COMPARATIVE EXAMINATIONS FOR THE EFFECTIVENESS OF MEDIA TO DETECT CLOSTRIDIA FROM CANNED MEAT PRODUCTS

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The routine examination of canned meat products consists of cheking the double seams on both end namely on canner's and maker's end and side seam of cans during production; controlling of cooling water for chlorine content; handling of cans after cooling process; incubation test; bacteriological examination; chemical examination of poisonous chemical materials, ingredients, adulteration; cheking of vacuum; pressure test for leakage; organoleptical examination and finally from time to time histological examination for detecting of adulteration.

To achieve the best results in bacteriological examination of canned meat products media used most frequently and primarily for detection of clostridia were studied.

Materials and Methods

Media: To the comparative examinations Cooked Meat Medium according to Holmann (CMM), Kitt-Tarozzi Liver Broth (LB), semisolid Sulphite Agar (SA), semisolid Glukose Deep Agar (GDA), Modified Cooked Meat Medium (MCMM) and Diagnostic Reinforced Clostridial Medium (DRCM) were used. The supplying of details about composition of media has been here disregarded.

Model strains: Spores of C.bifermentans ATCC 506, C.botulinum A ATCC 2012, C.botulinum B ATCC 3807, C.sporogenes ATCC 533, ATCC 276 and PA 3679 and cells of C.perfringens ATCC 8235 respectively were used.

MPN determination for effectiveness of media: To establish

the highest degree of dilution i.e. the greatest number of positive tubes of spore or cell suspension three-three of each medium and of each dilution were inoculated with 1,01, 0,01 ml of spore or cell suspension, respectively. The most probable test number (MPN) was with this method calculated. To MPN determination MPN table of ISO/TC 34/Sc 6/WG 2/UK-11/ was employed.

Preparation of inocula:

a.) Spore preparation; cleaning of spores: Strains were inoculated into 150 ml Cooked Meat Medium according to Holmann (CMM) in Erlenmeyer flasks. Flasks were incubated at 32°C for 20 days. After incubation cultures were filtered through sterile cheese cloth to keep back meat pieces of medium. Pieces of meat were thoroughly expressed before filtration. Filtrate was centrifuged at 6000 r.p.m. for 30 minutes. After centrifugation, the supernatant was discarded. Spores in precipitate were washed five times with distilled water. After each washing of spores they were resuspended in sterile distilled water and centrifuged for 20 minutes again. Finally the washed spores were stored in 50 ml sterile distilled water in steril flasks with glass beads and rubber cork in refrigerator at ± 4°C until the investigations.

The density of spore suspension was adjusted to the density of supersaturated barium chloride solution and the titre of spore suspension was established with dilution technique in CMM.

b.) CMM cultures, incubated at 32°C for 168 - 860 hours were taken and inoculated in media to be cheded at 80°C. Heat shock was carried out at 80°C for 20 minutes after inoculation and then cooled immediately in cold tap water.

Incubation of inoculated media: Tubes were incubated after inoculation at 32°C for 7 days.

C.perfringens suspensions were not heattreated. Incubation was happened at 37 and 39,5°C for 7 days.

Amount of spores: The amount of spores was 10^8- 10^9 in inoculum taken to the investigations.

Investigations were carried out at least three times.

Results.

According to the model investigations the best results in detection of clostridia has been achieved with Modified Cooked Meat Medium (MCMM), and Kitt-Tarozzi Liver Broth (LB). Cooked Meat Medium (CMM) according to Holmann, Diagnostic Reinforced Clostridial Medium (DRCM) semisolid Sulphite Agar (SA), and semisolid Glukose Deep Agar (GDA) was the further order of media in the study of effectiveness of clostridial media.

It could be established that the composition of media could greatly influence the results. The consistence, the glukose content, the incubation temperature has one by one influence on the results. Fluid and semisolid media give better results than solid media.

From the viewpoint of selective media used for detection of clostridia in general in routine bacteriological work semisolid Sulphite Agar (SA) with 0,1% glukose content may best be used. If clostridia are present since sulphite reduction blackening of media occurs within 48 hours. The fluid media have given in general better results than semisolid media, however, without any specific sign that Clostridia are present.

Conclusion

As conclusion of the model investigations can be established, that for bacteriological examination of canned meat products the

best results may be achieved with Modified Cooked Meat Medium (MCMM) or Kitt-Tarozzi Liver Broth (LB) from the fluid media, however, with the use of a combination of semisolid Sulphide Agar (SA). SA was indicated by blackening within 48 hours wether sulphite reducing clostridia were present because the most of clostridia that might have a role in spoilage of canned meat products had a sulphite reducing ability. If a blackening of SA was observed the two fluid media incubated at 28°C for a relevant time could be used for detection of C.botulinum without delay, SA should be incubated, however, at 32°C. Incubation for C.perfringens showed a better results at 39,5°C than at 32°C.

In many countries Glukose Deep Agar (GDA), is prescribed to the complementary bacteriological meat examination of meat animals for detecting clostridia in ill and emergency slaughtered animals. According to our model investigations, this medium has to be changed to Modified Cooked Meat Medium (MCMM) or Kitt-Tarozzi Liver Borth (LB) and to semisolid Sulphite Agar (SA) for achieving better results.

For detection and enumeration of mesophilic clostridial spores from meat and meat products diagnostic Reinforced Clostridial Medium (DRCM) has been suggested by ISO standard, however, according to our and Dutch investigations it was nor the best medium for this purpose. Our investigations could be taken into consideration in the diagnostic work and standards for clostridia.