Strains of *Staphylococcus* and *Bacillus* isolated from traditional sausages as producers of biogenic amines

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**INTRODUCTION**

Biogenic amines are basic nitrogen compounds usually formed by decarboxylation of precursor amino acids (Janz et al., 1983; Halász et al., 1994; Silla Santos, 1996).

Formation of biogenic amines in foods is important for health and also for unfavorable effects on flavor (Suzzi and Gardini, 2003). Biogenic amines affect blood pressure, and excessive quantities in food can trigger migraines, gastric and intestinal problems, and allergic responses in sensitive people (Smith, 1980; Taylor, 2003). Biogenic amines affect blood pressure, and excessive quantities in food can trigger migraines, gastric and intestinal problems, and allergic responses in sensitive people (Smith, 1980; Taylor, 2003). Biogenic amines affect blood pressure, and excessive quantities in food can trigger migraines, gastric and intestinal problems, and allergic responses in sensitive people (Smith, 1980; Taylor, 2003). Biogenic amines affect blood pressure, and excessive quantities in food can trigger migraines, gastric and intestinal problems, and allergic responses in sensitive people (Smith, 1980; Taylor, 2003). Biogenic amines affect blood pressure, and excessive quantities in food can trigger migraines, gastric and intestinal problems, and allergic responses in sensitive people (Smith, 1980; Taylor, 2003).

During ripening of meat products, the proteins undergo degradation processes; large peptides are first generated and then degraded into oligopeptides, and these are in turn degraded to free amino acids. The free amino acids are then catabolized, giving rise to different compounds such as ammonia, α-ketoacids, methylketones, and amines.

In meat products, formation of biogenic amines is largely associated with the activity of microorganisms present in meat (Ten Brink et al., 1990; Shalaby, 1996; Paulsen and Bauer, 1997). Ripening of sausages provides conditions that are very favorable for the production of biogenic amines, due to the active growth of microbial populations, acidification, and proteolysis.

Different measures have been taken with the aim of preventing or minimizing formation of biogenic amines during the manufacture of raw-cured sausages, such as improved hygiene in production plants, the use of starter cultures formed by lactic acid bacteria with acidifying capacity, and the use of certain preservatives (Buncic et al., 1993; Majala et al., 1993; Bover-Cid et al., 2000a,b; Suzzi and Gardini, 2003; Komprda et al., 2004; Lu et al., 2010). Although such practices usually reduce the production of biogenic amines, they do not totally prevent the production, and moreover, the increased proteolysis that results from the use of starter cultures may actually increase the availability of amino acids precursors.

Complete inhibition of biogenic amine formation during production of sausages, without any adverse effects, is desirable. However, production of biogenic amines is an extremely complex phenomenon that depends on several variables such as the growth kinetics of the microorganisms and their proteolytic and decarboxylase activities. In order to design strategies for specific inhibition of the production of these compounds, it is essential to obtain information about the potential production of biogenic amines by the microorganisms present in fermented meat products.

Of all the microbial groups present during the fermentation/ripening of the raw-cured sausages, the Enterobacteriaceae and the lactic acid bacteria have been abundantly studied as producers of biogenic amines. Information in the literature about the ability of the *Staphylococcus* species to produce biogenic amines is more reduced (Masson et al., 1996; Silla Santos, 1998; Martín et al., 2006; Drosinos et al., 2007; Bonomo et al., 2009; Even et al., 2010), and studies on the production of biogenic amines by *Bacillus* species are practically inexistent.

The objective of this research was to investigate the decarboxylase activity and the ability to produce biogenic amines “*in vitro*” by the species of *Staphylococcus* and *Bacillus* isolated from two Spanish traditional sausage varieties, with the aim of elucidating the role of the microorganisms belonging to these two genera in the production of biogenic amines during the manufacture of the fermented and ripened sausages.

