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Enumeration of lactic acid bacteria in meat products.

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Introduction

The ISO-expert group ISO/TC 34/SC 6/WG 15 on : Enumeration of lactobacteriaceae in meat and meat products is at present discussing which method should be proposed as an ISO-standard reference method.
In progress of the work evaluations of enumeration methods have been carried out in various meat institutes (Dr. A.F. Egan, Meat Research Laboratory, Brisbane, Dr. B.G. Shaw, Meat Research Institute, Bristol and Prof G. Reuter, Free University, Berlin). The work of prof. Reuter has been published in the International Journal of Food Microbiology vol. 2 (1 + 2) p. 55-68, in which the shortcommings of existing media used as general purpose selective media are underlined. Also in the ISO-expert group the view has been raised that a single medium would not be sufficient to cover all the different species of lactic acid bacteria. Therefore it has been decided to carry out further experiments with different media and to use a wider range of products as test material.
The objective of this work has been to evaluate the following media proposed by the ISO working group : de Man-Rogosa-Sharpe-agar, pH 6.2 (MRS 6.2), de Man-Rogosa-Sharpe-agar, pH 5.7 (MRS 5.7), Rogosa-acetate-agar, pH 6.2 (RA), Lactobacillus-sorbic-agar, pH 5.0 (LaS) and Nitrite-Actidione-Polymyxin-agar, pH 5.5 (NAP). As reference medium was used Trypotone-soya-yeast-glucose-agar, pH 6.7 (TSYG).

Materials and Methods

The materials used for this experiment were 95 samples of commercially available meat products. Most of the meat products were cured, sliced and either vacuum-packaged or packaged in controlled atmosphere (100% nitrogen or 60-80% nitrogen mixed with 20-40% carbon dioxide). The remaining meat products were vienna-type-sausages.
Further 15 samples of spices were tested, since these are important ingredients in these types of meat products. Spices are known to carry a very mixed bacterial flora, and therefore the testing of spices was anticipated to give useful information on the selectivity of the media. However, only 3 of the spices were tested on all 6 media.
From all samples traditional decimal-dilutions were made (dilution medium : 0.85% NaCl with 0.1% Bacto peptone) and from appropriate dilutions 0.1 cc was plated on the surface of all media with successive pouring of a cover-layer to create microaerophilic conditions. Incubation was carried out at 25°C for 4-5 days in an incubator.
For three of the samples a comparison was made between microaerophilic growth and anaerobic growth using the Oxoid jar-system in which a generation kit develops an atmosphere of 5-10% CO_2.

Results

In table 1 some results from various types of meat products are shown. Usually the figures are indicative of the age of the products and the very high figures - log 8 to 10 represent figures at the end or beyond the shelf-life

frequently associated with distinct organoleptic changes in smell. From the table it is seen that the highest counts of lactic acid bacteria were obtained on TSYG. These counts were significantly higher than counts on MRS 6.2, MRS 5.7 and RA, which again gave counts significantly higher than LaS and NAP. This is illustrated in table 2.
In table 3 is shown the log counts for lactic acid bacteria in different spices. It is seen that the best recovery has been obtained on the MRS-media, but due to the few results LSD.95 have not been calculated.
In table 4 is shown a comparison of log counts for lactic acid bacteria in sliced meat products between aerobic and anaerobic incubation. It is seen that no significant difference was found. It should be noted as mentioned earlier, that in this experiment aerobic incubation means microaerophilic growth conditions due to the cover-layer, and that in the anaerobic incubation system 5-10% CO_2 is provided.

