Identification of 129 Micrococcaceae strains isolated from food of animal origin

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Code words: Staphylococcus – nitrofurantoin – aurease – ID 32 STAPH – numerical identification

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 Micrococcus, 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 S. aureus (aurease+, Rapidec Staph technique*). 46 % were resistant to novobiocin (5 µg/ml), 13 to 44 % were identified with negative discordant tests using this micromethod. 44 % produced no acetoin, 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). *S. aureus* isolated from milk, cheese or other foods can produce enterotoxins which cause food poisoning in man (45, 48). French legislation therefore requires their detection and identification in food products.

Other species of staphylococci of human origin which are coagulase negative (15, 22, 25, 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. sciuri* (23), *S. intermedius* (16), *S. arlettae*, *S. klosii* and *S. equorum* (33). Some 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 Zebovitz 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 from food of animal origin. They have been studied in relation to their biochemical and ecological properties and on the basis of previous studies (10, 12).

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Material and methods

Preparation of the food

Food of animal origin (uncooked, frozen minced meat, cakes with confectioner's custard) are routinely analyzed in hygiene laboratories in accordance with the Decree of the 21st December 1979 (Journal Officiel Français of 19-01-80).

30 g of food (after thawing out) are ground in a grinder-homogenizer in buffed peptone water (Bacto-peptone 20 g, NaCl 5 g, monopotassium phosphate 1,5 g, disodium phosphate 9 g, distilled water 1000 ml, pH 7,2). It is obligatory to treat frozen food for 30 to 45 min at 20 °C.

Staphylococci isolation

This was done according to the AFNOR NF-V-08-014 standard for the enumeration of *S. aureus organisms* in food. 0.1 ml of food suspension was spread on Baird-Parker medium in a Petri dish. After 24 and 48 hours incubation 2 types of colonies were harvested:

– circular, convex, black and shiny colonies with a clear halo of eggyolk colour. These are considered as characteristic of coagulase positive *S. aureus* and are routinely enumerated after confirmation by a free coagulase test.

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

Reaction/ substrate	Test	Reaction/ substrate	Test
Urease	URE	Cellobiose (F)	CEL
Aginin dihydrolase	ADH	Acetoin (production)	VP
Ornithin decarboxylase	ODC	Nitrat (reduction)	NIT
Esculin (hydrolysis)	ESC	ß Galactosidase	ß GAL
Glucose (F)	GLU	Arginin arylamidase	ArgA
Fructose (F)	FRU	Alkaline phosphatase	PAL
Maltose (F)	MAL	Pyrrolidonyl Arylamidase	PyrA
Mannose (F)	MNE	Novobiocin (Resistance)	NOVO
Lactose (F)	LAC	Sucrose (F)	SAC
Trehalose (F)	TRE	N-Acetyl Glucosamine (F)	NAG
Mannitol (F)	MAN	Turanose (F)	TUR
Raffinose (F)	RAF	Arabinose (F)	ARA
Ribose (F)	RIB	ß Glucoronidase	ß GUR

(F) = Fermentation

– 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 (micrococci: (19)), as a function of the origin of their strains.

The colonies above are cultured on trypticase soya agar before being studied.

Differentiation tests for *Staphylococcus* and *Micrococcus*

The nitrofurantoin test (18) with 300 µg/ml discs (Diagnostics Pasteur) is preferred to other tests because it gives good differentiation of staphylococci and micrococci 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 if 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 strip 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 of other origins by 3 micromethods 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 *Micrococcaceae* identified as *S. aureus* by the ID 32 STAPH system were examined for free coagulase by a standardized micromethod: Rapidec Staph (bioMérieux s.a.). Aurease or free coagulase staphylococci were detected by a U.V. light fluorescent reaction after 2 hours incubation at 37 °C.

