Joosten and Northolt medium modified according to Maijala *et al.* (1993). All strains were subcultured in Brain Heart Infusion broth (BHI) (Unipath) and allowed to grow for 24–72 h at 37°C in aerobiosis.

DNA extraction and PCR methods

DNA extraction was carried out according to Sambrook *et al.* (1989). The 16S–23S rDNA intergenic spacer region was amplified with universal primers G1–L1, as reported by Jensen *et al.* (1993). RAPD PCR with primers OPL-01 (5′ GGCATGACCT 3′), OPL-02 (5′ TGGGCGTCAA 3′), OPL-05 (5′ ACGCAGGCAC 3′) and Hpy 1 (5′ CCGCAGCCAA 3′) was carried out with 0·5 μmol l^{-1} primer, 100 μmol l^{-1} dNTPs, 1·5 mmol l^{-1} $MgCl_2$, 0·5 U *Taq* DNA polymerase (Polymed), 1 μl 10× PCR buffer and about 10 ng target DNA in 10 μl reaction mixture. The amplification programme was unique and comprised initial denaturation at 94°C for 4 min, 45 cycles of denaturation at 94°C for 30 s, annealing at 35°C for 30 s and extension at 72°C for 1 min, final extension at 72°C for 5 min. Primers and amplification conditions applied allowed a reproducibility of the RAPD PCR profiles higher than 90%.

The amplification products were separated by electrophoresis at 100 V for 2 h on 1·5% agarose gel in 0·5× TBE buffer (45 mmol l^{-1} Tris/borate, 1 mmol l^{-1} EDTA, pH 8·3) stained with 0·5 mg l^{-1} ethidium bromide.

Clustering methods

The Systat software (SSPE Inc., Chicago, IL, USA) for Macintosh was used for physiological clustering; data were recorded as '1' or '0' if positive or negative, respectively. The similarity matrix was defined by the Simple Matching Coefficient (Ssm), and Unweighted Pair Group Method using Arithmetic Averages (UPGMA) was applied for clustering. RAPD PCR electrophoretic profiles with the

four primers were combined and compared by the GelCompar 4·0 software (Applied Maths, Kortrjik, Belgium) using the Pearson product moment correlation coefficient and UPGMA clustering method.

RESULTS

Figure 1 shows the counts on MSA during manufacturing and ripening in the six batches of 'Salsiccia sotto Sugna'. Gram-positive, coagulase-negative cocci occurred constantly and in high numbers. A higher standard deviation was registered at day 60, probably as a consequence of different drying extents among samples. Strains studied are listed in Table 1, separated according to producer and time of isolation.

Figure 2 shows examples of the profiles obtained by amplification of the 16S-23S rDNA spacer region. *Staphylococcus xylosus*, *Staph. simulans*, *K. varians*, *K. roseus* and *K. kristinae* were distinguished on the basis of size and number of amplified regions. Discrimination was not possible between *Staph. carnosus* subsp. *carnosus* and *Staph. carnosus* subsp. *utilis*. *Staphylococcus condimenti* (Probst *et al.* 1998) and *Staph. carnosus* showed very similar profiles but could be distinguished by the presence of an additional band of about 900 bp in the latter species. All isolates were genotypically identified as *Staph. xylosus* with the exception of isolate SS167, similar to *Staph. condimenti*.

