Detection without sample processing of yoghurt starters and DNA in milk products by PCR

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1. Introduction

Rapid progress in phylogenetic analysis has revealed in the last decade a permanently increasing number of oligonucleotides which can be used to identify species-specific lactic acid bacteria (LAB) applying nucleic acid probe technology or PCR technology (2,3,4; for a review see 1). Because traditional phenotypic identification of LAB is rather tedious and not always reliable, modern identification methods are designed that can replace the present ones.

It is known that quality of DNA extracted from complex matrixes influences strongly the performance of PCR technology. As components in many foods inhibit PCR, suitable sample preparation methods first have to be developed before this technology can be used more routinely in food industry.

In research, working with the Gram-negative microorganism *E. coli*, it is common use to transfer microbial cells from colony plates directly into the PCR reaction mixture e.g. to check cloned fragments or to screen gene banks. No DNA preparation is necessary. Cells are lysed and the nucleic acids released during the subsequent heating cycles serve as templates for the enzyme Taq-Polymerase. Although it is known that Gram-positive bacteria, e.g. *Streptococcus thermophilus* or lactobacilli, are much more difficult to lyse, we asked, whether it wouldn't be possible to use pure cultures directly in PCR and to achieve a successful and species-specific amplification and consequently identification in only a few hours. The next step was to find out, if fermented milk products itself (like yoghurts, yoghurt with fruits, probiotic products etc.) containing all their components can be used without preceding purification of the DNA in PCR to identify the starter cultures. As an example this was demonstrated for one of the yoghurt starter components *Streptococcus thermophilus.*

In the context of "risk assessment" of gene transfer between microorganisms - this also concerns genetically modified microorganisms – specific detection of free DNA in the food matrices is a matter of interest. It is assumed that DNA can be released (more or less intact) from starter bacteria – possibly due to processing procedures – and can be taken up by e.g. a contaminating flora. It was recently demonstrated that *Bacillus subtilis* naturally develops competence for uptake of free DNA when growing in milk products especially in chocolate milk (5, 6). To further characterize this system, we developed PCR conditions that allowed the direct monitoring of the model DNA pMG36enpr (7) in chocolate milk without any further purification. Furthermore, the detection limits of the single PCR assays as depending on the primer sets and target lenghts were determined.

2. Material and Methods

2.1 Yoghurt starter cultures

S. thermophilus was grown in LM17 (8) at 37°C or 40°C.

To propagate yoghurt cultures from different dairy companies skim milk (10 % w/v) was used after tyndallization. Cultures were incubated for 4-6 h at 40°C and stored at 4°C until use.

Yoghurt from different brands (specified in the single experiment) were stored at 4°C and used after the expiry date.

Chocolate milk, 1.8 % fat contents, UH-treated, Hansano, was used for the DNA experiments with plasmid pMG36enpr.

2.2 DNA preparation

Bulk DNA preparation for the PCR positive controls was carried out after Leenhouts et al. (9). Plasmid preparations of pMG36enpr were isolated from *Lactococcus lactis* according to Anderson et al. (10) followed by CsCl-gradient centrifugation (11). The amount of DNA was estimated on agarose gels (11) using a DNA mass ladder (Gibco BRL, Karlsruhe FRG).

2.3 Polymerase chain reaction (PCR)

2.3.1 PCR using pure cultures or yoghurt without further purification

The identification of *S. thermophilus* was carried out in a final reaction volume of 50 µl consisting of 5.0 µl 10 x PCR-buffer (200 mM Tris/HCl, pH 8.55, 160 mM $(NH_4)_2SO_4$, 15 mM $MgCl_2 \cdot 6H_2O$; 0.1 % (w/v) gelatin), each of the deoxynucleoside triphosphates at 0.05 mM, 1.0 µM of the *S. thermophilus* specific primers (Tab. 1), 0.1 % Tween 20 and 2.0 U Taq-Polymerase (Perkin-Elmer Applied Biosystems, Weiterstadt, FRG). A mastermix was prepared and kept on ice during distribution to the reaction tubes. 0.5 µl of overnight cultures (in liquid media), yoghurts or skim milk cultures, respectively, were directly pipetted into the PCR reaction mix.

An initial denaturation step of 10 minutes at 92°C was followed by a step down PCR program for higher specificity. The first 10 cycles were performed at a 10°C higher annealing temperature than the following 40 cycles. 10 cycles consisted of 20 seconds at 92°C primer denaturation, 1 minute 15 seconds at 63°C primer annealing and 30 seconds at 72°C template elongation, the subsequent 40 cycles were 20 seconds at 92°C, 1 minute at 53°C, 1 minute at 72°C, completed by a terminal elongation for 10 minutes at 72°C. For all PCR experiments the Gene Amp PCR system 9600 with hot top (Perkin Elmer Applied Biosystems, Weiterstadt, FRG) was used.