**MATERIALS AND METHODS**

**BACTERIAL STRAIN IDENTIFICATION AND MOLECULAR TYPING**

In this study, 38 strains of *Staphylococcus* (15 of *S. equorum*, 11 of *S. epidermidis*, 7 of *S. saprophyticus*, and 5 of *S. pasteuri*) and...
19 strains of *Bacillus* (13 of *B. subtilis* and 6 of *B. amyloliquefaciens*) were used. The strains were isolated from 20 units of Androlla sausage and from 15 units of Botillo sausage (two Spanish traditional sausage varieties) at the end of the manufacturing process. Manufacture process and features of these two sausages have been previously described (Lorenzo et al., 2000). The strains were initially identified by classical methods in previous researches (García Fontán et al., 2007a,b) and their identity was confirmed prior carrying out the present work by sequentiation of the 16S rRNA gene, comparing the obtained sequences with those available in the database GenBank (National Center for Biotechnology Information, Bethesda, MD, USA).

Strains were molecular typed by (GTG)₃-PCR fingerprinting techniques. Genomic DNA extracted from each strain was subjected to rep-PCR analysis using the single oligonucleotide primer (GTG)₃ (Iacumin et al., 2006). Reactions were carried out in a final volume of 25 µL containing 12.5 µL of 2 × ReddyMix 1.5 mM MgCl₂ (ABgene, Epsom, UK), 2 µL of extracted DNA, and 1 µM of (GTG)₃ primer. Amplifications were performed in a MyCycler thermal cycler (Bio-Rad, Hercules, USA). Initial denaturation (95°C, 2 min) was followed by 31 cycles of denaturation at 94°C for 3 s, a step at 92°C for 30 s, primer annealing at 40°C for 1 min and extension at 65°C for 8 min. The last cycle was followed by the final single extension step (65°C, 8 min).

Amplicons were separated by electrophoresis in a 1.5% agarose gel in buffer TBE 1× at 75 V for 2 h. After the run, gels were stained with ethidium bromide 1 µg/mL (Sigma-Aldrich, St. Louis, USA) for 30 min. The resulting fingerprints were visualized under UV light and digitally captured using the imaging system Gel Doc XR+ (Bio-Rad, Hercules, USA) and analyzed with the Quantity One software (Bio-Rad, Hercules, USA).

Strains were stored at −80°C in BHI broth (Oxoid Ltd., Basingstoke, Hampshire, UK), with 20% glycerol as a cryoprotective agent. Before use, the strains were reactivated by incubation in BHI broth at 37°C.

**PREPARATION OF INOCULA**

In order to prepare the inocula used in the quantitative analysis, firstly a correlation between the log CFU/mL and the Optical Density (at 650 nm) of the cultures was established for each strain by determining throughout the growth the O.D. and the log CFU/mL by plate counting in BHI agar (OXOID).

Samples of BHI broth cultures were collected after 24 h of incubation, the O.D. was measured (in order to calculate the number of CFU/mL), the cultures were centrifuged at 12000 × g and the cells were washed by resuspension in a solution of 0.85% NaCl and centrifugation at 12000 × g (three times). Finally, the cells were suspended in the 0.85% NaCl solution to provide inocula containing 10⁷ CFU/mL.

**PRELIMINARY QUALITATIVE TESTS FOR BIOGENIC AMINE PRODUCTION**

As a preliminary test of the capacity of the bacterial strains to produce biogenic amines, the method described by Joosten and Northolt (1987) was used. The culture medium used contained tryptophane (0.5%), yeast extract (0.5%), NaCl (0.5%), glucose (0.1%), Tween 80 (0.05%), MgSO₄ 7H₂O (0.02%), CaCO₃ (0.01%), MnSO₄ 4H₂O (0.005%), FeSO₄ 7H₂O (0.004%), bacteriological agar (2%), and purple bromocresol (0.006%) as pH indicator. The precursor amino acids of each biogenic amine (histidine, lysine, ornithine, and tyrosine) were added individually to the culture medium to a final concentration of 2%. The final pH was adjusted to 5.5 ± 0.1, the medium was sterilized and distributed in Petri dishes. Plates of the culture medium containing each one of the precursor amino acids were streaked, in order to obtain individual colonies, with each bacterial strain. The plates were incubated at 37°C and examined after 12, 24, 48, 72, and 120 h of incubation; a positive result was manifested by the appearance of a purple halo around the colonies.