Results

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

– 123 sensitive strains are staphylocci, but 3 of them are confirmed as micrococci (tab. 4)

– 6 resistant strains are micrococci

Biochemical identification of the 129 *Micrococcaceae* strains using the ID 32 STAPH system

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 %) are micrococci according to the nitrofurantoin test. But their identification on ID 32 STAPH shows that 3 of them, classified as *Staphylococcus* strains, have the biochemical profiles of *Micrococcus* (tab. 4). This has already been noted in an earlier study (11). On the other hand the 127 micrococci (collections, pathological products and the environment) studied by HEBERT and CAILLET (18) are resistant to nitrofurantoin. Thus 120 strains are staphylococci (tab. 2 and 3) and 9 are micrococci (tab. 4).

120 staphylococci isolated from food of animal origin

72 of the 120 staphylococci (60 %) are *S. aureus*, whilst 48 of them can be identified as of human origin (15, 22, 32). Although Baird-Parker medium was manufactured in 1962 for the detection of coagulase positive *S. aureus*, it permits the growth of other species which were unknown at the time and derived from colonies without a halo. The same has been observed with staphylococci isolated from raw milk (10) and deep-frozen vegetables (12)

72 *S. aureus* strains (tab. 2 and 3)

70 out of the 72 strains have free coagulase (aurease test, tab. 3). 2 strains have a negative reaction like those previously isolated from dry sausage (10).

These 72 staphylococci are identified as *S. aureus* of the data base by discordant tests such as the 5 µg/ml novobiocin test (NOVO):

– 33 *S. aureus* aurease positive (46 %) are resistant to this antibiotic, as against 1 % in the data base.

Tab. 2: Identification of 120 Staphylococcus strains isolated from food of animal origin

Staphylococcus species	Nb.	Number of strains T.P.	I.P./ T.A. 1	I.P./ T.A. 2 à 4	biochemical particularities
S. aureus	72	4	22	46	cf. Table 3
S. capitis	1			1	
S. chromogenes	4			4	
S. cohnii 1	1			1	
S. epidermidis 1	8		3	5	ADH-/5
S. epidermidis 2	1			1	
S. haemolyticus	1			1	
S. hominis 2	2			2	
S. schleiferi	2	1	1		
S. simulans	1			1	
S. warneri	21	2	9	10	PAL+/4 VP-/7
S. xylosus	1		1		
S. species	5			5	T.A. > 4
Total strains	120	7	36	77	

Nb. = Number
T.P. = typical biochemical profile
I.P. = identified profile
T.A. 1, T.A. 2 to 4 = tests against 1, 2 to 4 and > 4

Tab. 3: Identification of 72 S. aureus strains isolated from food of animal origin

S. aureus	Nb.	Number of strains T.P.	I.P./ T.A. 1	I.P./ T.A. 2 à 4	biochemical particularities
Aurease + Novobiocin resistance	33		5	28	NOVO R/33 IRE-/10 ADH-/5 LAC-/4 VP-/12 TUR-/8
Aurease + Novobiocin sensitive	37	4	17	16	URE-/15 ADH-/4 LAC-/6 VP-/18 TUR-/6
Aurease – Novobiocin sensitive	2			2	ADH-/2 LAC-/2 VP-/1
Total strains	72	4	22	46	NOVO R/33 URE-/25 ADH-/11 LAC-/11 VP-/31 TUR-/14

% of positive results of S. aureus (data base): NOVO 1 %, URE 84 %, ADH 83 %, LAC 95 %, VP 97 %, TUR 84 %
NOVO R: Novobiocin resistant

The 33 *S. aureus* strains of food origin and resistant to novobiocin behave like *S. saprophyticus* (1) or *S. cohnii* and *S. xylosus* (32), which are coagulase negative strains.

31 of the 72 *S. aureus* strains (44 %) do not produce acetoin (VP-test). 25 (35 %) have no urease (URE-test) and 11 (15 %) have no arginin dihydrolase (ADH-test), whilst 14 strains (19 %) do not ferment turanose (TUR-test) and 11 do not ferment lactose (15 %). The percentages of positive results for these tests lie between 83 % and 97 % (APILAB, Tab. 3). Here, being negative, they characterize 15 to 44 % of these strains.