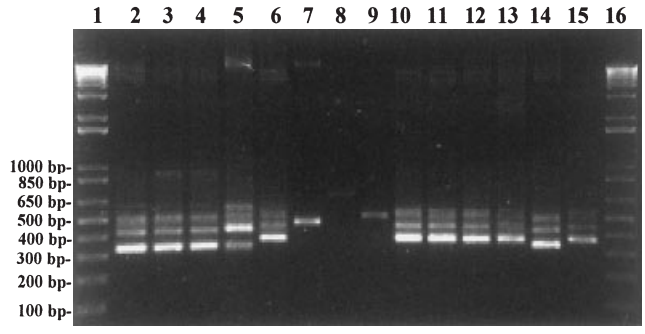


Fig. 2 Amplification profiles with universal primers specific for the 16S–23S rDNA spacer region. Lanes 1 and 16: 1 kb Plus DNA ladder (Gibco BRL); lanes 2–9: *Staphylococcus condimenti* DSM 11674^T, lane 3, *Staph. carnosus* subsp. *utilis* DSM 11676^T, *Staph. carnosus* subsp. *carnosus* DSM 20501^T, *Staph. simulans* 20322^T, *Staph. xylosus* 20266^T, *Kocuria varians* LMG 14231^T, *K. roseus* LMG 14224^T, *K. kristinae* LMG 14215^T; lanes 10–15: isolates SS200, SS49, SS30, SS81, SS167 and SS12

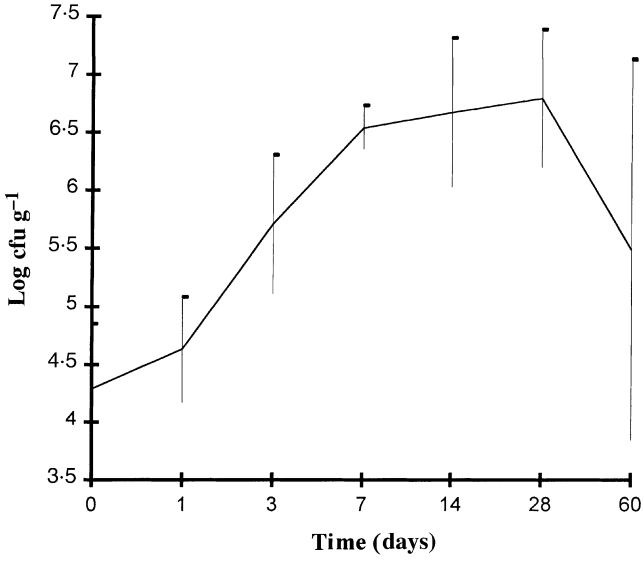
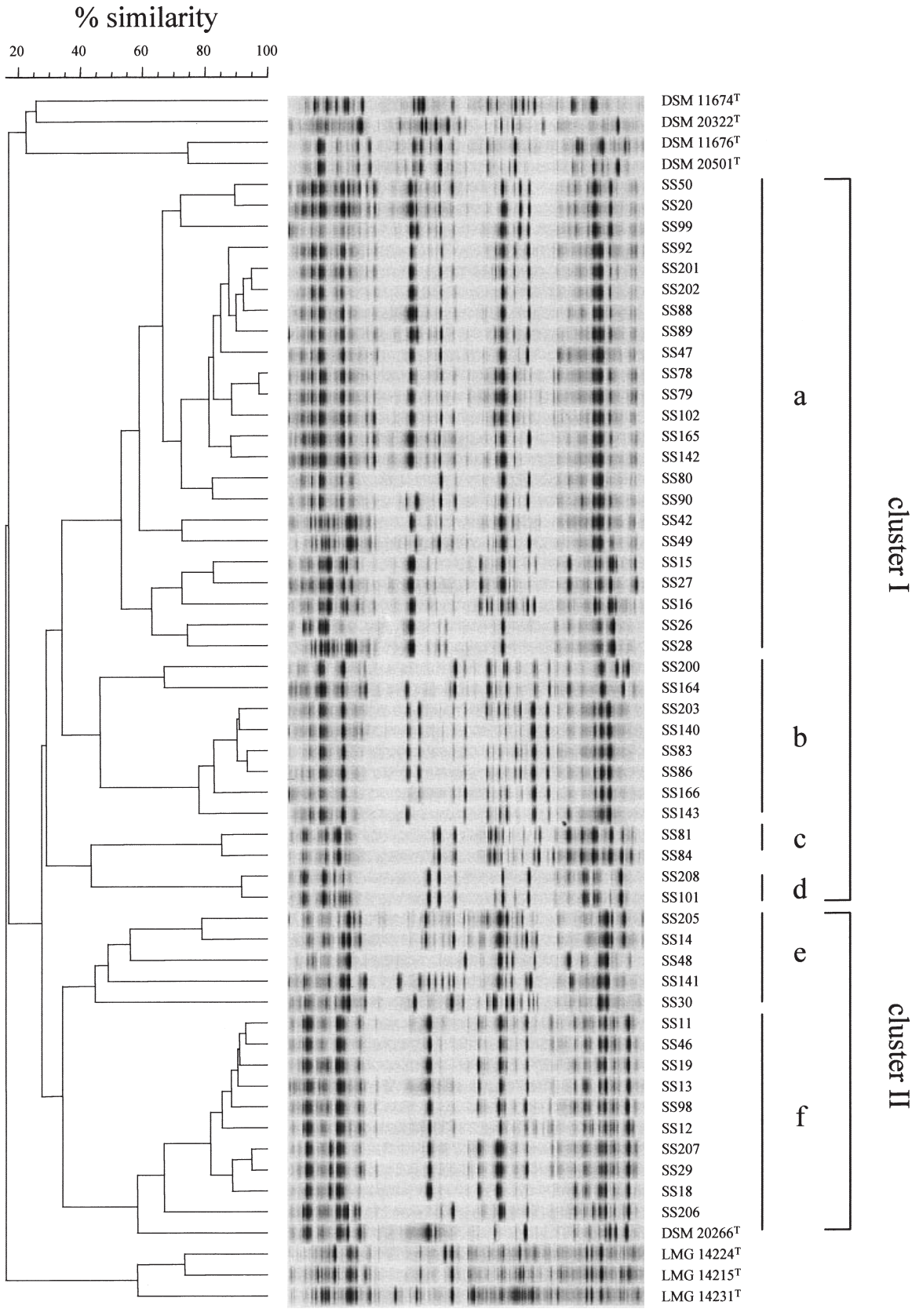