2.3.2 Direct detection of DNA in chocolate milk without further extraction procedure

A mastermix (reaction volume 100 µl) containing the following ingredients was prepared on ice: 10 µl of 10 x PCR-buffer (200 mM Tris/HCl, pH 8.8, 160 mM $(NH_4)_2SO_4$, 30 mM $MgCl_2 \cdot 6H_2O$; 0.1 % (w/v) gelatin), deoxynucleoside triphosphates 0.1 mM, 1µM of appropriate primers (Tab. 1), 0.2 % Tween 20 and 2, 4 or 8 U of a blend of 100 U:1 U Ampli-Taq-Polymerase (Perkin Elmer Applied Biosystems, Weiterstadt, FRG) and Pfu-

DNA-Polymerase (Stratagene, Amsterdam, NL), respectively, as it is described in the text. The time temperature program was as follows: an initial denaturation step was carried out for 5 minutes at 92°C. The subsequent 35 cycles consisted of 90°C for 20 seconds, 62°C or 52°C for 30 seconds (depending on the primers, given in Tab. 1) and 72°C for 5 minutes. The program was finished by a terminal polymerization step at 72°C for 10 minutes.

Tab. 1: Primers used in PCR experiments

Primer	Specificity	Sequence 5'-3'	Annealing Temperature (°C)	Direction	Reference
16k	*S. thermophilus*	CGA ACA GCA TTG ATG TTA	53	>>>	(12)
16m	*S. thermophilus*	CAC TAT GCT CAG AAT ACA	53	<<<	(12)
72	pMG36enpr	GTT ACT TTG GAT TTT TGT GAG C	52	>>>	(7)
72a	pMG36enpr	ATG GGT CGA TCG AAT TCG GTC CTC GGG	62	>>>	(7)
73/1	pMG36enpr	GTG GCA GCT GCC GTT GAA GCG	62	>>>	(7)
74	pMG36enpr	CCT TTA GAT TAT GGT TTG AGG	52	>>>	(7)
74/2	pMG36enpr	GTT TGA GGG CAA TTA TCA GTG TGG	62	>>>	(7)
75	pMG36enpr	GAG GAT GAA GAG GAT GAG G	52	<<<	(7)
75/2	pMG36enpr	GAC GAA GAG GAT GAA GAG GAT GAG GAG GC	62	<<<	(7)
76	pMG36enpr	CCT CAA ACC ATA ATC TAA AGG	52	<<<	(7)
77/1	pMG36enpr	CAG ACT TTG CAA GCT TGC ATG CCT GC	62	<<<	(7)

3. Results

3.1 Direct identification of *S. thermophilus* by PCR without DNA preparation

Our question was, if starter cultures could be identified by PCR when pure cultures, the yoghurt product itself, or a similar fermented milk product is directly added to the reaction mixture without preparing the nucleic acids and removing substances which may inhibit PCR. Identification of pure cultures of *S. thermophilus and Lactobacillus delbrueckii* ssp. in the appropriate liquid media were usually carried out using 0.5 µl or less from an overnight culture (results not shown).

15 yoghurts of a number of dairy companies containing different fruits, flavours, additives, and of different fat contents (1.5 %, 1.8 %, 3.5 %, 10 %) were examined about two weeks after the expiry date. For a detailed list of the products see Fig. 1. In Fig. 1B it can be seen that from 15 yoghurts examined 12 (including lane 12 where only a very weak band is visible) exhibited a single 968 bp amplification fragment after agarose gel electrophoresis showing species-specificly the presence of *S. thermophilus*.

Assuming that more DNA targets may be accessible for the enzyme, if freshly grown cultures are used – they may be lyzed more easily during the repeated heating steps – the starters were subcultured in skim milk for 4-6 h. Of 13 of the mixed cultures the amplicon of the expected size could be obtained (Fig. 1 A).

Interestingly when using the original milk product that did not result in a successful amplification reaction (Fig. 1 B, lane 3, 12 weak, 13, 14) the expected PCR product could be seen after adding the respective skim milk culture and vice versa (Fig. 1 A, lane 1, 5).

