Analitycal methods

Posters B-39-B-62.1

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Poster session and workshop 6

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 - a soft mount and blue cheese

Analitycal methods

Results and discussions

During 1996-1997 we isolated \$2 Listeria sp. strains from toodstuffs Listeria spp. were mostly isolated from meal products was prevaled in faw theat processing (new salami), from ripening cheese and mechanicaly recovered poultry meal. L. monocytogenes was revealed in faw treat products (neetwurst salami), from ripening cheese and mechanicaly recovered poultry meal. L. monocytogenes was revealed in faw treat products (neetwurst salami), form ripening cheese and mechanicaly recovered poultry meal. L. monocytogenes was revealed in faw treat products (neetwurst salami), dry and furnented subsage and pate (Table 1). We succeeded to isolate 17 strains of Listeria fifth from 30 examined samples of mechanically recovered poultry meat. On the basis of further diagnostic tests 16 isolated strains were identified in one case.

As many as 65 strains of Listeris sop, were investigated will Strains L. monocytogenes were revealed in 17 %. The fragm 750 pb in case of L. monocytogenes. The PCR method above innocua which meat samples were contaminated experimentime interval of several days were identical.

Conclusions

From our experiments and from literature data it mainly the matter of the products with a short (in of starter cultures. From our results it follows the spp. and three sumples contained even L monocy

Isolated strains of L. monocytogenes were identifi sensitivity and specificy is a great advantage of the samples too.

Pertinent literature

Me Lauchlin, J. 1993. Listeriosis and Listeria monocytogenes. Environmental Policy and Practic lay, LM, 1996. Prevalence of Listeria spp. In meat and poulity products. Food Control 7, 209-Salamina, G., Donne E. D., Miccolini, A. and et al. 1996. A floodborne outbreak of gastrounter Foldemiol. Infect 117: 429-436.

Rijpens, N. P. Jannes, G. and Herman L.M.F. and turkey products determined by polymerase o



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Tuesday, September 1st 11:15h-12:45h

B-39

Occurrence of Listeria monocytogenes in meat products and identification by applied PCR method

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Background

Listeria belongs to the microorganisms whose monitoring deserves a great attention due to serious course of the disease. The way of transfer is of high importance in epidemiology of listeriosis. Consumption of contaminated food is the most frequent way of transfer. Fresh meat, raw meat products and ready to eat dishes are a significant source of Listeria spp. In case of disease the way of transfer shall be determined.

Objectives

Our study was aimed at monitoring of occurrence of Listeria monocytogenes in meat and meat products and at molecular identification of isolated strains with help of PCR methods.

Methods

During 1996 – 1997 we were taking samples from selected kinds of foodstuffs and monitoring occurrence of Listeria spp. Our attention was drawn mainly to:

- meat products without heat processing (raw salami)
- heat processed meat products
- mechanicaly recovered poultry meat
- soft, ripening and blue cheese

Taken samples were examined according to standard USDA methods. For certain samples of foodstuffs the quick screening test of the Clearview (Oxoid) was applied for identification of Listeria spp. Selected strains were identified by using the PCR method. From certain available methods the PCR method was chosen, as it is based on gene sequence for listeriolyzin O. Two oligonucleotide primers LP1 and LP2 were used. PCR products were subject to electrophoretic analysis on 1% agarose gel and detected by ethidium bromide staining by applied UV transillumination.

Results and discussions

During 1996-1997 we isolated 52 Listeria sp. strains from foodstuffs. Listeria spp. were mostly isolated from meat products without heat processing (raw salami), from ripening cheese and mechanically recovered poultry meat. L. monocytogenes was revealed in raw meat products (meetwurst salami), dry and fermented sausage and paté (Table 1). We succeeded to isolate 17 strains of Listeria spp. from 30 examined samples of mechanically recovered poultry meat. On the basis of further diagnostic tests 16 isolated strains were identified as L. innocua; L. monocytogenes was identified in one case.

As many as 65 strains of Listeria spp. were investigated with PCR methods. A majority of them was identified as L. innocua (83%). Strains L. monocytogenes were revealed in 17%. The fragments yielded with help of the described PCR method reached the size of 750 pb in case of L. monocytogenes. The PCR method above enabled to identify L. monocytogenes even in the mixed culture with L innocua which meat samples were contaminated experimentally by. The results reached by classical examination carried out in the time interval of several days were identical.

Conclusions

From our experiments and from literature data it follows that raw meat products represent a hazard of listeriosis for the people. It is mainly the matter of the products with a short time of ripening where the technological barriers cannot by applied, and without use of starter cultures. From our results it follows that more than 30 % of examinated raw salami (meetwurst salami) contained Listeria spp. and three samples contained even L. monocytogenes.

