Identification of 129 Micrococcaceae strains isolated from food of animal origin

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129 strains of Micrococcaceae were isolated from food of animal origin (minced meat, cakes with confectioner's custard) on Baird-Parker medium.

120 were Staphylococcus and 9 Micrococcaceae, according to the ID 32 STAPH System (1989). The results were analyzed using a computer program.

60 % of these 120 Staphylococcus strains of animal origin were coagulase positive St. aureus (aurease, Rapidet Staph technique). 46 % were resistant to novobiocin (5 µg/ml), 13 to 44 % were identified with negative discordant tests using this method. 44 % produced no aceton, 35 % had no urease and 15 % no arginine dihydrolase.

48 Staphylococcus strains (40 %) were identified as human coagulase negative Staphylococcus species (strains: 39; species: 9) or as animal species (strains: 4; species: 1). 5 were Staphylococcus sp. S. epidermidis and S. warneri represented 63 % of these Micrococcaceae with particular biochemical characteristics.

Micrococcaceae, particularly staphylococci, with strains of coagulase positive S. aureus have been detected in food products for 20 to 30 years. Many papers have dealt with their enumeration and identification (3, 8, 19, 27, 29).

Other species of staphylococci of human origin which are coagulase negative (15, 22, 32) are not researched in food. They are potentially pathogenic and this finds expression in various infections in man (6, 7, 30). Some of them may also produce enterotoxins (31).

Staphylococci species of animal origin have been described during the past ten years, i.e. S. au­reus, S. intermedius (16), S. arlet­i­na, S. klo­si­ii and S. equorum (33). Some of them may also be pathogenic to man.

In food control laboratories, coagulase positive S. aureus organisms are usually isolated on Baird-Parker medium (2) but many other media have been suggested from Zehovit medium (35) to modified Baird-Parker media (26) and comparative studies have been made of the media (3).

During obligatory bacteriological controls Micrococcaceae have been isolated on Baird-Parker medium (36) from food of animal origin during the past ten years, i.e. S. auri­e­us, S. inter­me­di­us, S. arleti­na, S. klo­si­ii and S. equor­um (33). Some of them may also be pathogenic to man.

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Table 1: List of 26 biochemical tests in the ID 32 STAPH-system

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive substrate</th>
<th>Negative substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease</td>
<td>LPR</td>
<td>CEL</td>
</tr>
<tr>
<td>Agarase</td>
<td>ADR</td>
<td>VP</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>ODC</td>
<td>NIT</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>EGC</td>
<td>BAL</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>ESY</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>GLU</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>GLU</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>FRU</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>MAL</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>LAC</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>TRA</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>MAIN</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>RA</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Ornith decarboxylase</td>
<td>RFR</td>
<td>S. aureus</td>
</tr>
</tbody>
</table>

- similar colonies, but without halos. Some of them correspond to coagulase positive S. aureus (10) but most of them are identified with other species of staphylococci (14, 12) or other bacteria (Micrococc: (19)), as a function of the origin of their strains.

The colonies above are cultured on tryptic soy agar before being studied.

Differentiation tests for Staphylococcus and Micrococcus

The nitrofurantoin test (18) with 300 µg/ml disks (Diagnostics Pasteur) is preferred to other tests because it gives a good differentiation of staphylococci and micrococc of food origin (11). It was carried out in Mueller-Hinton (D.P.) medium, the antibiogramme technique being used for these 129 strains.

After 24 hours incubation at 37 °C the diameter of the zone of inhibition is measured. If it is more than 15 mm the strain is sensitive (Staphylococcus) but it is less than 15 mm it is resistant (Micrococcus).

Biochemical study

A micromethod was used here. The new system ID 32 STAPH (5) with its 26 biochemical tests (tab.1) intended mainly for the identifica­tion of the genera Staphylococcus and Micrococcus is the one in question.

Each strain was inoculated with a suspension into 2 ml of sterile dis­tilled water, the opaqueness of which is equal to 0.5 MacFarland units. The procedures recommended by the manufacturer are fol­lowed for inoculation and for the reading of the biochemical tests after 24 hours incubation at 37°C.

A comparison of the identification of staphylococci of food origin and other organisms by 3 micrococc has shown that the ID 32 STAPH system gives the best results (13). Micrococcaceae strains from international collections which have already been studied with this system were not reintroduced into the present work (13).

Numerical identification

The results of the 26 biochemical tests for each strain are expressed in + and − signs and then in a numerical profile with 9 numbers in accordance with the manufacturer's coding system.