Fig. 1 Counts of coagulase-negative staphylococci during ripening of Salsiccia Sotto Sugna with relative standard deviations

Forty-six isolates were identified, also phenotypically, as *Staphylococcus* spp. as they were susceptible to lysostaphin and furazolidone, and were coagulase-negative and β-haemolysis-negative. The identification of most isolates as *Staph. xylosus* was confirmed. However, exceptions to characters defined as species- or genus-specific (Kloos and Schleifer 1986; Kloos *et al.* 1992) did not allow an unequivocal identification of some strains. Three strains were able to grow on FTO agar and two were resistant to lysostaphin. Therefore, they could not be clearly differentiated from *Kocuria* and *Micrococcus*. Further exceptions were observed for characters reported as stable in the species

Table 1 Time of isolation and provenance of strains

Ripening time (days)	Isolates	
	Producer 1	Producer 2
0	SS11, SS12, SS13, SS14, SS15	SS83, SS84, SS86
1	SS16, SS18, SS19, SS20	SS78, SS79, SS80, SS81, SS88, SS89, SS90, SS92
3	SS26, SS27, SS28, SS29, SS30	SS42
7	SS46, SS47, SS48, SS49, SS50	SS200, SS201, SS202, SS203
14	SS98, SS99, SS101, SS102	SS140, SS141, SS142, SS143
28	SS205, SS206, SS207, SS208	SS164, SS165, SS166, SS167