**QUANTITATIVE ANALYSIS OF THE BIOGENIC AMINES PRODUCED BY THE BACTERIAL STRAINS**

In a previous study (Lorenzo et al., 2008), the different biogenic amines were quantified in the sausage units from which the microbial strains tested in the present work were isolated. We observed that in these sausages the putrescine and cadaverine were by far the major biogenic amines. In order to quantify the production of each biogenic amine (putrescine and cadaverine) by the different bacterial strains, in each bacterial strain, and for each individual precursor amino acid (ornithine and lysine), 2 tubes (5 mL each) of the culture medium (Joosten and Northolt, 1987) containing 2% of the corresponding individual precursor amino acid were each inoculated with 0.1 mL of a solution (0.85 g NaCl/L), containing 10⁸ CFU. The tubes, with a final concentration of 2 × 10⁷ CFU/mL, were incubated at 37°C for 72 h (previously, quantification of the biogenic amines was performed along 96 h of growth, showing that for most strains maximum accumulation took place after 72 h of incubation). After incubation, the O.D. was measured in one tube, and the corresponding biogenic amine was determined in the other. Firstly, 1 mL of 2 N HCl was added to the tube in order to stop microbial growth and decarboxylation. The content of the tube was then placed in a 25 mL volumetric flask, 1 mL of 1,7-diaminoheptane (internal standard) was added, and the final volume was made up with a 0.6 N HClO₄ solution. An aliquot (0.5 mL) of the mixture was then immediately placed in a tube, and 100 µL of 2 N NaOH (to make the solution more alkaline), 150 µL of a saturated solution of NaHCO₃, and 1 mL of dansyl chloride, were added consecutively. The tube was shaken gently, and placed in a water bath at 40°C for 45 min. In order to remove residues of dansyl chloride, 50 µL of ammonia were then added and the mixture was left to stand for 30 min. Finally, the volume was made up to 2.5 mL with acetonitrile and the mixture was filtered (0.25 µm).

Separation, identification, and quantification of the biogenic amines were carried out by HPLC, following the procedure described by Eerola et al. (1993), using the equipment and chromatographic conditions reported by Lorenzo et al. (2010).

A standard solution containing appropriate amounts of agmatine, tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine, and 1,7-diaminoheptane (as internal standard) was used to quantify the biogenic amines present in the samples.

All the samples and standards were injected at least in duplicate in different days. Repeatability tests were performed by injecting
RESULTS

MOLECULAR TYPING OF THE STAPHYLOCOCCUS AND BACILLUS STRAINS ISOLATED FROM TRADITIONAL SAUSAGES

The obtained resulting fingerprints of the strains of Staphylococcus and Bacillus used in the present study demonstrated that the strains belonging to the same species were different. This is important, because some strains belonging to the same species came from the same sausage unit and could be the same.

DECARBOXYLASE ACTIVITY OF THE STAPHYLOCOCCUS AND BACILLUS STRAINS ISOLATED FROM TRADITIONAL SAUSAGES

The decarboxylase activity of the Staphylococcus strains tested is shown in Table 1. In this table, results reported in the literature for the same species were summarized. The Staphylococcus strains displayed a great variability in the histidine decarboxylase activity, with 7 of the 15 strains of S. equorum (46.67%), 9 of the 11 strains of S. epidermidis (81.82%), 6 of the 7 strains of S. saprophyticus (85.71%), and 4 of the 5 strains of S. pasteuri (80%) displaying this activity. The tyrosine decarboxylase, ornithine decarboxylase, and lysine decarboxylase activities were less variable. All strains of S. saprophyticus displayed tyrosine, ornithine, and lysine decarboxylase activities, whereas the frequency of the presence of these activities was slightly lower in strains of S. equorum, S. epidermidis, and S. pasteuri. Lysine was the amino acid decarboxylated with a higher frequency; the 100% of the strains of S. equorum and S. saprophyticus, the 91% of the strains of S. epidermidis and the 80% of the strains of S. pasteuri were able to decarboxylate this amino acid.

The decarboxylase activity observed in the Bacillus strains tested is shown in Table 2. Of the 13 strains of B. subtilis studied, 10 displayed histidine decarboxylase activity (76.92%), 12 tyrosine decarboxylase activity (92.31%), 8 ornithine decarboxylase activity (61.54%), and 8 lysine decarboxylase activity (61.54%). The strains of B. amyloliquefaciens showed less variable behavior; of the six strains studied, four were able to decarboxylate histidine (66.72%), and six were able to decarboxylate tyrosine, ornithine, and lysine (100%).