They are interpreted with a com­puter program which gives information such as: the number and nature of the discordant tests with the percentages of positive results for the relevant species.

Aurease test for S. aureus strains

The Micrococcus identified as S. aureus by the ID 32 STAPH system were examined for free or bound aurease by a standardised micromethod: Rapide Staph (B. MeRieux s.a.). Aurease or the coagulase staphylococci were de­tected by a U.V. light fluorescent reaction after 2 hours incubation at 37°C.

Results

The 129 Micrococcaceae strains of the present study were found. They submitted to a 300 µg/ml nit­rofurantoin test, to be divided as follows:

- 123 sensitive strains are staph­ylococi, but 3 of them are confirmed as micrococc (tab.4)

- 3 S. aureus

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120 strains isolated from these foods and classified as Staphylococcus strains are identified with various species of the data base (tab. 2 and 3) and only 9 strains with micrococci (tab. 4).

The results show the number of strains with a typical biochemical profile (discordant tests: 0), the strains with profile identified by discordant tests (1, 2 to 4) and their biochemical details. They are analyzed and discussed.

Discussion

129 Micrococcaceae strains isolated from food of animal origin

Of the 2 types of colonies harvested on Baird-Parker medium: 123 (95 %) are presumed to be staphylococci, whilst 6 (5 %) have a biochemically defined profile (discordant tests: 0), the strains with profile identified by discordant tests (1, 2 to 4) and their biochemical details. They are analyzed and discussed.

The 48 staphylococci isolated from food of animal origin have been biochemically defined (24). These are identified with STAPH-ID system by 1 to 4 atypical biochemical characteristics.

The 48 staphylococci other than S. aureus (tab 2) can be identified by atypical biochemical profile with 0 or 1 discordant test on the ID 32 STAPH strip.

9 micrococci isolated from food of animal origin (tab. 4)

These are identified with 6 of the 6 species which are differentiated in the ID 32 STAPH system, whilst the genus Micrococcus has 9 which have been biochemically defined (24).

6 micrococci have a biochemical profile with 0 or 1 discordant test on the ID 32 STAPH strip.

3 micrococci are identified as species with 2 to 4 atypical tests. Thus 5 of the 6 M. luteus from foods are phosphate positive whilst only 10 % of the strains have this enzyme (data base).

The M. luteus strains from collections and of various origins (water, air), have given between 90 and 100 % of phosphate positive tests on the API STAPH strip (10).

The M. roseus strain has a white pigment here although M. luteus usually produces a pink or red pigment (24).

Conclusions

Of the 129 strains of Micrococcus:

- 8 species are rarely found in these foods: S. capitis, S. chromogenes, S. haemolyticus, S. hominis, S. simulans, S. xylosus and S. schleiferi. They were originally isolated from the human skin (15, 22, 32) but can also be found in milk products and meat products (8, 9, 27) and in cheese (39).

They are identified in the ID 32 STAPH system by 1 to 4 atypical tests. The 8th species S. chromogenes (17) of animal origin: pigs, cows (bovine mastitis, poultry) is represented by 4 strains which can be identified by atypical positive tests.

- 2 other species: S. epidermidis 1 and 2 (9 strains) and particularly S. warneri (21 strains) make up 63 % of these staphylococci of food origin other than S. aureus.

S. epidermidis, which is rede fined by SCHLISLER and KLOOS (32) and is isolated from the human skin, can be transmitted to domestic animals by man or by his activities (21). 5 out of the 9 strains have no arginin dichromobase.

S. warneri which is sometimes isolated from the human skin (22) and often from bat of other primates (21) is the second species here, numerically, after S. aureus. 4 of these 4 strains are phosphate positive, although this test is always negative in the data base.

S. warneri strains from collections may give a weakly positive reaction (22).
Their animal origins: resistance to 5 µg/mL novobiocin and other typical characteristics of S. aureus and other coagulase-negative species.

11 staphylococci species, particularly S. aureus, S. epidermidis and several others, are also coagulase-negative staphylococci were found in these foods. The specific pathogenic potential of 2 of these species was tested in various clinical studies (7, 15, 28, 30). But in bacteriological food control only the species S. aureus is still determined and enumerated in foods, according to French food law. The other species are not taken into consideration although found in these foods or in others (10, 12, 29).

References


3. BARKER, V., J. BULLEN, J.C. MANN (1989): Characterization of coagulase-negative Staphylococci isolated from minced meat and other foods or in others (1

4. BARKER, V., J. BULLEN, J.C. MANN (1989): Characterization of coagulase-negative Staphylococci isolated from minced meat and other foods or in others (1