% similarity
20
40
60
80
100
DSM 11674T
DSM 20322T
DSM 11676T
DSM 20501T
SS50
SS20
SS99
SS92
SS201
SS202
SS88
SS89
SS47
SS78
SS79
SS102
SS165
SS142
SS80
SS90
SS42
SS49
SS15
SS27
SS16
SS26
SS28
SS200
SS164
SS203
SS140
SS83
SS86
SS166
SS143
SS81
SS84
SS208
SS101
SS205
SS14
SS48
SS141
SS30
SS11
SS46
SS19
SS13
SS98
SS12
SS207
SS29
SS18
SS206
DSM 20266T
LMG 14224T
LMG 14215T
LMG 14231T
a
b
c
d
e
f
cluster I
cluster II

◀

Fig. 3 Genotypic clustering based on comparison of RAPD PCR profiles

Staph. xylosus, i.e., xylose, melezitose, fructose, trehalose and mannitol fermentation, urease activity, pigmentation, and ability to grow at 45°C.

Safety aspects were also tested. None of the isolates was β-haemolytic and 25 strains were able to decarboxylate tyrosine, tryptophan and ornithine.

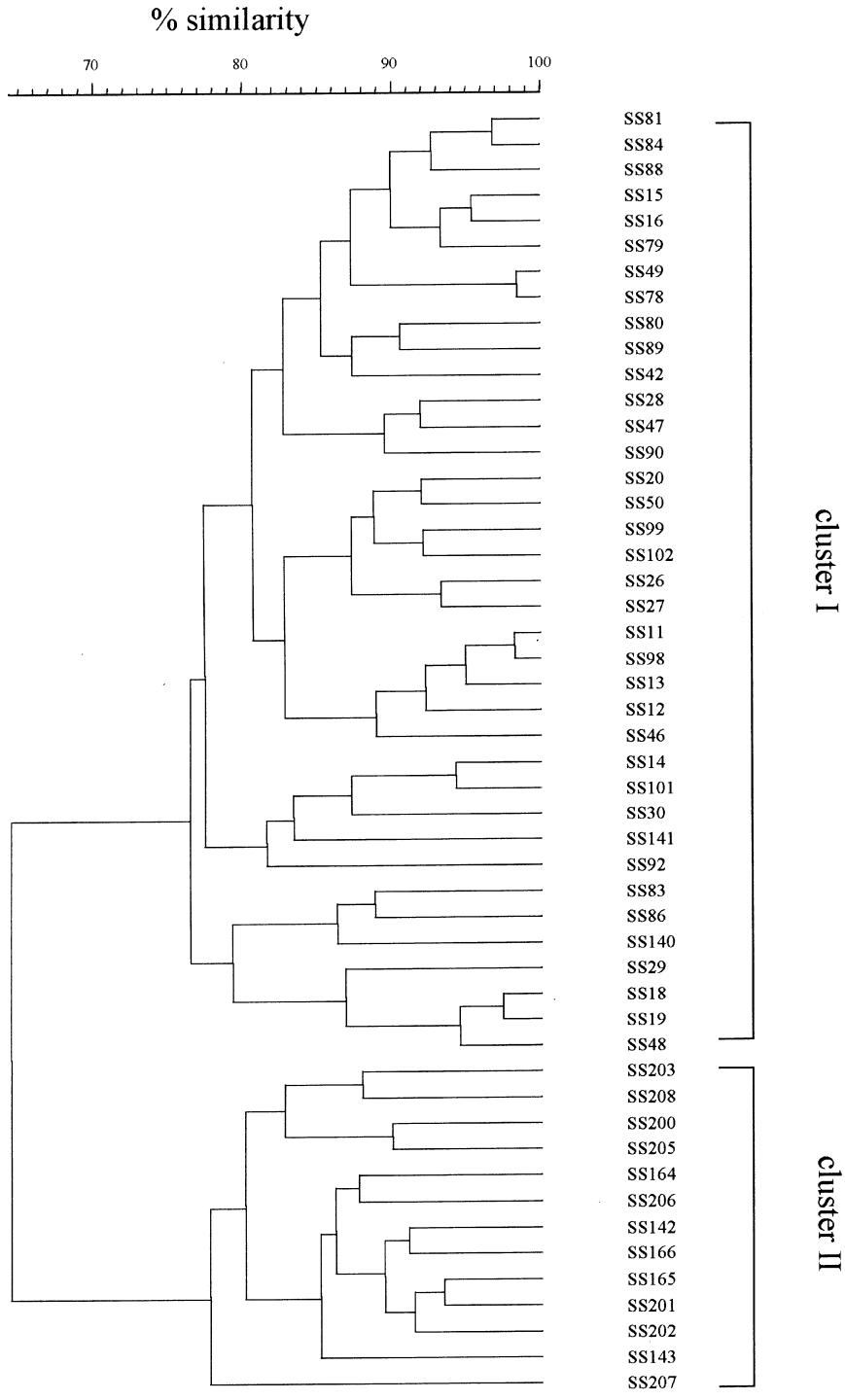


Fig. 4 Clustering based on physiological similarities

Additional technologically-important characters such as skim milk, tributyrin and lard hydrolysis were assayed; none of the isolates hydrolysed skim milk, only five isolates, i.e., SS18, SS81, SS84, SS101 and SS167, hydrolysed lard, and none hydrolysed tributyrin.

Figure 3 and Fig. 4 show *Staph. xylosus* strains grouping by numerical analysis of genotypic and phenotypic profiles.

As shown in Fig. 3, the cluster comprising the type strain DSM 20266^T and all *Staph. xylosus* isolates merges at an overall similarity of 27%.

The distribution of single physiological features within the RAPD PCR clusters was analysed. With respect to nitrate reduction, two main intraspecies clusters were recognized. Cluster I comprised strains able and unable to reduce nitrates, but these were well separated. Subclusters *a* and *c* comprised only strains unable to reduce nitrates, and subclusters *b* and *d* included only nitrate reducers. Cluster II included only strains able to reduce nitrates. Distribution of decarboxylase activity was similar; almost all strains able to reduce nitrate also decarboxylated amino acids, except strains SS206 and SS141 in cluster II (Fig. 3). All other characters were distributed randomly within the genotypic clusters.

