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ЕВРОПЕЙСКИЙ КОНГРЕСС РАБОТНИКОВ НИИ МЯСНОЙ ПРОМЫШЛЕННОСТИ

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DATA CONCERNING THE DIFFERENTIATION OF THE MICROFLORA OF CANNED HAM

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Many literature data are available on the microbiological characteristics, the questions of examination and qualification of the preserv meatproducts, among others that of canned ham. In spite of this fact there are many unsolved details. We delt also in various respects with the quality questions of canned ham and in this study we present a problem which we consider important from theoretical as well practical viewpoint considering the microbiological qualification of semi conserves.

We desired to clear the systematical proper place of the members of the residual flora of our canned ham product. It was also our aim to see. wether there exists any relation between the place of the sample-taking and the total number of living microbes in one and the same product.

We examined microbiologically without previous incubation I5 customary /3 - 5 kg/ oval shaped canned hams. The

samples were prepared on the whole with the generally known usual technology. We mention as the most important fact from the viewpoint of our investigations, that after the vacuumising, the sealed cans were cooked in waterbath on 74°C for 55 minutes per one kg product, then after the usual cooling they were stored on +4°C till they were used for the examinations. From each can we took under steril conditions always from the same 9 places /see figure I./ altogether 135 samples and determined the total number of the aerob and anaerob microbes, per g of meat.

Figure I. The predetermined points for taking the samples.

To save place we don't give the detailed results of the counts gained with diluting resp. plate method, We only mention that the numbers of microbes - as it could have been foretold - didn't show any regularity neither at samples taken from the same place and slice of various hams nor from various places /slices/ of the same hams. One cause of the great dispersion beside others is, that in spite of the same technology the product cannot be considered from microbiological viewpoint as homogen lot. The causes of this phenomenon are well-known.

The highest aerob microbe number was $9,3.10^{1}$/g and beside some steril sample the lowest value was 10^{1}/g. The largest anaerob microbe number was 10^{3}, the least 10^{1}/g. These latter values we got from the majority of the samples, i.e. the number of the anaerob microbes was always considerably less than this of the aerob residual population.

From the mixed cultures we determined after Bergey and

Skerman /I,2/ the following bacteria species:

Aerococcus viridans /syn. Pediococcus homari/

Streptococcus faecalis

Leuconostoc mesenteroides

Pseudomonas /fluorescens?/

Micrococcus conglomeratus

Bacillus megaterium /cereus?/

Bacillus subtilis

Xanthomonas sp.

Bacillus sp.

Beside the enumerated aerob /facultativ anaerob/ species /genera/ we also izolated some obligate anaerob strains, the systematical determination of which is on course. The biochemical and other characteristics of the members of the aerob group we do not give here, we only mention that they are the members of the usual residual bacterial flora known from the literature data of the other investigations too.

We want to draw the attention to the question of Aerococcus and Enterococcus /Streptococcus/ group, which we consider as important from the viewpoint of microbiological diagnostics and also from viewpoint of practical estimation of the canned ham-products. We give shortly our opinion as follows.

In the studies of the microbiological problems of preserv meat-products there are very scarce if any data about the Aerococcus, described first by Williams /3/, and this subgenus and the pertaining species are obviously referred as members of the Enterococcus group. Further we are convin-

ced that the Aerococcus cultures /colonies/ which can be precisely differentiated with adequate methods, are counted by most of the laboratories, to make things easier, to the genus Micrococcus. This failure of systematical classification is easy to commit, because the differentiation needs more attentive and careful work, partly this problem is considered by most of the laboratories negligable.

But we consider this question essential, because the Aerococcus should be qualified from point of view of hygiene quite differently as the microbes belonging to the Enterococcus /Streptococcus/ group. Namely the latter together with the E-coli serve as indicator organism, thus we must quite differently estimate the presence of Aerococcus /Pediococcus/ in preserv product as that of Enterococcus, or more strictly of Streptococcus faecalis. More attention must be paid to this question, because lately there were made many suggestions to consider instead of E-coli the Str. faecalis as indicator microorganism. We also favour this view.

According to our investigations, although the Aerococcus viridans is in many respects similar to the members of the Enterococcus group, their routine differentiation is quite possible /see table I. and 2./

Table I and 2.

The most striking morphological difference is, that the Aerococcus never forms chain, the cells are never elongated /oval/, they are always spherical. On the other hand the chainformation and the elongated cells are characteristics to the Streptococcus faecalis /figure 2, 3/. Here we must

draw the attention to the fact, that the stained-fixed preparations are not reliable, because of the making those the cells become deformed in various degree. But the reliable detection of the morphological characteristics by the phase contrast method gives a good guide for the systematical orientation as well.

From biochemical point of view the Aerococcus in contrast to the Streptococcus faecalis does not hydrolise the arginine, does not reduce the methylenblue-milk, it acidifies the litmusmilk /but does not coagulate it/, the ultimate pH value is greater in 1% glucose-broth and at least it does not react with the Lancefield D serum, which precipitates characteristically the Str. faecalis. Beside these differentiating characteristeics there are also many similar ones causing frequently false Enterococcus /Streptococcus/ diagnosis on course of the routine work at the microbiological qualification of the preserv products.