Table 1 | Decarboxylase activity of the Staphylococcus strains isolated from traditional sausages in this work and from different sausages and other sources in previous studies.

| Species | No. of strains | No. of strains positive | % | Bonomo et al. (2009) | Drosinos et al. (2007) | Even et al. (2010) | Martin et al. (2006) | Masson et al. (1996) | Silla Santos (1998) |
|---------|----------------|------------------------|---|---------------------|----------------------|---------------------|---------------------|---------------------|---------------------|
| **HISTIDINE DECARBOXYLASE ACTIVITY** |
| Staphylococcus equorum | 15 | 7 | 47 | 0 | 0 | NT | NT | NT |
| S. epidermidis | 11 | 9 | 82 | NT | NT | 3 | 14 | NT | NT |
| S. saprophyticus | 7 | 6 | 86 | 0 | 7 | 0 | NT | 0 | <70 |
| S. pasteuri | 5 | 4 | 80 | 0 | NT | NT | NT | NT | NT |
| **TYROSINE DECARBOXYLASE ACTIVITY** |
| S. equorum | 15 | 14 | 93 | 0 | 0 | NT | NT | NT | NT |
| S. epidermidis | 11 | 8 | 73 | NT | NT | nt | 0 | NT | NT |
| S. saprophyticus | 7 | 7 | 100 | 0 | 50 | nt | NT | 100 | 100 |
| S. pasteuri | 5 | 5 | 100 | 0 | NT | nt | NT | NT | NT |
| **ORNITHINE DECARBOXYLASE ACTIVITY** |
| S. equorum | 15 | 14 | 93 | 0 | 0 | 0 | NT | NT | NT |
| S. epidermidis | 11 | 10 | 91 | NT | NT | 3 | 21 | NT | NT |
| S. saprophyticus | 7 | 7 | 100 | 0 | 16 | 3 | NT | NT | <70 |
| S. pasteuri | 5 | 4 | 80 | 0 | NT | NT | NT | NT | NT |
| **LYSINE DECARBOXYLASE ACTIVITY** |
| S. equorum | 15 | 15 | 100 | 86 | 0 | 0 | NT | NT | NT |
| S. epidermidis | 11 | 10 | 91 | NT | NT | 3 | 21 | NT | NT |
| S. saprophyticus | 7 | 7 | 100 | 50 | 50 | 3 | NT | NT | <70 |
| S. pasteuri | 5 | 4 | 80 | 100 | NT | NT | NT | NT | NT |

(NT) Species not tested in the corresponding study. (nt) Decarboxylase activity not tested in the corresponding study.
also produced variable quantities of putrescine, with concentrations ranging between 1.19 and 12.39 mg/L. The strains of *S. saprophyticus* generally produced less putrescine, at less variable concentrations ranging from 0.43 to 1.91 mg/L.

Regarding the production of cadaverine, the quantities accumulated were again very variable within the strains of *S. equorum* (from 0.25 to 5.31 mg/L) and *S. epidermidis* (from 0.46 and 36.52 mg/L). A considerable variability was also registered within the strains of *S. saprophyticus* (from 0.58 to 4.79 mg/L) and *S. pasteuri* (from 0.40 to 4.33 mg/L).

The species that accumulated the highest amounts of putrescine and cadaverine were *S. equorum* and *S. epidermidis*. One of the strains of *S. equorum* accumulated 1415.05 mg/L of putrescine and one of the strains of *S. epidermidis* displayed a high aminogenic ability, accumulating 977.13 mg/L of putrescine or 36.52 mg/L of cadaverine after 72 h of growth in the culture medium.

The Bacillus strains in the present study also displayed a high degree of variability in their ability to produce putrescine and cadaverine. In the strains of *B. subtilis*, the quantities accumulated ranged from 0.39 to 18.43 mg/L for putrescine and from 0.43 to 4.29 mg/L for cadaverine. In the strains of *B. amyloliquefaciens*, the quantities ranged from 0.76 to 3.07 mg/L for putrescine, and from 0.53 to 3.07 mg/L for cadaverine.

**DISCUSSION**

Information in the literature concerning the amino acid decarboxylase activity in strains of the genus *Staphylococcus* is scarce and shows in general that microorganisms belonging to this genus are not significant possessors of these activities. Nonetheless, Silla Santos (1998) reported a high frequency of histidine, tyrosine, ornithine, and lysine decarboxylase activity in strains of *S. xylosus* and *S. saprophyticus* isolated from Spanish fermented sausages, which is in agreement with our results. Furthermore, Martin et al. (2007) observed ornithine and lysine decarboxylase activities in 57% of the strains of *S. xylosus* isolated from Iberian dry-cured sausages.