The RAPD PCR analysis permitted a partial distinction of strains on the basis of the manufacturing plant of origin (Table 1, Fig. 3). Subcluster *b* and cluster II were entirely constituted by strains from the same producer, whereas in the remaining subclusters, isolates from the two producers were intermixed.

Figure 4 shows that in very few cases, isolates were phenotypically associated according to their genotypes. Two main clusters were distinguished; cluster II comprised only strains isolated after 7 days of ripening which were not genotypically closely related.

DISCUSSION

The results confirm that strains belonging to *Staph. xylosus* prevail in traditional Italian salami, being dominant over other CNS and *Kocuria* species (Pirone and Manganelli 1990; Comi *et al.* 1992). Very recent studies on Spanish Chorizo, carried out on 426 strains, provided similar data; no strains were identified as *Kocuria* spp. and 94·6% of isolates belonged to the species *Staph. xylosus* (García-Varona *et al.* 2000). The presence of *Kocuria* spp. in ripened sausages from other countries (Fischer and Schleifer 1980) is probably due to climatic factors and diverse production practices.

Phenotypic identification of the *Staph. xylosus* strains under study was problematic due to exceptions in some characters officially accounted for as genus- or species-specific. These findings are common to those of other studies (Sierra *et al.* 1995) and demonstrate the necessity of confirmation by molecular methods.

The rDNA 16S–23S spacer region amplification permits the identification of strains belonging to *Staph. xylosus*, *Staph. simulans*, *Staph. carnosus* or closely-related species, and of *Kocuria* species (Fig. 1). This facilitates the correct identification of such components of sausage microflora, and confirms the advantage of application of such a technique for the differentiation of bacterial species.

The only partial genotypic distinction of *Staph. xylosus* strains of different provenance indicates the existence of biotypes of wider diffusion, as also observed by García-Varona *et al.* (2000).

