DETEKCE ATYPICKÝCH KMENŮ BAKTÉRIÍ RODU *SALMONELLA*
DETECTION OF UNTYPICAL *SALMONELLA* STRAINS

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Abstract: Cultivation method and polymerase chain reaction coupled with immunomagnetic separation (IMS-CM and IMS-PCR) are new techniques for the detection of bacteria in foodstuffs. The purpose of the present study was to investigate the application of various sample preparation method for the final identification of *Salmonella* strains. The presence of PCR inhibitors in some food products (milk powder) can be cause of false-negative results in PCR without IMS in target cells. It was also demonstrated that IMS-PCR was successfully used for identification and quick confirmation of untypical *Salmonella* strains isolated from human stool samples and rabbit meat.

Key words: *Salmonella*, untypical strain, PCR, immunomagnetic separation

INTRODUCTION

The most important alimentary infections are caused by *Salmonella* cells. The incidence of salmonellosis has significantly increased in several European countries and also in the Czech Republic (Threllfall et al., 1998; Šrámková et al., 1997). The culture methods for indentification *Salmonella* strains are laborious and time-consuming requiring some steps of identification and confirmation. Therefore new alternative methods reducing time of analysis have been studied.

Polymerase chain reaction (PCR) techniques can speed up the detection of *Salmonella* cells. PCR methods suitable for identification of *Salmonella* have been reported using a variety of primers (Aabo et al, 1993, 1995; Mahon et al., 1994; Soumet et al., 1997). PCR is very effective when analysing pure microbial cultures.

In some cases *Salmonella* cells present in processed foods (dried foods) are not in the same physiological conditions as they are in pure laboratory cultures. Using immunomagnetic separation (IMS), these cells were detached from the competitive microflora environment. The IMS of bacterial cells implies the use of a magnetic beads-antibody system causing particles to be selectively attached to target cells when added to a cell suspension. The IMS technique has been used for extraction of target microbial cells from biological samples and for selective enrichment in many cases (Ripabelli et al., 1997; Šafařík and Šafaříková, 1999). This technique can be effectively combined with the other methods of microbial cells identification, such as standard cultivation or PCR. Another advantage of IMS is the separation of analysed cells from substances which can inhibit PCR.

The aim of this work was the identification and characterisation of untypically growing *Salmonella* cells isolated from human stool samples and rabbit meat.

MATERIAL AND METHODS

Salmonella cells were isolated from different food samples according to ČSN ISO 6579. The *Salmonella* cells were isolated using immunomagnetic particles Dynabeads M280 anti-*Salmonella* from the food samples. Magnetic particles were separated using a Dynal MPC-M magnetic particle concentrator (Dynal, Oslo, Norway). Various differentiation cultivation media (RV-BGA, SC-XLD, Rambach) were used for cultivation of enriched bacterial cultures.

PCR was performed using primers ST11 and ST15 (Aabo et al., 1993) which enables to amplify a 429 bp long unique DNA fragment specific to the *Salmonella* genus. The cells captured on immunomagnetic particles were used for isolation of purified DNA (phenol extraction method) and for preparation of cell lysates (10 minutes of boiling). Both purified DNA and the DNA from cell lysates were used as DNA matrices in PCR.

The PCR mixture contained 5 pmol of primers, 10 mM dNTP, 25 mM $MgCl_2$, 0.5 U Taq polymerase (TopBio, Czech Republic). Amplification was carried out after a hot start in 30 cycles on an MJ Research 100 cycler (USA): 94 °C/ 40 sec, 57 °C/ 40 sec, 72°C/ 80 sec (except the last cycle in which the time was prolonged to 10 minutes). PCR products were identified by ethidium bromide-agarose gel electrophoresis in TBE buffer. Gels were photographed with a Polaroid camera CD34 on a TT667 film.

RESULTS AND DISCUSSION

The group of analysed samples contained selected human isolates of *Salmonella* and one suspect isolate of rabbit meat. These strains did not grow typically on differentiation media or did not give typical confirmative biochemical reactions (Table 1). It has been known that *Salmonella arizonea* does not give typical red colonies but gives blue or blue-violet colonies on Rambach agar (Kuhn et al., 1994). We have observed that colonies grown on Rambach agar after immunomagnetic separation also lacked the typical red colour in the case of uncertain *Salmonella* from rabbit meat and four stool samples with *Salmonella salamae* and *Salmonella arizonae*. Strains of *Salmonella salamae* (14/98) and *Salmonella arizonae* (4/98) have grown atypically on three differentiation media (Table 1). The biochemical confirmation reactions were also untypical.

IMS-PCR was proven for confirmation of all these bacteria. The untypical *Salmonella* cells were captured on immunomagnetic particles and 5 µl of boiled lysates were used in the polymerase chain reaction. Amplification products were obtained in all cases. The results are given in Table 2 and Figure 1. Amplification products were also obtained if amplification was carried out with purified DNA (phenol extraction method) without IMS.