However, Drosinos et al. (2007) analyzed 300 staphylococci strains isolated from traditional fermented Greek sausages and observed that only a low proportion of strains displayed amino acid decarboxylase activity. The species with the highest proportion of strains that displayed histidine, tyrosine, ornithine, or lysine decarboxylase activity were *S. saprophyticus*, *S. simulans*, and *S. xylosus*, but within each species the proportion of strains that were positive for a specific amino acid decarboxylase activity was never

**Table 2 | Decarboxylase activity of the Bacillus strains isolated from traditional sausages in this work.**

| Species                  | No. of strains | No. of strains positive | %   |
|--------------------------|----------------|-------------------------|-----|
| HISTIDINE DECARBOXYLASE ACTIVITY |                |                         |     |
| *Bacillus subtilis*      | 13             | 10                      | 77  |
| *B. amyloliquefaciens*   | 6              | 4                       | 67  |
| TYROSINE DECARBOXYLASE ACTIVITY |                |                         |     |
| *B. subtilis*            | 13             | 12                      | 92  |
| *B. amyloliquefaciens*   | 6              | 6                       | 100 |
| ORNITHINE DECARBOXYLASE ACTIVITY |            |                         |     |
| *B. subtilis*            | 13             | 8                       | 62  |
| *B. amyloliquefaciens*   | 6              | 6                       | 100 |
| LYSINE DECARBOXYLASE ACTIVITY |             |                         |     |
| *B. subtilis*            | 13             | 8                       | 62  |
| *B. amyloliquefaciens*   | 6              | 6                       | 100 |

*Range of values of the strains.

**Table 3 | Values of accumulation of putrescine (mg/L) and cadaverine (mg/L) in the culture medium after 72 h of growth of the Staphylococcus strains isolated from traditional sausages.**

| Species                  | Putrescine | Cadaverine |
|--------------------------|------------|------------|
|                          | No. of strains | Range of values* | Average | SD | No. of strains | Range of values* | Average | SD |
| *Staphylococcus equorum* | 14         | 1.46–1415.05  | 112.67  | 374.92 | 15           | 0.25–5.31        | 1.85    | 1.59 |
| *S. epidermidis*         | 10         | 0.37–977.13   | 100.58  | 308.01 | 10           | 0.46–36.52       | 5.83    | 10.91 |
| *S. saprophyticus*       | 7          | 0.43–1.91     | 1.13    | 0.49  | 7            | 0.58–4.79        | 2.11    | 1.88 |
| *S. pasteuri*            | 4          | 1.19–12.39    | 4.47    | 5.29  | 4            | 0.40–4.33        | 2.35    | 1.76 |

*Range of values of the strains.

**Table 4 | Values of accumulation of putrescine (mg/L) and cadaverine (mg/L) in the culture medium after 72 h of growth of the Bacillus strains isolated from traditional sausages.**

| Species                  | Putrescine | Cadaverine |
|--------------------------|------------|------------|
|                          | No. of strains | Range of values* | Average | SD | No. of strains | Range of values* | Average | SD |
| *Bacillus subtilis*      | 8          | 0.39–18.43  | 3.14    | 6.19 | 8            | 0.43–4.29        | 1.30    | 1.50 |
| *B. amyloliquefaciens*   | 6          | 0.76–3.27   | 1.89    | 0.94 | 6            | 0.53–3.07        | 1.40    | 1.11 |

*Range of values of the strains.
greater than 50%. Martín et al. (2006) studying 239 *Staphylococcus* strains isolated from fermented sausages reported that only the 14.6% (35 strains) were able to decarboxylate one or more amino acids. In this same way, Even et al. (2010) working with 129 strains of coagulase-negative staphylococci isolated from various environments including cheeses and fermented sausages, observed that only 5 strains (≈6%) were able to produce detectable amounts of biogenic amines.