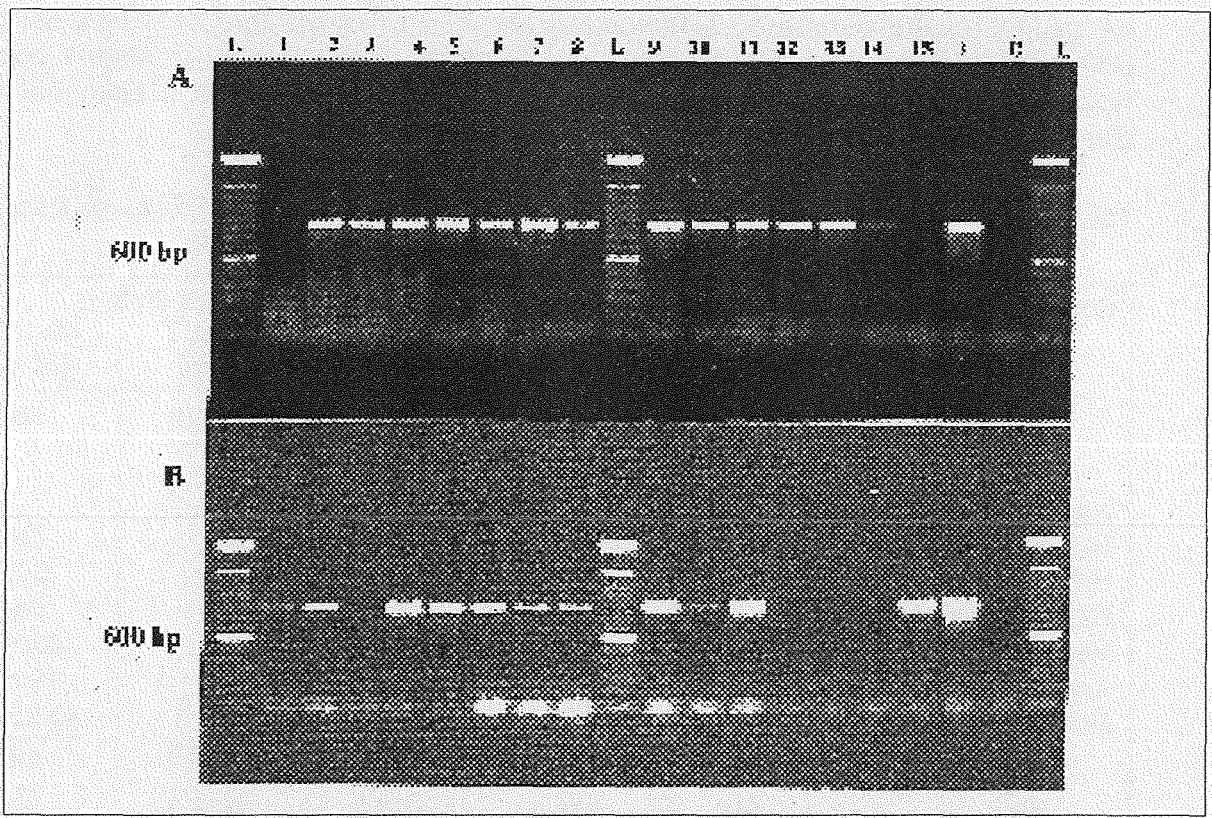

Fig. 1: Detection of *Streptococcus thermophilus* (968 bp fragment) without extracting DNA by adding 0.5 µl of yoghurt (B) or the respective skim milk culture (A, incubated for 4-6 h at 40°C) directly to the PCR reaction mixture (final volume 50 µl). 15 different yoghurts containing different fruits, flavours and additives were examined.
Lane 1: Plain yoghurt, company A, fat contents 3.5 %; 2: Plain yoghurt, company B, fat contents 10 %; 3: Yoghurt with lemon flavour, fat contents 3.5 %; 4: Yoghurt wirth pears and wheatgrains, fat contents 3.5 %; 5: Yoghurt with strawberries and rhubarb, fat contents 1.8 %; 6: Yoghurt with raspberries and vanilla flavour, fat contents 3.5 %; 7: Plain yoghurt, company C, fat contents 3.5 %; 8: Yoghurt with strawberries, fat contents 3.5 %; 9: Mixed milk product with herbs (St. Johns wort and melissa), fat contents 3.5 %; 10: Yoghurt with nuts and nougat, fat contents 3.5 %; 11: Yoghurt with cherries, fat contents 10 %; 12: Yoghurt with cherries, fat contents 3.5 %; 13: Yoghurt with plums, fat contents 3.5 %; 14: Plain yoghurt, company D, fat contents 1.5 %; 15: Yoghurt drink, fat contents 2.0 %; +: positive control, containing DNA extracted from *S. thermophilus*; C: water control, without any DNA; L: 100 bp ladder as molecular weight marker

3.2 Direct detection of DNA in chocolate milk without further extraction procedure

Investigating transformation in milk products, the free DNA contents, physical stability and availability of the model plasmid pMG36enpr for possible uptake by microorganisms was of interest. A PCR system directly using the milk product containing the DNA templates without further DNA extraction should help to monitor (semi-)quantitatively the DNA contents, stability or possible degradation of DNA over longer periods of time.

Various primer combinations were chosen flanking DNA segments of increasing lengths (1.6, 2.4, 3.3, 4.2, and 5.8 kb) of the plasmid pMG36enpr. The detection limits of each single primer pair were determined using 1:10 serial dilutions of pMG36enpr in chocolate milk (Tab. 2). In Fig. 2 differences in detection sensitivity were demonstrated by comparing two primer combinations 74 and 75 (21-mer, 19-mer, respectively), and 74/2 and 75 (24-mer, 19-mer, respectively) both addressing nearly the identical DNA sequence of 1.6 kb. Applying the latter primer set a PCR product could be obtained when

only 0.01 pg/µl plasmid DNA was added (corresponding to 0.01 ng of nucleic acids per ml of chocolate milk). However, the detection limit was two orders of magnitudes lower when the primers 74 and 75 were involved (Fig, 2 A).