To avoid failures we propose for the differentiation the Micrococcus, Enterococcus and Aerococcus genera on course of microbiological routine work, beside the phase contrast microscopical examinations the catalase-test, because this separates the members of the Micrococcus genus from the Enterococcus and Aerococcus groups /5/. The separation of the Streptococcus from the also catalase-negative Aerococcus can be achieved best partly on basis of morphological characteristics /no chainformation, round cell-form/, partly examining the arginine hydrolisis and ultimate pH value.

The pickled meat gets a greenish colour from the Aero-

coccus viridans, this also renders possible to mistake it for the Streptococcus, producing also peroxide and not having characteristic features from the chainbuilding point of view.

The presence of Aerococcus viridans in preserv products is a very interesting feature for itself, but it must be estimated from the viewpoint of hygiene quite differently, than as an Enterococcus /Streptococcus/ contamination.

S u m m a r y

After vacuum-treating the sealed canned hams /I5 off 3-5 kg/ were cooked in waterbath on 72°C for 55 minutes per one kg product. The composition of microflora was determined in samples taken from the same 9 places from every can. We didn't found any correlation between the number of microbes determined on the same spots of various hams. Beside the great number of steril samples, the highest number of aerob microorganisme was $9,3.IO^{I}$/g and that of anaerob microbes - IO^{2}/g.

The following pure genera were separated from mixed cultures and identified: Aerococcus viridans /syn. Pediococcus homari/, Streptococcus faecalis, Leuconostoc mesenteroides, Pseudomonas /fluorescens?/, Micrococcus conglomeratus, Bacillus megaterium /cereus?/, Bacillus subtilis, Xanthomonas sp., Bacillus sp.

The present study was aimed with the exact differentiating of Aerococcus and Enterococcus groups, more exactly of the genus Streptococcus faecalis. This was considered essential, because more and more authors suggest to take the Str. faecalis as indicator organism for hygienic estimation of preserv meatproducts. As many morphological and biochemical

features of Str. faecalis /members of the Enterococcus group/ correspond with those of the ubiquiter Aerococcus and in consequence are frequently mixed up, the tested sample or lot is easily mistaken for fecal contaminated.

In the tables are given the similar and differing characteristics of both bacteria. As a basis for their differentiating are suggested their capacity of chainbuilding, the testing by arginine hydrolisis, the determination of ultimate pH value and perhaps the precipitating test with Lancefield D serum.

Literature

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Ist table

Identities between the biochemical characteristics of Aerococcus viridans and Streptococcus faecalis.

Tests	Aerococcus	Streptococcus
Nitrate reduction	-	-
Lecitinase test	-	-
Gelatin stab	-	-
Lysis of meat protein	-	-
Indol formation	-	-
H_2O_2 formation	+	+
Greening of meat pigments	+	+
Growth in both prepared with $NH_4H_2PO_4$ as solely N source	-	-
Growth in 6,5% NaCL broth	+	+
Gram staining	+	+
Surface of bouillon	no change	no change
Fluorescence	-	-
Urease test	-	-
Growth on +6 C^o	+	+
" +45 C^o	+x	+
" in 40% bile	+	+
" on pH 9,6	+	+
Survival of 60 C^o 30 minutes	+	+
Growth on $kTeO_4$ I:2500 agar	+	+
Growth in anaerobic conditions	+	+
Colony formation on agar	tiny, translucid, greenish	tiny, translucid, greenish
Motility	-	-
Sporeformation	-	-
Presence of catalase	-	-
Starch hydrolysis	-	-
Growth in broth containing sodium citrate as solely C source	-	-

x Our results are differing from the literature data.

The split of dextrin, dulcitol, esculin, fructose, glucose, glycerol, lactose, maltose, mannose, mannitol, raffinose and sucrose gave the same results with both strains.

2nd table

Differences between the biochemical characteristics of Aerococcus viridans and Streptococcus faecalis

Tests	Aerococcus	Streptococcus
Litmus milk	ac.	ac.+red.
Final pH	4,9-5,I	3,9-4,I
Reduction of O,I % met.blue milk	-	+
Hydrolysis of arginine	-	+
Chain formation	-	+
Prec. with Lancefield D serum	-	+
Configuration	in pairs,tetrades	in pairs,chains
Shape of the cells	spherical	oval
Formazane formation on TTC agar	-	+x
Split of arabinose	+	-
Split of sorbitol	-	+

x It regards only to the Str.faecalis; Str.faecium forms colonies very similar to those of Aerococcus.

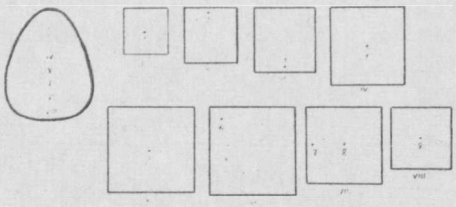

Fig.I The predetermined points for taking the samples.