Bonomo et al. (2009) did not find any tyrosine or ornithine decarboxylase activity in any of the 37 *Staphylococcus* strains tested, and only observed histidine decarboxylase activity in two strains of *S. warneri*. As observed in the present study, lysine was the amino acid most frequent decarboxylated, and 62% of strains, belonging mainly to the *S. equorum* and *S. xylosus* species, were able to decarboxylate this amino acid. In the latter study the highest proportion of lysine-decarboxylating strains were in the *S. pasteuri* and *S. succinus* species.

Masson et al. (1996) did not observe histidine decarboxylase activity in any of the tested strains of *S. carnosus*, *S. xylosus*, *S. warneri*, and *S. saprophyticus* isolated from sausages. They observed tyrosine decarboxylase activity in all these strains, but the amounts of tyramine produced never achieved 40 μg/mL. Bover-Cid et al. (2001) did not observe any decarboxylase activity in any of the staphylococci strains tested.

Information regarding the amino acid decarboxylase activity of *Bacillus* strains isolated from meat products is very scarce. Roig-Sagüés et al. (1996) analyzed four strains of *Bacillus* spp. isolated from *salchichón* (a Spanish traditional sausage) and found that some of the strains displayed histidine decarboxylase activity and were able to produce histamine, although in very low quantities (about 0.5 μg/mL).

There is some information about *Bacillus* strains isolated from salted and ripened Spanish anchovies. Hernández-Herrero et al. (1999) reported that *B. pumilus* was able to produce histamine, but in low quantities (12–17 μg/mL) and at low environmental NaCl concentrations (0.5–3% NaCl); this ability disappeared at higher concentrations of NaCl (10 and 20%).

Rodríguez-Jerez et al. (1994) analyzed 16 strains of *Bacillus* spp. isolated from Spanish salted semi-preserved anchovies and observed that none of the strains displayed ornithine or lysine decarboxylase activity; the percentage of strains that displayed histidine decarboxylase activity ranged from 75 to 81.25% depending on the culture medium (Niven or modified Niven) used in the test. The quantities of histamine produced ranged from 0 to 10.54 μg/mL.

The aminogenic potential of the *Staphylococcus* and *Bacillus* strains analyzed in the present study was generally low (quantities produced lower than 25 mg/L for putrescine and lower than 5 mg/L for cadaverine), with the exception of one strain of *S. epidermidis* and one of *S. equorum*, which produced higher quantities. These results therefore confirm that the amino acid decarboxylase activities are not particularly high in species of the genera *Staphylococcus* and *Bacillus*, especially when compared with other microbial groups such as Enterobacteriaceae or lactic acid bacteria, present in the fermented meat products (Bover-Cid et al., 2001; Lorenzo et al., 2010).

In the literature, there is little available information on the production of putrescine or cadaverine by *Staphylococcus* species. Martin et al. (2006) observed the production of variable quantities of putrescine (from 25 to >1000 mg/L) and cadaverine (from 25 to 1000 mg/L), being the strains of *S. epidermidis* the main producers. Even et al. (2010) also reported the production of variable quantities of putrescine (from 7 to 1499 mg/L) and cadaverine (from 3 to 140 mg/L).

In the literature, there is no information about putrescine and cadaverine production by species of the genus *Bacillus*, either from meat products or other different origin.

The high variability in biogenic amine production within strains belonging to the same species confirms previous findings (Bover-Cid and Holzapfel, 1999; Martín et al., 2006; Even et al., 2010) and again shows that the amino acid decarboxylase activity is a strain-dependent property. Although the aminogenic capacity of these two bacterial genera is not usually very high, since they can reach high counts in the sausages it is important to reduce their counts by the implementation of rigorous hygienic measures, in order to reduce the risk of accumulation of biogenic amines in the final products.

**CONCLUSION**

(a) Histidine, lysine, ornithine, and tyrosine decarboxylase activities were present in most of the strains of *Staphylococcus* and *Bacillus* isolated from Spanish traditional sausages. However, some variability was observed between strains, even within the same species.

(b) The production of putrescine and cadaverine by the species of *Staphylococcus* and *Bacillus* isolated from Spanish traditional sausages was in general low, with amounts accumulated lower than 25 mg/L for putrescine and lower than 5 mg/L for cadaverine. Only a strain of *S. equorum* produced 1415 mg/L of putrescine and a strain of *S. epidermidis* accumulated 977 mg/L of putrescine and 36 mg/L of cadaverine.

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