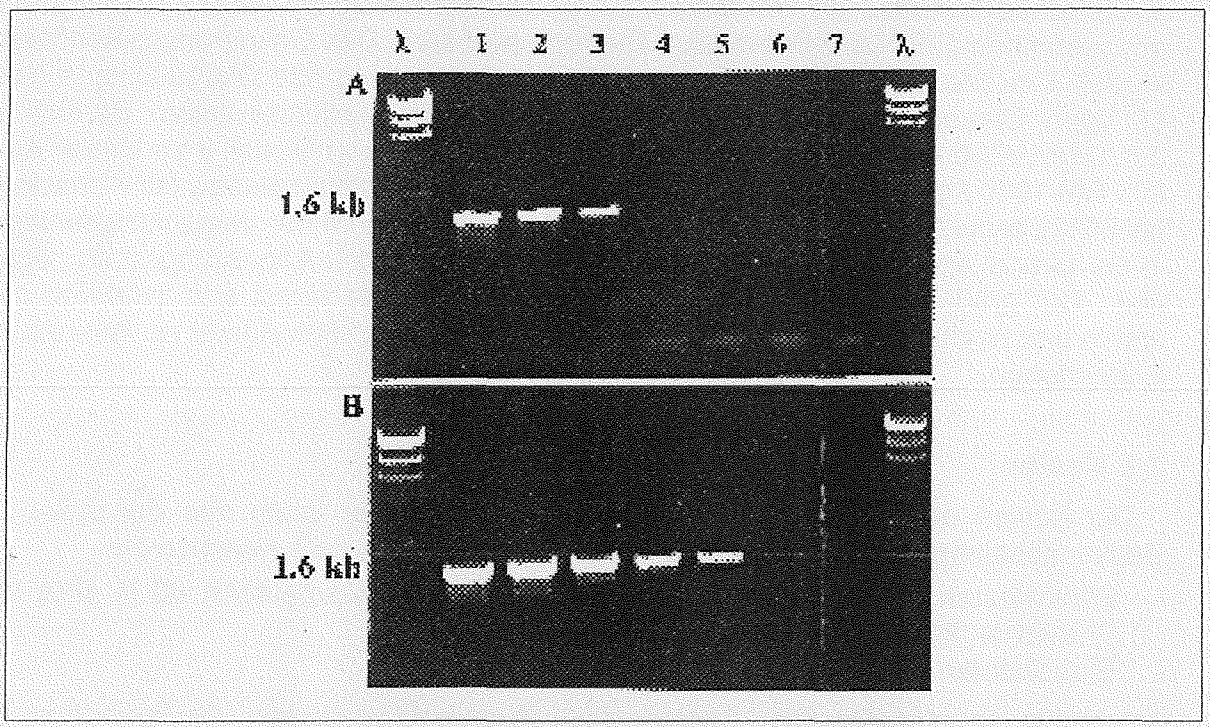

Fig. 2: Detection sensitivities of DNA in chocolate milk by PCR depending on primer design and length. Different amounts of DNA were diluted stepwise in chocolate milk, 1.0 µl of each dilution was directly added to the PCR reaction mixture (final volume 100µl).
A: primer combination 74 and 75 (21-mer, 19-mer, respectively) was used,
B: primer combination 74/2 and 75 (24-mer, 19-mer, respectively) was added, resulting each in a 1.6 kb amplification product.
Lane 1: 1.0 µl chocolate milk containing 1.0 ng/µl plasmid DNA pMG36enpr; 2: 1.0 µl chocolate milk containing 0.1 ng/µl plasmid DNA pMG36enpr; 3: 1.0 µl chocolate milk containing 0.01 ng/µl plasmid DNA pMG36enpr; 4: 1.0 µl chocolate milk containing 0.001 ng/µl plasmid DNA pMG36enpr; 5: 1.0 µl chocolate milk containing 0.0001 ng/µl plasmid DNA pMG36enpr; 6: 1.0 µl chocolate milk containing 0.00001 ng/µl plasmid DNA pMG36enpr; 7: 1.0 µl chocolate milk containing 0.000001 ng/µl plasmid DNA pMG36enpr; λ: λ-*Hin*d III digest as molecular weight marker

Tab. 2: Detection limits of plasmid DNA diluted in chocolate milk

Primers	74+75	74/2+75	74/2+75/2	72+77	72a+77/1	73/1+75/2	72+76	72+75	72a+75/2
Primer length	21-mer 19-mer	24-mer 19-mer	24-mer 29-mer	22-mer 20-mer	27-mer 26-mer	21-mer 29-mer	22-mer 21-mer	22-mer 19-mer	27-mer 29-mer
Template length (kb)	1.6			2.4		3.3	4.2	5.8	
Detection limit of DNA diluted in chocolate milk (µg/ml)	0,001 (4 U Taq-Polym.-blend)	0,00001 (4 U Taq-Polym.-blend)	0,00001 (4 U Taq-Polym.-blend)	0,001 (4 U Taq-Polym.-blend)	0,00001 (4 U Taq-Polym.-blend)	0,0001 (4 U Taq-Polym.-blend)	0,1 (4 U Taq-Polym.-blend)	(1,0 weak) (8 U Taq-Polym.-blend)	1,0 (4 U Taq-Polym.-blend)