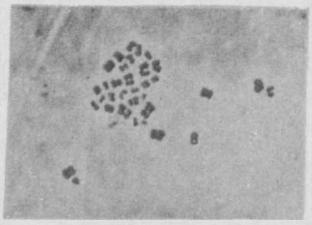

Fig.2.
Aerococcus viridans : no chainformation, sphaerical diplococci, tetrade forms, Phasis contrast microphoto, 2000x

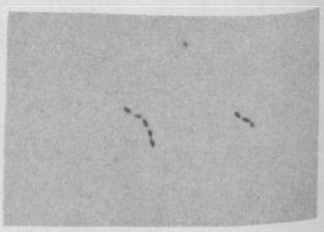

Fig.3.
Streptococcus faecalis: chain formation, elongated, oval cell form. Phasis contrast microphotot, 2000x

ANGABEN ZUR DIFFERENZIERUNG DER MIKROFLORA VON DOSENSCHINKEN

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Es wurde die Zusammensetzung der Mikroflora von 15 Dosenschinkenerzeugnissen mit einem Gewicht von 3 - 5 kg - die nach Evakuierung verschlossen und im Wasserbad bei 72° C 55 min pro kg gekocht wurden - untersucht. Aus jeder Dose wurden 9 Proben von denselben Stellen der Schinken entnommen. Zwischen den Keimzahlen der von gleichen Stellen der verschiedenen Schinken entnommenen Proben konnte kein Zusammenhang festgestellt werden. Neben zahlreichen sterilen Proben betrug die grösste aerobe Keimzahl $9{,}3.10^{1}$/g und die grösste anaerobe Keimzahl 10^{2}/g.

Aus den gemischten Kulturen könnte man folgende reine Stämme identifizieren: Aerococcus viridans /syn. Pediococcus homari/, Streptococcus faecalis, Leuconostoc mesenteroides, Pseudomonas /fluorescens?/, Micrococcus conglomeratus, Bacillus megaterium /cereus ?/, Bacillus subtilis, Xanthomonas sp., Bacillus sp.

Die eigentliche Zielsetzung der Untersuchung war die exakte Isolierung der Gruppen Aerococcus und Enterococcus

bzw. die des Streptococcus faecalis. Dies halten wir deshalb für wichtig, da der Streptococcus faecalis von immer mehreren Autoren zur hygienischen Beurteilung der preservartigen Fleischerzeugnisse als Indikatormikroorganismus empfohlen wird. Da aber der Streptococcus faecalis /die Glieder der Enterococcus Gruppe/ bezüglich so der morphologischen, als auch vieler biochemischen Eigenschaften mit dem ubiquitären Aerococcus übereinstimmt und da sie miteinander oft verwechselt werden, kann sehr leicht eine fekale Kontamination für die untersuchte Probe bzw. für die Herstellungspartie festgestellt werden, obwohl sie nicht vorliegt.

Die gleichen und abweichenden Eigenschaften dieser zwei Bakterien wurden in einer Tabelle zusammengestellt. Zur Isolierung werden die Kettenbildung, die Argininhydrolyse, die Bestimmung des End-pH-Wertes, eventuell die Präzipitationsprobe mit dem Lancefield D-Serum empfohlen.

DONNÉES SUR LA DIFFÉRENCIATION DE LA MICROFLORE DU JAMBON EN BOITE

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Nous avons étudié la composition de la flore microbienne de 15 produits de jambon en boîte, fermés après un traitement par le vide et cuits dans le bainmarie durant 55 minutes à la température de 72° C. Les échantillons ont été prélevées dans chaque boîte en 9 points différemment situés. Nous n'avons pu constater aucune corrélation entre les nombres de germe des échantillons prélevées dans des lieus identifiques des jambons différents. Le nombre maximum des germes aérobies est de $9,3.10^7$/g, celui des germes anaérobies de 10^2/g, en dehors de nombreuses échantillons stériles.

Nous avons pu identifier des cultures mixtes les souches pures suivantes: Aerococcus viridans /syn. Pediococcus homari/, Streptococcus faecalis, Leuconostoc mesenteroides, Pseudomonas /fluorescens?/ Micrococcus conglomeratus, Bacillus megaterium /cereus?/, Bacillus subtilis, Xanthomonas sp. Bacillus sp.

Le but proprement dit de notre étude était de séparer

exactement les groupes Aerococcus et Enterococcus, plus précisément, le Strèptococcus faecalis. Nous croyons cette séparation importante parce que le Str. faecalis est recommandé, par des auteurs de plus en plus nombreux, comme microorganisme indicateur, pour apprécier, au point de vue hygiénique, les produits de charcuterie ayant un caractère de préserve. Puisque le Str.faecalis est conforme, en configuration et en nombreuses propriétés biochimiques, à l'Aerococcus ubiquiter et puisqu'on prend même souvant l'un pour l'autre, il se peut que la contamination fécale soit erronnément constatée sur une échantillon examinées ou sur on lot de production.

En exposant les carractères semblables et différents de ces bactéries, nous avons proposé, afin de les séparer, la formation des chaines, l'hydrolise d'arginine, le dosage du pH terminal et, éventuellement, l'essai de précipitation par le sérum Lancefield D.


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