The 48 staphylococci other than *S. aureus* (tab 2)

43 strains can be identified with 10 species of coagulase negative staphylococci. 5 strains have not been classified in the taxonomy of the data base and are designated *Staphylococcus* sp.

– 8 species are rarely found in these foods: *S. capitis, S. cohnii, S. haemolyticus, S. hominis* 2, *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 (29).

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 with 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 was redefined by SCHLEIFER 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 dihydrolase.

S. warneri which is sometimes isolated from the human skin (22) and often from hat of other primates (21) is the second species here, numerically, after *S. aureus*. 4 of these strains are phosphatase positive, although this test is always negative in the data base. *S. warneri* strains from collections may give a weakly positive reaction (22).

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

These are identified with 4 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 phosphatase positive whilst only 10 % of the strains have this enzyme (data base). *M. luteus* strains from collections and of various origins (water, air), have given between 90 and 100 % of phosphatase 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)

Tab. 4: Identification of 9 Micrococcus isolated from food of animal origin

Micrococcus species	Nb.	Number of strains T.P.	I.P./ T.A. 1	P.I./ T.A. 2 à 4	biochemical particularities
M. kristinae	1			1	
M. luteus	6	1	3	2	Nitro S/2 PAL+/5
M. nishinomiyaensis	1		1		
M. roseus	1	1			Nitro S white colour
Total strains	9	2	4	3	

Nitro S = sensitive nitrofurantoin

Conclusions

Of the 129 strains of *Micrococ-*

caceae isolated from minced meat and cakes on Baird-Parker medium, 120 are staphylocci and only 9 micrococci on the basis of the selective properties of this medium.

In the ID 32 STAPH system which was developed for *Micrococcaceae* of clinical and animal origin, they can be identified as various species of *Staphylococcus* (except for 5 *Staphylococcus* sp.) and of *Micrococcus* (5). But in the present study some strains of these two genera have biochemical properties related to their animal origins: resistance to 5 µg/ml novobiocin and/or atypical characteristics of *S. aureus* and other coagulase negative species.

11 staphylococci species, particularly *S. aureus, S. epidermidis* and *S. warneri,* and also 4 species of micrococci were found in these foods. The specific pathogenic power of *S. aureus* or the potential pathogenic power of coagulase negative staphylococci or micrococci has been observed 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).

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Opportunities and problems with the export of beef and pork to Italy

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Code words: beef – pork – exportation – branded meat

For the German beef and pork producer the Italian market is an almost classical export objective. Relatively large quantities of these two kinds of meat are consumed in Italy but the degree to which the country is self-sufficient in this respect is well below 70 %. Two thirds of the beef exported is forequarters and the rest hindquarters. Beef sides and other forms of beef do not feature at all. Three quarters of the pork exported is in the form of hams, the rest being mainly pork sides. In recent years Italian consumers have begun to follow new trends which will influence the structure of German exports. To determine these market trends an enquiry was undertaken amongst experts in 8 Italian and 3 German firms in this sector with the support of the Central Marketing Association of the German agricultural industry (CMA). Here interest was centred on the requests made by Italian wholesalers and retailers to German export firms.

The results of this enquiry show that, in the case of beef, the demand is for younger carcases with the best conformation, a good fat deposit and a comparatively bright colour. The end product expected by the consumer is light, tender meat with no visible fat deposit and not too strong an aroma. In the case of pork exports to Italy are chiefly concentrated on hams for processing and the product criteria for the sale of fresh meat therefore only play a subordinate role here. Generally speaking the Italian market is interested in having the product really fresh which means that as far as beef is concerned there is only a limited opportunity for longer ripening.

These results, and the fact that German meat is produced very close to the Italian market, offer certain opportunities for German quality meat. The promotion of these types of products on the Italian market would seem to be particularly important. In view of the problems discussed the reputation of Germany as regards meat quality and the ability to maintain it is poor as compared with competing EU member states. It could only be upgraded by actual performance.

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