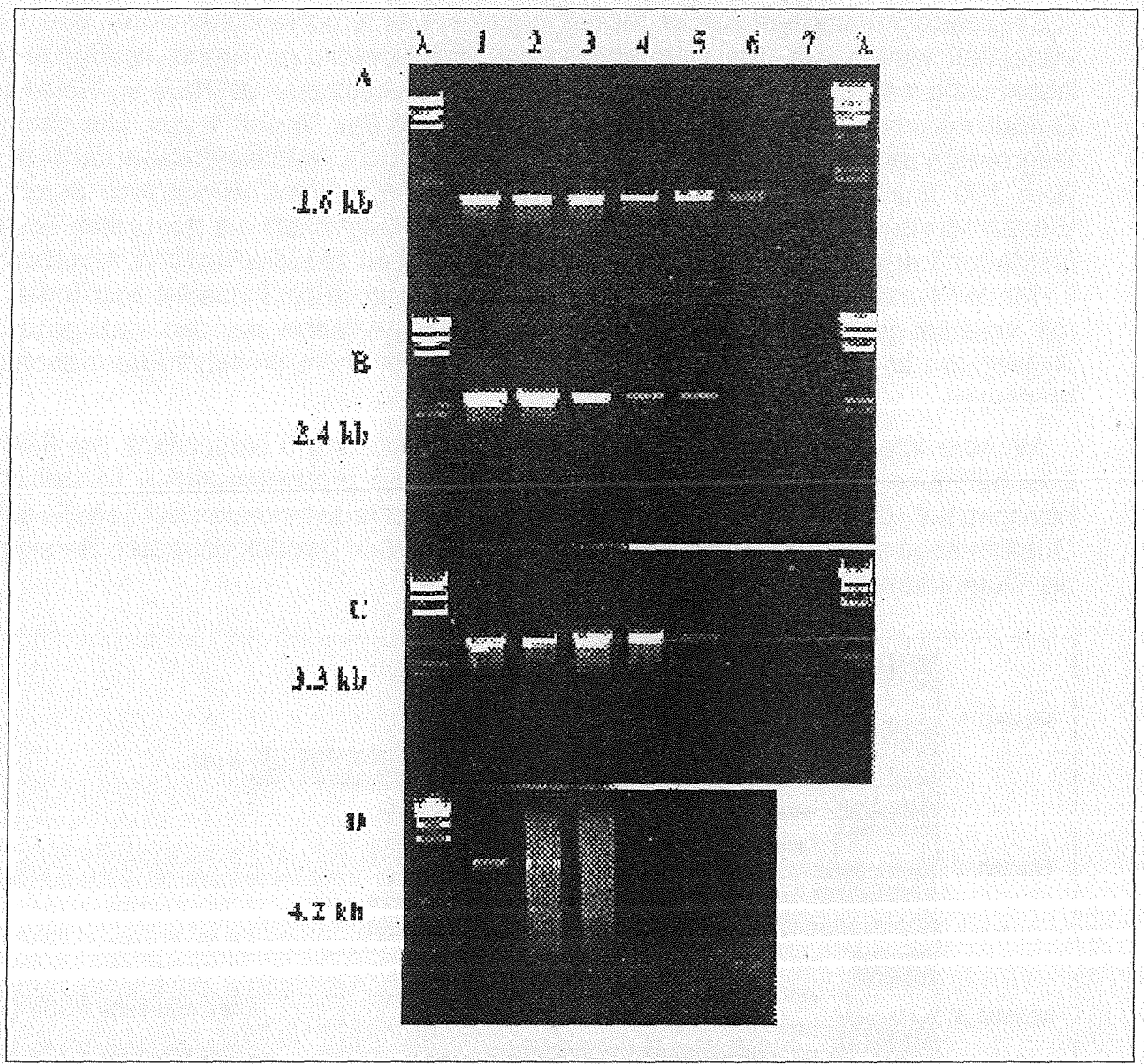

Fig. 3: Differences in detection sensitivity of PCR depending on the target length. DNA was stepwise diluted 1:10 in chocolate milk, 1.0 µl of each dilution was directly added to the PCR reaction mixture (final volume 100 µl) without any further purification.
Dilution series: Lane 1: 1.0 µl chocolate milk containing 1.0 ng/µl plasmid DNA pMG 36enpr; 2: 1.0 µl chocolate milk containing 0.1 ng/µl plasmid DNA pMG36enpr; 3: 1.0 µl chocolate milk containing 0.01 ng/µl plasmid DNA pMG36enpr; 4: 1.0 µl chocolate milk containing 0.001 ng/µl plasmid DNA pMG36enpr; 5: 1.0 µl chocolate milk containing 0.0001 ng/µl plasmid DNA pMG36enpr; 6: 1.0 µl chocolate milk containing 0.00001 ng/µl plasmid DNA pMG36enpr; 7: 1.0 µl chocolate milk containing 0.000001 ng/µl plasmid DNA pMG36enpr; A: primer combination 74/2 and 75/2 was used resulting in a 1.6 kb amplimer. For PCR reactions analyzed in lanes 1-4, 2 U of Taq-Polymerase blend were added, for PCR reactions in lanes 5 and 6, 4 U were added.; B: primer combination 72a and 77/1 was used resulting in a 2.4 kb amplimer*; C: primer combintion 73/1 and 75/2 was used resulting in a 3.3 kb amplimer*; D: primer combination 72 and 76 was used resulting in a 4.2 kb amplimer*; λ: λ-*Hin*d III digest as molecular weight marker

* The Units of Taq-Polymerase used are given in Tab. 2.