Discussion

TSYG is an excellent medium for recovery of lactic acid bacteria from meat products, but its use cannot be recommended for routine work due to concurrent growth of non-lactics. Thus in this experiment growth of moulds, Bacillus and Brochothrix thermosphacta often made counting on TSYG extremely difficult.
B. thermosphacta, which together with lactic acid bacteria is the most important spoilage organism in this type of Danish meat products, did not grow on any of the 5 media tested due to various inhibitors (acetate, sorbic acid, polymyxin and low pH).
The media of choice for these types of Danish meat products are MRS 6.2, MRS 5.7 and RA. Although not significant there was some indication that MRS gives a higher recovery than RA. On the other hand the interesting observation was made, that with RA leuconostoc could be identified separately due to a slime-formation around the colony. Thus dealing with a spoilage problem like "blowers" in flexible package RA could be the medium of choice.
As far as the examination of spices is concerned many plates with TSYG, MRS 6.2. MRS 5.7 and RA could not be read after 4-5 days of incubation due to mould-growth. In these cases LaS and NAP gave the best performance due to their ability to inhibit mould growth. Thus when moulds are expected LaS and NAP could be the media choice. On such occasions it should also be considered to use anaerobic incubation in MRS, since moulds are strictly aerophilic organisms.
These experiments with LaS and NAP support some earlier observations on raw minced meat, that when high selectivity is needed to enumerate lactic acid bacteria, NAP is more satisfactory medium than MRS. Thus in raw minced meat gram-negative bacteria on MRS, but not NAP due to inhibition from nitrite, polymyxin and low pH.
Regarding colony-morphology it should be mentioned that NAP in this experiment had a disadvantage in producing smaller colonies (pin-point) than the other media tested. This made the reading of plates more difficult. This disadvantage was not noted in earlier examinations of raw minced meat. The reason for this difference might be that minced meat provides a more favourable environment than the cured meat products.

Conclusions

TSYG is an excellent medium for enumeration of lactic acid bacteria from meat products carrying almost pure cultures.
For routine examination of cured heat-treated, sliced, vacuum-packaged and controlled atmosphere-packaged meat

products MRS is recommended. Microaerophilic growth conditions give satisfactory results.
RA can be used for enumeration of leuconostocs.
MRS in combination with anaerobic incubation is recommended for examination of spices, but using NAP in a microaerophilic system gives satisfactory results.

Table 1

Meat products	Media					
	$MRS_{6,2}$	$MRS_{5,7}$	NAP	RA	LaS	TSYG
Cooked pork loin	5,6	5,8	5,4	5,6	5,5	5,6
Sliced bacon	8,2	8,0	7,5	8,0	7,7	8,2
Cooked ham	5,7	5,5	5,1	5,4	5,3	6,0
Cooked salted beef	6,5	6,5	6,3	6,3	6,1	7,0
Cooked ham	8,3	8,4	7,4	8,4	7,9	8,4
Cooked rolled pork	8,3	8,3	8,3	8,3	8,3	8,4
Smoked pork loin	9,0	9,0	8,9	9,0	8,1	9,3
Smoked pork loin	4,6	4,5	4,5	4,7	4,5	5,0
Cooked meat sausage	7,2	7,2	7,0	7,1	7,1	7,2
Vienna sausage	3,8	3,7	3,4	3,5	3,3	3,8

Log counts of lactic acid bacteria in different meat products.

Table 2

Media	TSYG	$MRS_{6,2}$	$MRS_{5,7}$	RA	LaS	NAP
Average log count	7,14	6,99	6,97	6,93	6,74	6,68
$LSD._{95}$ = 0,115						

Average log count and least significant difference ($LSD._{95}$) for lactic acid bacteria in different meat products.

Table 3

Spices	Media					
	$MRS_{6,2}$	$MRS_{5,7}$	NAP	RA	LaS	TSYG
Black pepper	5,4	5,1	4,2	4,5	3,5	5,2
Dried parsley	4,5	4,4	4,3	3,9	4,0	4,9
Dried onions	5,8	5,7	5,5	5,5	5,4	5,6
Average log count	5,23	5,07	4,67	4,63	4,30	5,23

Log counts for lactic acid bacteria in different spices.

Table 4

Sliced meat products	Media							
	$MRS_{6,2}$		RA		LaS		NAP	
	A	AN	A	AN	A	AN	A	AN
Smoked pork loin	8,0	8,5	8,1	8,4	7,9	7,9	7,9	8,3
Cooked ham	10,1	10,0	9,6	10,0	9,2	9,5	8,5	10,2
Smoked pork loin	7,6	7,7	7,8	7,7	7,7	7,7	7,7	7,7

Comparison of log counts for lactic acid bacteria in sliced meat products between aerobic (A) and anaerobic (AN) incubation.