In some strains, the quantity of amplification product creation was higher if a smaller amount of cell lysates (2 µl) was used in PCR in comparison with a larger amount (5 µl). This can be explained by the presence of intracellular PCR inhibitors in *Salmonella* cell lysates. The results agree with the observation made by Gibson and McKee (1993). According to these authors the post-PCR degradation was caused by a very thermostabile endogenous nuclease present in *Salmonella* cells. On the basis of the above mentioned assumption it is possible to deduce that intracellular inhibitors were present in some samples (Table 2). High amounts of inhibitors with expressive activity were present in *Salmonella* strain from rabbit meat sample and from *Salmonella arizonae* strains.

CONCLUSIONS

The above mentioned results have demonstrated that both immunomagnetic separation – cultivation (IMS-CM) and the immunomagnetic separation – polymerase chain reaction (IMS-PCR) are suitable methods for the identification of *Salmonella* strains isolated from human stool samples and from rabbit meat. Both methods are more specific in sample analysis than the conventional cultivation procedures. The immunomagnetic separation – polymerase chain reaction (IMS-PCR) is more specific than the immunomagnetic separation – cultivation method (IMS-CM) than the cultivation method without immunomagnetic separation.

Table 1: Identification of *Salmonella* strains according their growth on differentiation media.

Salmonella strain	Growth on differentiation solid media			Unusual biochemical reactions
	RA	XLD	BGA	
75/98 *enterica* ser. *Typhimurium*	+	+	+	Lys – Ino +
94/98 *enterica* ser. *Typhimurium*	+	+	+	Dul -
187/98 *enterica* ser. *Typhimurium*	+	+	+	Lys – Ino +
LT2-18 *enterica* ser. *Typhimurium*	+	+	+	
CCM 4420 *enterica* ser. *Enteritidis*	+	+	+	
78/98 *enterica* ser. Java	+	+	+	Dul -
13/98 *salamae*	-	+	+	Mal + Ino +
14/98 *salamae*	-	-	-	Mal +
4/98 *arizonae*	-	-	-	ONPG + Mal +
18/98 *arizonae*	-	+	+	ONPG + Mal +
strain from rabbit meat	-	+	+	ONPG + Lys -; Orn -

Growth: + positive *Salmonella* culture on selective agar media, - negative *Salmonella* culture on selective agar media. **Biochemical difference:** + positive reaction, - negative reaction.

Table 2: The amplification of specific DNA fragment.

Salmonella strain	Presence of PCR product		
	(A) 5 µl	(B) 5 µl	(B) 2 µl
75/98 *enterica* ser. *Typhimurium*	+	+	+
94/98 *enterica* ser. *Typhimurium*	+	+	+
187/98 *enterica* ser. *Typhimurium*	+	+*	++
LT2-18 *enterica* ser. *Typhimurium*	+	+*	++
CCM 4420 *enterica* ser. *Enteritidis*	+	+*	++
78/98 *enterica* ser. Java	+	+*	++
13/98 *salamae*	+	+	+
14/98 *salamae*	+	+	+
4/98 *arizonae*	+	-*	-
18/98 *arizonae*	+	-*	-
strain from rabbit meat	+	-*	-

Presence of PCR product in IMS-PCR: +, ++ band of good or very good intensity, - no band. (A) IMS-PCR performed with 5 µl of IMS extract after cell lysis; (B) IMS-PCR performed with 5 µl and 2 µl of IMS after 5 days of storage IMS extract in refrigerator; * presence of putative PCR inhibitor in cell lysate.

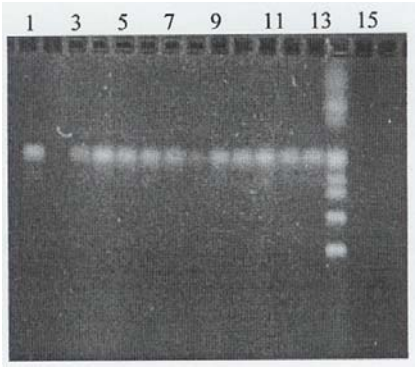

Figure 1: Agarose gel electrophoresis of PCR products obtained after IMS-PCR of selected *Salmonella* strains. Lanes: (1)-positive control with purified DNA *Salmonella*; (2)-negative control with DNA *E. coli;* 3-13 different *Salmonella* strains – (3)-187/98; (4)-75/98; (5)-94/98; (6)-LT2-18; (7)-CCM 4420; (8)-78/98; (9)-13/98; (10)-14/98; (11)-4/98; (12)-18/98; (13)-strain from rabbit meat; (14)-DNA standards (970, 750, 595, 544, 447, 305, 239, 194 and 155 bp); (15) control without DNA.

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