Differences in metabolic profiles between strains isolated at different times during ripening were also observed by García-Varona *et al.* (2000), who reported that most isolates from the late ripening stage were able to produce acetoin, and showed higher proteolytic and lipolytic activities.

No agreement exists between intraspecies grouping by the two clustering methods, but these appear complementary in separating strains with technologically-important properties, i.e., nitrate reduction and amino acid decarboxylation abilities, and tendency to develop in late ripening phase.

The clustering methods adopted here can help the selection of autochthonous bacterial strains to be used as starters in typical productions instead of commercial cultures. Isolates representing distinct genotypic or phenotypic clusters can be further tested for safety attributes and used in manufacturing trials for the evaluation of their suitability in exalting the sensory properties of local productions.

REFERENCES

Amato, D., Di Gennaro, P., Lanorte, M.T., Caruso, M., Suzzi, G. and Laurita, C. (1999) Caratterizzazione dei prodotti tipici lucani. 'La salsiccia sotto sugna'. *Industrie Alimentari* **38**, 1–9.

Barrière, C., Montel, M.C. and Talon, R. (1998) Production of catalase and superoxide dismutase by *Staphylococcus carnosus* and *Staphylococcus xylosus*. In *44th International Congress of Meat Science and Technology* ed. Diestre, A. and Monfort, J.M. pp. 794–795. Barcelona.

Berdagué, J.L., Monteil, P., Montel, M.C. and Talon, R. (1993) Effect of starter cultures on the formation of flavour compounds in dry sausages. *Meat Science* **35**, 229–240.

Comi, G., Citterio, B., Manzano, M., Cantoni, C. and De Bertoldi, M. (1992) Evaluation and characterization of *Micrococcaceae* strains in Italian dry fermented sausages. *Fleischwirtsch* **72**, 1679–1683.

Coppola, S., Iorizzo, M., Saotta, R., Sorrentino, E. and Grazia, L. (1996) Characterization of micrococci and staphylococci isolated from Soppressata Molisana, a Southern Italy fermented sausage. *Food Microbiology* **12**, 1–6.

Demeyer, D.I., Hoozee, J. and Mesdom, H. (1974) Specificity of lipolysis during dry sausage ripening. *Journal of Food Science* **39**, 293–296.

Fischer, U. and Schleifer, K.H. (1980) Vorkommen von staphylokokken un mikrokokken in Rohwurst. *Fleischwirtsch* **60**, 1046–1049.

García-Varona, M., Santos, E.M., Jaime, I. and Rovira, J. (2000) Characterisation of *Micrococcaceae* isolated from different varieties of chorizo. *International Journal of Food Microbiology* **54**, 189–195.

Hierro, E., de la Hoz, L. and Ordóñez, J.A. (1997) Contribution of microbial and meat endogenous enzymes to the lipolysis of dry fermented sausages. *Journal of Agricultural and Food Chemistry* **45**, 2989–2995.

Hierro, E., de la Hoz, L. and Ordóñez, J.A. (1999) Contribution of the microbial and meat endogenous enzymes to the free amino acid and amine contents of dry fermented sausages. *Journal of Agricultural and Food Chemistry* **47**, 1156–1161.

Huggins, A.R. and Sandine, W.E. (1984) Differentiation of fast and slow milk-coagulating isolates in strains of lactic streptococci. *Journal of Dairy Science* **67**, 1674–1679.

Irlinger, F. and Bergère, J.-L. (1999) Use of biochemical tests and analyses of ribotype patterns for classification of micrococci isolated from dairy products. *Journal of Dairy Research* **66**, 91–103.

Jensen, M.A., Webster, J.A. and Strauss, N. (1993) Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphysm. *Applied and Environmental Microbiology* **59**, 945–952.

Johansson, G., Berdagué, J.-L., Larsson, M., Tran, N. and Borch, E. (1994) Lipolysis, proteolysis and formation of volatile components during ripening of a fermented sausage with *Pediococcus pentosaceus* and *Staphylococcus xylosus* as starter cultures. *Meat Science* **38**, 203–218.

