EVALUATION OF THREE AGAR MEDIA TO DETECT HISTAMINE- AND TYRAMINE-FORMING BACTERIA IN RIPENED SAUSAGES

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Background and objectives

Niven and col. (5) developed an agar medium for qualitative detection of histamine-forming bacteria based in the colour change of bromo cresol purple due to change in pH. To adapt this medium to different purposes, modifications have been described by Joosten and Northolt (1) and Maijala (4). These mediums have been employed to detect histamine- and tyramine-forming bacteria in different foods. However, some reports (6,7) have described false-positive reactions in some of these mediums due to the formation of different alkaline compounds or false-negative results as a result of the fermentative activity of some bacteria, such as lactic acid bacteria, over carbohydrates (4).
The aim of this work has been to evaluate the effectiveness of these three agar mediums as a rapid and reliable method to detect histamine- and tyramine-forming microorganisms in ripened sausages.

Methods

Eight samples of ripened sausages elaborated in Spain were analysed. Total aerobic mesophyllic microorganisms (PCA at 30ºC 48 h), Lactic acid bacteria (LAB) (MRS pH 5.5, at 30ºC 4 days), micrococci (MSA at 30ºC 72 h), enterococci (KF Streptococcus Agar at 37ºC 48 h), enterobacteria (VRBG at 37ºC 24 h), spore-forming aerobic microorganisms (PCA after heat treatment at 80ºC 1 min (PCA-HT), at 30ºC 24h) and pseudomonas (CFC, at 20ºC 72 h) were enumerated (7).

Histidine- and tyrosine-decarboxylase bacteria (HDB and HDT respectively) were determined by the Most Probable Number (MPN) method using 3-tubes series of Tryptic Soy Broth with 1% of L-histidine (TSBH) or 1% of L-tyrosine (TSBT) (pH 5.3), incubated at 30ºC 48h. Histamine formation was determined in each tube by an enzymatic method (3) and tyramine by Thin Layer Chromatography (TLC) after mixing 1 ml of broth with 0.5 ml of 0.4M perchloric acid (2).
A total of 175 isolates were obtained from the different media. One strain of LAB (*L. curvatus*, H+ T+ (7)) from the author's collection was used as a control. After purification, the strains were tested in the agar media described by Niven and col (5), Joosten and Northolt (JNM)(1) and Maijala (MDA)(4), with a 2% of L-Histidine or L-tyrosine (pH 5.3). Plates were incubated at 30ºC for 96h, with lectures each 24h. Colonies surrounded by a purple or a transparent ring suggested a positive reaction for histidine and tyrosine decarboxylation respectively. All the positive strains were confirmed by the aforementioned enzymatic and TLC methods in TSBH and TSBT broths.

Results and discussion

HDB and TDB counts were detectable in all the analysed samples but TDB were significatively higher than HDB (Table 1). The 95% of isolates obtained from VRBG showed a positive reaction to histamine in the decarboxylation agars, being all confirmed by the enzymatic method. However, none of the positive isolates obtained from the CFC, MSA and PCA-HT media were confirmed (Figure 1). These high number of false positive results have previously been described in Niven (6) and JNM (7). None of the isolates obtained from PCA and MRS was positive neither the decarboxylation agars nor by the enzymatic method. The strain used as control did not show a positive reaction in any of the agar media, but was positive in the enzymatic method.

Isolates obtained from CFC and VRBG showed the highest percentage of positive results in the decarboxylation agars with tyrosine, but they were not confirmed by TLC. All the positive isolates obtained from MRS and KF media were confirmed by TLC. In this case JNM was more effective and faster than Niven and MDA (Figure 2), detecting all the confirmed strains obtained from KF and failing only with one isolate obtained from MRS.

Some of the confirmed isolates were identified. All the histamine-positive strains identified belonged to the Enterobacteriaceae family (Table 2). Most of them were obtained from sample 1, which presented the highest HDB counts, and also from samples 2 and 3. However, HDB counts were also detected in the other samples despite any histamine-forming isolate was obtained. Tyramine-positive isolates were identified as enterococci or LAB. All the tyramine-forming LAB isolates were obtained mainly from the samples 2, 3 and 7, which presented the highest TDB counts.

Conclusions

None of the tested decarboxylation agar medium would be a good choice as a single method to detect HDBs or TDBs. The enzymatic and the TLC methods offer a rapid and easy alternative, being more specific for detecting HDB and TDB.

Table 1. Bacterial counts (Log CFU/g) and pH value obtained from the analyzed samples

	N° Sample							
	1	2	3	4	5	6	7	8
PCA	8.10	8.69	8.36	8.85	8.77	8.71	8.40	8.61
MRS	7.93	8.60	8.15	7.67	8.78	8.56	8.32	8.56
MSA	5.69	5.72	6.13	6.00	6.02	5.20	5.72	6.09
KF	5.91	6.01	5.74	6.47	3.50	5.00	5.37	3.02
PCA-HT	2.18	1.70	2.18	1.30	1.65	1.54	3.12	4.08
VRBG	5.06	2.13	2.33	-	-	-	-	-
CFC	-	1.70	3.02	-	-	-	-	-
MPN-HDB	5.63	3.63	2.36	2.36	2.36	3.18	3.63	1.36
MPN-TDB	4.63	8.04	7.66	4.63	5.97	4.97	7.66	3.63
pH	5.8	5.3	5.9	5.9	5.4	5.2	5.4	5.7

(-) Not detected

Table 2. Identification of some of the confirmed amino acid decarboxylase isolates

	Identified	Histamine +	Tyramine +
E. faecalis	9	-	9
E. faecium	14	-	14
L. curvatus	14	-	14
L. delbrueckii	1	-	1
S. liquefaciens	5	5	-
H. alvei	3	3	-
K. oxytoca	1	1	-
K. pneumoniae	1	1	-
Not identified	7	6	1

Figure 1. Percentage of positive isolates in the decarboxylation agars with histidine and by the enzymatic method

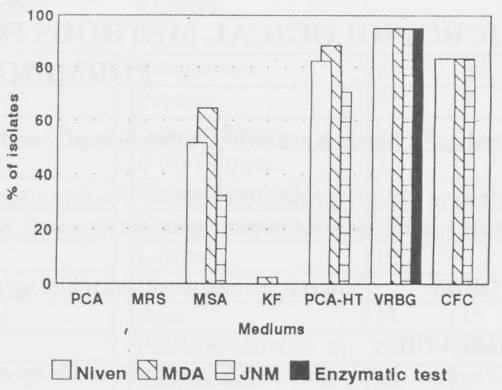
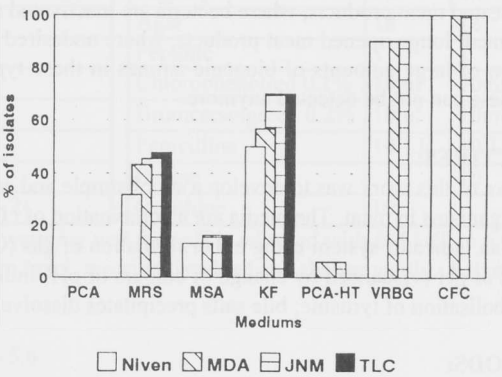


Figure 2. Percentage of positive isolates in the decarboxylation agars with tyrosine and by TLC



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