MICROBIOLOGICAL METHODS FOR THE DETECTION OF MICROORGANISMS FORMING BIOGENIC AMINES

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BACKGROUND:
Biogenic amines mainly are formed from amino acids by microbial decarboxylation. In fermented food, they appear as "undesired by-products of a desired microbial activity". Besides the toxicological and pharmacological aspects, biogenic amines have gained interest as possible indicators for food freshness and hygiene. Especially types of food, in which the contaminant bacteria are inactivated or outnumbered during the production process, are of interest. Such products, for instance, are
- heat treated meat products, where bacteria are inactivated and killed by heating, as frankfurter-type sausage and cooked ham
- fermented, long- ripened meat products, where undesired bacteria are outnumbered by lactobacilli in the first weeks of ripening. The detection of large amounts of biogenic amines in these types of food shows the previous activity of undesired microorganisms even when these can not be detected anymore.

OBJECTIVES:
Objective of this work was to develop a set of simple and easily applicable microbiological methods for the detection of amine forming microorganisms in meat. The media are a combination of (1) nutrients with selective/ elective additives, (2) a substrate (i.e. amino acid) and (3) an indicator system using either formation of gas (CO_2 from decarboxylation of amino acids, collectable in Durham- tubes) or changes of pH (visualised by change of colours of pH- indicators) or disappearance of precipitates (tyrosine precipitates disappear due to metabolisation of tyrosine; bile salts precipitates dissolve due to rising of the pH value...) to indicate a positive reaction.

METHODS:
Composition and preparation of nutrient media for the detection of microorganisms forming cadaverine (cad.), putrescine (put.) and tyramine (tyr.) are listed in table 1 and drawings of positive results are given in figure 1. Incubation time and temperature were: 42°C, 2 days for enterococci; 37°C, 1 day for enterobacteriaceae; 37°C, 2 days for lactobacilli; 25°C, 3 days for pseudomonads and 30°C, 3 days for total aerobic count. Detection of histamine (his.) - forming microorganisms was made according to KRANNER and SCHOPF (1992). Reliability and specifity of these methods were tested in several ways: (a) specified amine forming strains obtained from DSM („Deutsche Stammsammlung von Mikroorganismen") were inoculated and the results were compared with the DSM- specifications given in table 2 and (b) field strains of meat samples were inoculated, and -in case of enterobacteriaceae and pseudomonas spp.- positive colonies were identified by Enterotubell® or OxyFerm® kits. In all cases, the pH values of positive and negative reaction zones were measured. The reaction zones were punched out, and biogenic amines were extracted with 5% (w/v) trichloroacetic acid under gentle warming. The extract was filtrated, alkalised to pH 10-11 and then derivatised with dansylchloride under heating (70°C 10 minutes). After centrifugation, the supernatant was analysed by means of RP- HPLC. Chromatographic conditions were as described by PAULSEN (1994).

RESULTS and DISCUSSION:
All DSM - strains plated out on the media for the detection of cadaverine, putrescine and tyramine showed the results expected. Although all strains were specified as histamine- forming, using the method of KRANNER and SCHOPF (1982), only DSM 5987 was found to be positive. The reason of this discrepancy is not cleared yet. Analysis of positive halos and negative agar zones (see above) confirmed all these results.
Field strains: For enumeration of positive colonies, it was found to be important to use only plates with less than 50, evenly spread colonies to avoid counting negative colonies masked by the halos of positive colonies. For the same reason, if more than one dilution is applied on the plate, it is advisable to use plates with compartiments (e.g. Bibby Sterilin 503V; 3 compartiments).

CONCLUSIONS:
Although further validation and modifications will be necessary, the methods presented in this paper are useful and easy to apply in the field of meat microbiology, enabling rapid estimation of the amine- forming potential in meat.

A list of **PERTINENT LITERATURE** can be obtained from the authors.

Table 1: Composition and preparation of the media

ENTERO-BACTERIA-CEAE	Cad.	Put.
Components:	g/l	g/l
Peptone	7	7
Yeast extract	3	3
NaCl	5	5
Glucose	6	2
Bile salt	1.5	1.5
N.red 0.1%	60ml	60ml
C.violet 0.1%	2ml	2ml
Lysine	7	
Ornithine		10
Agar	25	25
pH	5.8± 0.2	5.8± 0.2

TYROSINE - MEDIUM	Entero bact.	Entero cocci	Lacto-bacilli 2)	Pseudo-monas
Components:	g/l	g/l	g/l	g/l
Base layer:				
Peptone	10	10		10
Yeast extract	1	1		1
Sorbitol	2	2		2
Tyrosine	5	5	3.3	5
Bile salt	1.5			
C.violet 0.1%	2ml			
Aesculine		1		
Fe-NH_4-citrate		0.5		
Agar	15	15	20	15
Kanamycine suppl. 3)		10ml		
Penicilline				100000 I.E.
MRS- base 4)			50	
Top layer 1)				
Tyrosine	15	15	15	15
All other	com-	po-	nents	as
listed above.	Also	see	notes	1)- 2)

TOTAL AEROBIC COUNT 5)	Cad.	Put.
Components	g/l	g/l
Peptone	5	5
Yeast extract	3	3
d-(+)-Glucose	1	1
Chlorophenolred 0.1%	10ml	10ml
Bromcresolgreen 0.2%	10ml	10ml
Lysine	10	
Ornithine		10
Agar	15	15

PSEUDOMONAS 5)	Put.	Cad.
Components	g/l	g/l
Glucose	1	1
KH_2PO_4	2	2
$MgSO_4$	0.5	0.5
Agar	18	18
Peptone	1	1
Chlorophenolred 0.1%	10ml	10ml
Bromcresolgreen 0.2%	10ml	10ml
Penicilline	10^5I.E	10^5I.E
Lysine		10
Ornithine	10	

1) Preparation of the top layer: tyrosine is solved in 2M NaOH under gentle warming und then pH is adjusted with 5M HCl to 6.0-6.2. To the so produced milky suspension, dissolved warmed base layer medium (excl. tyrosine) is added and shaken gently. A thin layer (~1- 2mm) of this mixture is poured on the solidified base layer.

2) pH is adjusted to 5.4 ± 0.02 with lactic acid.

3) OXOID SR 092E **4)** MERCK 10661 **5) pH** is adjusted to 5.6

N.red = neutral red; **C.violet** = crystal violet

Table 2: Bacterial strains used for testing (specifications as given by DSM)

Species	Strain (s)	Formation of			
	DSM - Nr.	cad.	put.	his.	tyr.
Lactobacillus buchneri	5987			+	
Pseudomonas fluorescens, biovar 1	50071, 50091	+	+	+	+
Pseudomonas fluorescens, biovar 3	50117, 50124			+	
Pseudomonas putida	1693,2112,3226,3601,6414,50257,50906		+	+	+
Pseudomonas putida, biovar A	50198, 50201, 50208	+	+	+	+
Pseudomonas chloroaphis	50082, 50083, 50139	?	+	+	

Figure 1:

Enterobacteriaceae: appearance of putrescine/ cadaverine forming colonies

Total aerobic count: appearance of putrescine/ cadaverine forming colonies

Tyrosine- agar: appearance of tyramine forming colonies