Kloos, W.E. and Schleifer, K.H. (1986) The genus *Staphylococcus*. In *Bergey's Manual of Systematic Bacteriology* ed. Sneath, P.H.A., Mair, N.S. and Sharpe, M.E. pp. 1013–1035. Baltimore: Williams & Wilkins.

Kloos, W.E., Schleifer, K.H. and Gotz, F. (1992) The genus *Staphylococcus.* In *The Prokaryotes, a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications* 2nd edn. pp. 1367–1420. Balows, A., Treuper, H.E., Dworkin, M., Harder, W. and Schleifer, K.-H. New York: Springer Verlag.

Liepe, H.U. (1983) Starter cultures in meat production. *Biotechnology* **5**, 400–424.

Maijala, R.L., Eerola, S., Aho, M.A. and Hirn, J.A. (1993) The effect of GDL-induced pH decrease on the formation of biogenic amines in meat. *Journal of Food Protection* **56**, 125–129.

Moschetti, G., Mauriello, G. and Villani, F. (1997) Differentiation of *Staphylococcus xylosus* strains from Italian sausages by antibiotyping and low frequency restriction fragment analysis of genomic DNA. *Systematic Applied Microbiology* **20**, 432–438.

Pirone, G. and Manganelli, E. (1990) Caratterizzazione di *Micrococcaceae* isolate da salami tipo Napoli. *Industria delle Conserve* **65**, 220–223.

Probst, A.J., Hertel, C., Richter, L., Wassill, L., Ludwig, W. and Hammes, W.P. (1998) *Staphylococcus condimenti* sp. nov., from soy sauce mash, and *Staphylococcus carnosus* (Schleifer and Fischer 1982) subsp. *utilis* subsp. nov. *International Journal of Systematic Bacteriology* **48**, 651–658.

Rodríguez, M., Núñez, F., Córdoba, J.J., Sanabria, C., Bermúdez, E. and Asensio, M.A. (1994) Characterization of *Staphylococcus* spp. & *Micrococcus* spp. isolated from Iberian ham throughout the ripening process. *International Journal of Food Microbiology* **24**, 329–335.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual* 2nd edn. Ford, N., Nolan, C., Ferguson, M. and Irwin, N. New York: Cold Spring Harbor Laboratory.

Schleifer, K. and Kloos, W.E. (1975) A simple test system for the separation of staphylococci from micrococci. *Journal of Clinical Microbiology* **1**, 337.

Seager, M.S., Banks, J.G., Blackburn, C. and Board, R.G. (1986) A taxonomy study of *Staphylococcus* spp. isolated from fermented sausages. *Journal of Food Protection* **48**, 295–297.

Sierra, M., Gonzles Fandos, M.E., García, M.C., García, M.L. and Moreno, B. (1995) Numerical taxonomy of an 'atypical' population of Gram-positive cocci isolated from freshly dressed lamb carcasses. *International Journal of Food Microbiology* **24**, 363–373.

Simonetti, P. and Cantoni, C. (1983) Coagulase negative staphylococci for dry sausage ripening. *Industrial Alimentaria* **22**, 262–264.

Stahnke, L.H. (1994) Aroma components from dried sausages fermented with *Staphylococcus xylosus*. *Meat Science* **38**, 39–53.

Tiecco, G. (1992) Stafilococchi coagulasi negativi. *Microbiologia Degli Alimenti Di Origine Animale*. pp. 473–506. Bologna: Edagricole.

Torriani, S., Di Bucchianico, R., Pattarini, F., Zabeo, G. and Dellaglio, F. (1994) Presenza e caratterizzazione biotecnologica di batteri lattici e *Micrococcaceae* negli insaccati abruzzesi. *Industria delle Conserve* **69**, 3–9.