Different primer arrangements were tested and those selected that achieved the highest sensitivity in detecting the plasmid DNA in the PCR assay (Tab. 2, Fig. 3). As it was expected, the sensitivity decreased with length of the target sequence. Tab. 2 shows the minimum amounts of plasmid DNA which could at least be amplified and detected by

agarose gel electrophoresis. With primer set 72a and 77/1, flanking a 2.4 kb DNA segment 0.01 pg DNA/µl chocolate milk could be amplified. With primer set 73/1 and 75/2 resulting in a 3.3 kb fragment, 0.1 pg DNA/µl could be detected. With primer set 72 and 76, resulting in a 4.2 kb fragment, 0.1 ng DNA/µl was identified and using primers 72a and 75a at least 1.0 ng DNA/µl of a 5.8 kb segment was determined. The smear observed in Fig. 3 D (lane 2,3) was probably caused by the unspecific action of the enzyme Taq-Polymerase (which was added in relative high amounts) lacking suitable target sequences.

4. Discussion

It can be concluded that pure cultures of *S. thermophilus* (and *L. delbrueckii* ssp.) as well as fermented milk products (containing mixed bacterial cultures) itself can be used in the PCR reaction mix, provided that bacterial numbers are high enough. Calculating that a normal yoghurt contained about 10^8-10^9 *S. thermophilus* per ml (even less at the expiry date), in 0.5µl of the products still 10^4-10^5 microbial counts were expected to be present. Those numbers appeared to deliver sufficient targets accessible for Taq-Polymerase that amplification products could be formed without preparation of DNA. However, the addition of more than 1.0 µl of pure culture or yoghurt products often leads to an inhibition of PCR, so that we preferred to use 0.5 µl or even less. If bacterial counts are below a critical limit which may be the case at the expiry date, some of the bacteria may be still viable and can be repropagated and detected using a fresh skim milk culture (compare Fig.1 A,B, lanes 3, 12,13,14). Concerning the results in Fig. 1 A: lane 1 and 15 it must be assumed that *S. thermophilus* still present in the original product had died off before it was transferred into the new media and thus no amplification product was formed using the skim milk culture. A possible explanation for one of the two samples (the plain yoghurt in lane 1) may be that this product was fermented with a proper *L. delbrueckii* subsp. *bulgaricus*. This led to a much lower pH than with a "yoghurt mild" culture and therefore to less survival of *S. thermophilus* in the final product.

0.5 µl of plain yoghurts not containing any additional ingredients were usually examined without problems (Fig. 1A; 1B, lane 1,2,3). In order to assess interference of the PCR reaction by milk constituents and/or cell debris, 0.5 µl of 1:10 dilutions of fresh skim milk culture in TE-buffer (9) was added directly to the PCR reaction mix. In relation to results with purified DNA, a reduction of the PCR reaction efficiency by 2-3 orders of magnitude can be assumed. Furthermore, we suppose that fat contents do not inhibit PCR under the conditions described (Fig. 1B, lane 2, 11, 10 % fat contents). Another question was, whether different kinds of fruits and ingredients in fermented products would inhibit the PCR or whether such products can be used in the same way in PCR as plain yoghurts cannot be answered conclusively. From 10 yoghurts investigated that contained flavours and/or fruits eight exhibited a species-specific amplification (one of them only a weak reaction). In order to be able, to attribute PCR-inhibitory substances to specific fruits, a much more detailed analysis would be required, using identical yoghurts supplemented with different fruit mixtures.

Although pure cultures of *L. delbrueckii* ssp. (MRS, skim milk) were also sucessfully identified (not shown), problems may arise using mixed cultures due to lesser target numbers per colony forming units and thus to a markedly lower detection sensitivity as it was previously described (13).

Free DNA present in another milk product like chocolate milk can also be detected in PCR without purification. A final reaction volume of 100 µl was needed to dilute the substances that interfered with the enzyme and to achieve reliable amplification using not

more than 1µl of the sample. Moreover it is necessary to use a higher amount of Taq-Polymerase. To obtain amplification products, especially longer ones (4.2kb, and 5.8kb), the use of a blend of two enzymes (additionally Pfu-Polymerase with a 3'-5'proofreading activity) was applied, as it was described by Barnes (14). The detection sensitivity decreased with the lengths of the target sequences from 0.01pg/µl chocolate milk (addressing a 1.6kb segment) down to 1.0 ng/µl (for a 5.8kb segment) of the chosen plasmid pMG36enpr (Tab. 2). 0.01pg correspond to 10^3 molecules of the plasmid which demonstrated the inhibiting effects of the milk substances – usually 1-10 molecules are considered as sufficient to perform a successful PCR. Referring to the results of a previous publication (15) the detection limits of free DNA in milk were found to be 10ng/ml (or 10pg/µl) by hybridization after preceding purification. However, for a continued monitoring of DNA- contents and physical stability over extended time periods only a more convenient and sensitive method without time consuming extractions procedures must be applicable.