Isolated strains of L. monocytogenes were identified with help of PCR method and used oligonucleotides LP1 and LP2. High sensitivity and specifity is a great advantage of the method. L. monocytogenes and L. innocua may be differentiated in the mixed samples too.

Pertinent literature

Mc Lauchlin, J. 1993. Listeriosis and Listeria monocytogenes. Environmental Policy and Practice 3: 201-214.

Jay, J.M. 1996. Prevalence of Listeria spp. In meat and poultry products. Food Control 7: 209-214.

Salamina, G., Donne E. D., Niccolini, A. and et al. 1996. A foodborne outbreak of gastroenteritis involving Listeria monocytogenes. Epidemiol. Infect 117: 429-436.

Rijpens, N. P., Jannes, G. and Herman L.M.F. 1997. Incidence of Listeria spp. and Listeria monocytogenes in ready-to eat chicken and turkey products determined by polymerase chain reaction and line probe assay hybridization. J. Food Protection 60: 548-550.



Table I

Frequency of Listeria spp. in meat products

Product	No. samples	No. (%) Listeria spp.	No. (%) L. innocua	No. (%) L. monocytogenes
Raw salami	20	9 (45)	7 (35)	2 (10)
Paté	60	5 (8)	3 (5)	2 (3)
Cooked Salami	19	3 (16)	3 (16)	0
Mechanically recovered poultry meat	30	17 (6)	16 (5)	1 (1)
TOTAL	185	52 (28)	44 (24)	8 (4)

bacteria by a chemical procedure (Hoffman and Winwon, 1987) which also degraded the polycurbonate membrane. The extracted DNA was amplified using a FCE assay and primers (Thomas et al. 1984). The primers amples efficace spicific for the genetic sequence of informitally windence factor in L memocytogenes. The template DNA gene initially demuted at 95°C for 5 min and then 35 cycles of FCE amplification is moder the following conditions : derivatation at 94°C for 1 min. pynar annealing at 80°C for 5 min and then 35 cycles of FCE amplification is moder the following conditions : derivatation at 94°C for 1 min. pynar annealing at 80°C for 5 min and then 35 cycles of FCE amplification is moder the following conditions : derivatation at 94°C for 1 min. pynar annealing at 80°C for 1 min and then 35 cycles of FCE amplification is moder the following conditions : derivatation at 94°C for 1 min. pynar annealing at 80°C for 1 min and then 55 cycles of FCE for 2 min is moder the following conditions : derivatation at 94°C for 1 min. pynar annealing at 80°C for 1 min and DNA extension at 72°C for 2 min is moder to the sequence of the second using gel discuspleates at the method was emploid to inoculated with a standard cultural procedure for the licitertion of Listeria memorytogenes (Daffy et al. 1994). This method was applied to inoculated both culture, inoculated mean culture and

Reaths and discussions Surface adhesion onto a polycurbonate membrane was successfully used to isolate bacteria from the minicited [062 maniple. This simple and last extraction procedure has previously been employed in conjunction with immunofluotescence (bhardan et al. [097] It is considerably firster and gives similar yields to immunomagnete argunation (Duffy *et al.*, 1997.) The DNA was obtracted from calls eleminative, This advantage of dus procedure was that it niso degraded the polycarbonate membrane. This study showed that the listeryolysis of prime yielded a \$20 kp product. This primer proved to be flightly epscific for *L. monographeres* only. In inoculated brochs the surface advanprime yielded a \$20 kp product. This primer proved to be flightly epscific for *L. monographeres* only. In inoculated brochs the surface advanprime yielded a \$20 kp product. This primer proved to be flightly epscific for *L. monographeres* only. In inoculated brochs the surface advandesting the culture was necessary to produce a visible PCR product band (\$200b). For the 120 retail samples analysed using structed cultural technique and the Surface adhestion - PCR method, a total of 23 samples tested positive for *L. monographere* dusting the structed winds the Surface adhestion - PCR method, a total of 23 samples tested positive for *L. monographere* using the structed winds the Surface adhestion - PCR method, a total of 23 samples tested positive for *L. monographere* using the structed was approximately lag₁₆ 2 -3 cft ad¹⁶ which is below the detection limit of the FCR test. A lagger cardinated into adverse the product Thirspecthod tools approximately 24b to curry out and is considerably factor than provingly described PCR methods (Beaseern et al., 1900.) This predicted tools approximately 24b to curry out and is considerably factor than provingly described PCR methods (Beaseern et al., 1900.) This predicted tools approximately 24b to curry out and is considerably factor than provingly described PCR methods (B

Conclusions : The membrane surface adhesion assay is a rapid, acrustive and specific method for the detection of L monocytogenes in 1999, semisive and case in completed within a 24 hour time period.

References

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