Because the amount of target DNA accessible has been recognized as an important factor for reaction specificity, some authors (16) deal with the question of how many cycles are needed for a PCR. It is known from experience that the efficiency of a PCR amplification is 1.0 only in theory. In practice, amplifying intact and purified DNA the average amplification efficiency is close to 0.7 [$y = a (1+ F)^n$ with a = concentration of targets, F = efficiency of PCR amplification (0.0-1.0), n = number of amplification cycles, y = detection limit, 10ng]. If DNAs are investigated that are not purified at all and contain remnants of inhibiting substances the amplification efficiency is lowered remarkedly (to 0.35 or even less). This may be compensated for by applying increased cycle numbers – in general 30-35 cyles are used – what may be not sufficient for such purposes. Since the technique itself allows 60-70 or more cycles, we chose at least 50 cycles for the identification of yoghurt starters. Thus, it may well be that the detection sensitivity for DNA in chocolate milk can also be increased.

Components in milk are known to interfere with PCR. Powell et al. S(17) found that PCR was inhibited at some milk concentrations but not at others, when fresh milk was used. With UH-treated milk, either skimmed, semi-skimmed or full-fat, no inhibition of the PCR was apparent below 10 % milk contents in the PCR reaction mix – what it is in good accordance with our results. As a possible explanation for those findings it was supposed that a proteinase inhibiting the PCR by degrading the Taq-Polymerase was sensitive to UH-treatment. On the basis of the types of inhibitors that were active against that type of proteinase it was assumed that the protease was plasmin.

From our results it can be concluded that milk products like chocolate milk, which is commercially available as UHT-product, can be used without further purification in PCR assays, if detection sensitivity is considered as sufficient for the experimental purpose. The detection limits for unpurified DNA are certainly higher than for purified DNA in PCR, but losses during purification would also have to be considered.

Starter cultures from yoghurt can also be identified using the original product (containing all the fruit components) or a fresh skim milk culture (if an older product has to be examined) without any purification in PCR, provided that a sufficient number of the respective microorganisms were present. The higher target numbers have to compensate for the components interfering with the reaction. Because the milk is usually heated to 80-90°C before the starters are added, one can speculate that this procedure may inactivate the inhibitors mentioned above.

Acknowledgements

We like to thank Kirsten Zenz for some initial experiments and Annegret Laudy for typing the manuscript.

This work was supported in part by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (Grant No. 0311049).

5. References

(1) Schleifer, K.-H., Ehrmann, M., Beimfohr, C., Brockmann, E., Ludwig, W., Amann, W.: Int. Dairy J. **5** 1081-1094 (1995).
(2) Tilsala-Timisjärvi, A., Alatossava, T.: Int. J. Food Microbiol. **35** 49-56 (1997).
(3) Yamamoto, T., Morotomi, M., Tanaka, R.: Appl. Environ. Microbiol. **58** 4076-4079 (1992).
(4) Kaufmann, P., Pfefferkorn, A., Teuber, M., Meile, L.: Appl. Environ. Microbiol. **63** 1268-1273 (1997).
(5) Bräutigam, M., Hertel, C., Hammes, W.P.: FEMS microbiol. Lett. **155** 93-98 (1997).
(6) Zenz, K., Neve, H., Geis, A., Heller, K.J.: System. Appl. Microbiol. **21** 28-32 (1998)
(7) van de Guchte, M., Kodde, J., van der Vossen, J., Kok, J., Venema, G.: Appl. Environ. Microbiol. **56** 2606-2611 (1990).
(8) Krusch, U., Neve, H., Luschei, B., Teuber, M.: Kieler milchw. Forsch. Ber. **39** 155-165 (1987).
(9) Leenhouts, K.J., Kok, J., Venema, G.: Appl. Environ. Microbiol. **56** 2726-2735 (1990).
(10) Anderson, D.G., McKay, L.L.: Appl. Environ. Microbiol. **46** 549-552 (1983).
(11) Sambrook, J., Fritsch, E.F, Maniatis, T.: Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989)
(12) Lick, S., Keller, M., Bockelmann, W., Heller, K.J.: System. Appl. Microbiol. **19** 74-77 (1996).
(13) Lick, S., Heller, K.J.: Milchwissenschaft **53** 671-675 (1998).
(14) Barnes, W.M.: Proc. Nath. Acad. Sci. **91** 2216-2220 (1994)
(15) Casey, M., Frere, J., Novel, M.: Schweiz. Milchw. Forschung **21** 76-77 (1992)
(16) Rameckers, J., Hummel,. S., Herrmann, B.: Naturwissenschaften **84** 259-262 (1997).
(17) Powell, H.A., Gooding, C.M., Garrett, S.D., Lund, B.M., McKee, R.A.: Lett. Appl. Microbiol. **18** 59-61 (1994).

6. Summary

Lick, S., Schneede, K., Wittke, A., Heller, K.J.: **Detection without sample processing of yoghurt starters and DNA in milk products by PCR.** Kieler Milchwirtschaftliche Forschungsberichte **51** (1) 15-25 (1999)

26 PCR without sample processing (yoghurt, starter identification, free DNA in milk products)

The development of DNA-based methods (hybridization and PCR technology) for the specific and sensitive identification of microorganisms opened a new area in the field of food microbiology. However, suitable sample preparation procedures have at first to be developed for the single food matrix before these techniques can be applied in routine food analysis. We showed that yoghurt starters like *S. thermophilus* and *L.delbrueckii* ssp. can be identified using overnight cultures of the appropriate liquid media without DNA purification. For *S. thermophilus* it was demonstrated that different kinds of yoghurt (plain yoghurts or yoghurts containing fruits) could also be used in the PCR reaction mix to identify this starter without sample processing, provided that enough microorganisms were present (about10^4cfu/µl). In order to identify free DNA it could be shown for another milk product that 0.01µg DNA of a model plasmid pMG36enpr was detected in 1ml of chocolate milk when the sample was added without any further purification of the nucleic

acids. The use of different primer sets that addressed targets of increasing lengths (1.6-5.8 kb) made it possible to monitor the DNA contents of the product during, e.g. transformation experiments. As expected, the detection limits changed with primer design and decreased with increasing template lengths from 0.01 pg/µl (1.6 kb) down to 1.0 ng/µl (5.8 kb) of chocolate milk.

Zusammenfassung

Lick, S., Schneede, K., Wittke, A., Heller, K.J.: **Identifizierung von Starterkulturen und DNA mit PCR in Milchprodukten mit PCR ohne vorherige DNA-Preparation.** Kieler Milchwirtschaftliche Forschungsberichte **51** (1) 15-25 (1999)

26 PCR ohne DNA-Preparation (Joghurt, Identifizierung von Starterkulturen mit PCR, Detection von freier DNA in Milchprodukten)

Die Entwicklung von molekularbiologischen Methoden (insbesondere von Hybridisierung und PCR) zur spezifischen und sensitiven Identifizierung von Mikroorganismen eröffnet neue Perspektiven in der Lebensmittelmikrobiologie. Jedoch werden zunächst geeignete Methoden zur Aufarbeitung der einzelnen Lebensmittelmatrices benötigt, bevor diese Technologien weitere Verbreitung in der Lebensmittelroutine finden können. Wir zeigen hier, daß eine Artidentifizierung der Gram-positiven Joghurtstarter *Streptococcus thermophilus* und *Lactobacillus delbrueckii* subsp. *bulgaricus* mit Hilfe spezies-spezifischer PCR möglich ist - ohne die DNA zu extrahieren - wenn nur die Kultursuspension (Übernachtkultur) direkt dem PCR-Reaktionsgemisch beigefügt wird. Am Beispiel *S. thermophilus* wird demonstriert, daß sich sogar mit dem ungereinigten Produkt Joghurt selbst (Naturjoghurt oder Joghurt mit diversen Früchten) spezies-spezifische Amplifikate erhalten lassen, wenn eine genügend hohe Anzahl von Mikroorganismen (etwa 10^4 cfu/µl) vorhanden sind. Für die Detektion freier DNA in einem anderen Milchprodukt (Kakao) konnte - für das ungereinigte Produkt - eine Nachweisgrenze von 0.01 pg/µl ermittelt werden. Die Nachweisgrenzen hängen aber erwartungsgemäß stark vom Primerdesign und von der Länge des Amplifikates ab: für ein 1.6 kb Fragment: 0.01 pg/µl, im Gegensatz dazu für ein 5.8 kb Fragment: 1.0 ng/µl.

Résumé

Lick, S., Schneede, K., Wittke, A., Heller, K.J.: **Identification par technique de PCR de levains et d'ADN dans des produits laitiers sans préparation préalable des ADN.** Kieler Milchwirtschaftliche Forschungsberichte **51** (1) 15-25 (1999)

26 Technique de PCR sans traitement préalable des échantillons (yaourt, identification de levains, d'ADN libéré dans des produits laitiers)

Par le développement de méthodes sur base d'ADN (hybridisation et technologie de PCR) pour l'identification spécifique et sensible de microorganismes, une nouvelle époque dans la recherche de la microbiologie alimentaire a été entamée. Cependant, des méthodes adéquates de préparation d'échantillons doivent tout d'abord être développées pour la matrice alimentaire individuelle avant que ces techniques puissent être utilisées pour des analyses de routine des aliments. Nous avons pu démontrer que des cultures de levain de yaourt comme *S. thermophilus* et *L.delbrueckii* ssp. peuvent

être identifiées à l'aide de la méthode PCR tout en n'utilisant que leur culture de suspension sans en avoir extrait l'ADN. Pour *S. thermophilus,* on a pu démontrer par l'utilisation directe dans la réaction PCR, qu'il est possible d'identifier le levain dans du yaourt (yaourt au lait entier ou aux fruits), sans en avoir extrait préalablement l'ADN, sous condition qu'il y ait un nombre suffisant de microorganismes (env. 10^4 cfu/µl). Lors de l'identification d'ADN libéré avec la méthode PCR, on a pu démontrer que 0,01µg ADN peuvent déjà être identifiées sans purification préalable des acides nucléiques. Tout en utilisant des combinaisons primaires différentes s'adressant à des séquences de 1,6-5,8 kb, il a été possible de déterminer et de retenir pendant un essai de transformation la teneur en ADN d'un produit laitier (cacao) pour une période plus longue. Comme on s'y attendait, les limites de détection sont étroitement liées à la combinaison primaire et à la longueur de l'ADN à amplifier (baisse de 0,01pg/µl (1,6 kb) et de 1,0 ng/µl (5,8 kb)).