Improved detection of Salmonella through the optimized use of DNA polymerases in real-time PCR

Löfström, Charlotta; Fachmann, Mette Sofie Rousing; Josefsen, Mathilde Hasseldam; Al-Habib, Zahra Fares Sayer; Hoorfar, Jeffrey

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Improved detection of *Salmonella* through the optimized use of DNA polymerases in real-time PCR

Charlotta Löfström, Mette S.R. Søndergaard, Mathilde H. Josefsen, Zahra Fares Al-Habib, Jeffrey Hoorfar

National Food Institute, Technical University of Denmark, Søborg. E-mail: chalo@food.dtu.dk

Aim

To investigate the performance of different DNA polymerases in real-time PCR and their resistance towards inhibitors in a food model system. As a model detection of *Salmonella* in meat samples with a validated real-time PCR method was used.

Introduction

To improve food safety it is important to pursue fast, well performing and low-cost methods for detection of foodborne pathogens. Though real-time PCR offers several advantages compared with classical microbiology, the choice of a suitable DNA polymerase has been shown to optimize method performance considerably[1].

Screening:

- Standardized *Salmonella* qPCR assay[2]
- Detecting a 10-fold dilution series of purified DNA
- 13 DNA polymerases and 2 DNA polymerases in kits were included

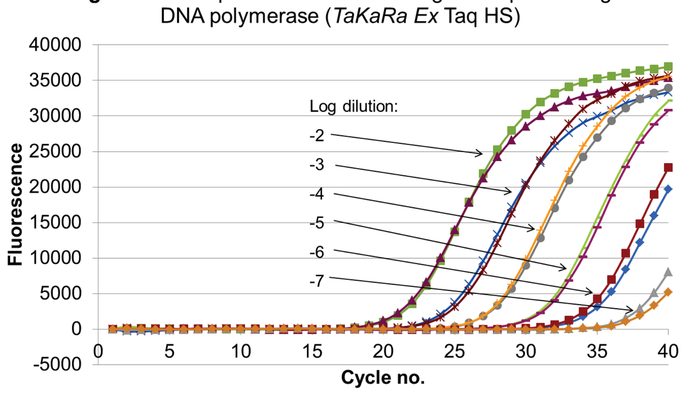
Conclusions

- The performances of the tested DNA polymerases varied considerably, reinforcing the importance of careful selection of an appropriate DNA polymerase for the PCR assay and sample type in question.
- Under the tested conditions for detecting *Salmonella* in minced meat samples, VeriQuest and *TaKaRa Ex* Taq HS were found to be the two best performing alternatives DNA polymerases.
- The experiments will be repeated using another target bacterium and sample type to assess the generality of the results.

Good performance:

- AmpliTaq 360
- FastStart Taq
- HotMaster *Taq*
- HotStarTaq Master Mix Kit
- *TaKaRa Ex* Taq HS
- Tth
- VeriQuest Probe qPCR Master Mix

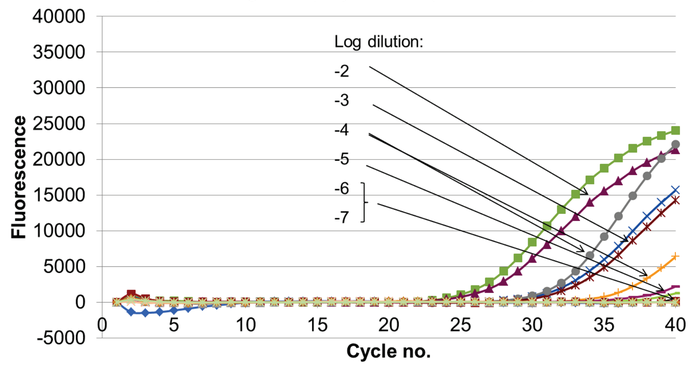
Figure 1. Example of PCR results using a well performing DNA polymerase (*TaKaRa Ex* Taq HS)



Intermediate performance:

- MyTaq
- MyTaq HS
- Phusion High-Fidelity
- PicoMaxx High Fidelity PCR System

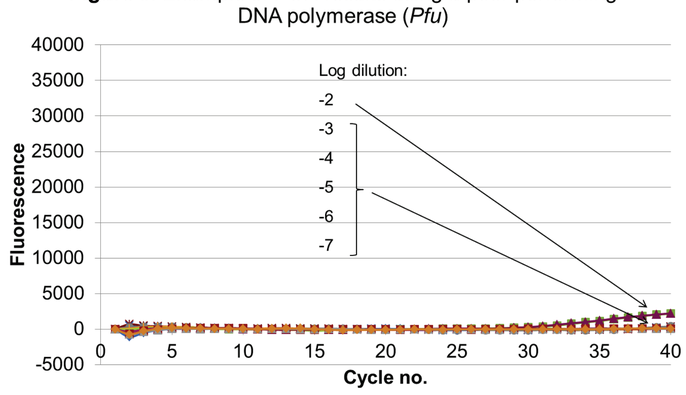
Figure 2. Example of PCR results using an intermediate performing DNA polymerase (PicoMaxx)



Bad performance:

- Herculase II Fusion
- One*Taq*
- *Pfu*
- TITANIUM Taq

Figure 3. Example of PCR results using a poor performing DNA polymerase (*Pfu*)



Further evaluation:

- 5 selected well performing DNA polymerases (Table 1)
- Standardized *Salmonella* qPCR assay[2]
- Minced meat samples artificially contaminated with *Salmonella* (10^1-10^6 CFU/ml) analyzed in triplicate after DNA extraction
- Ct values, amplification efficiency (AE), linear range and linearity of standard curve (R^2) were calculated

Table 1. Results from qPCR using different DNA polymerases when analysing minced meat samples enriched in BPW for 18 h followed by artificial contamination with *Salmonella*.

DNA polymerase	Ct values (mean of triplicates[a]) from qPCR for indicated no. of *Salmonella* (CFU/ml)						AE	R^2	Linear range (CFU/ml)
	10^1	10^2	10^3	10^4	10^5	10^6			
Tth	ND[b]	ND	35.4	32.6	27.3	25.4	0.99	0.909	10^3-10^6
VeriQuest Probe qPCR Master Mix	ND	39.3[c]	33.5	32.1	27.8	25.6	1.10	0.953	10^3-10^6
FastStart Taq	ND	ND	36.3	35.7	29.5	29.3	1.45	0.874	10^4-10^5
*TaKaRa Ex*Taq HS	ND	ND	36.6	34.9	29.3	27.3	0.97	0.929	10^3-10^6
AmpliTaq 360	ND	ND	35.4	32.0	27.3	25.5	0.94	0.842	10^4-10^5

[a] Standard deviation 0.1-1.9 in the trial for the different triplicate analyses
[b] ND: Not Detected
[c] Detection in 1/3 replicates

Materials and Methods

13 commercially available DNA polymerases and 2 polymerases in kits were included:

- AmpliTaq 360 (Applied Biosystems)
- FastStart Taq (Roche)
- Herculase II Fusion (Stratagene)
- HotMaster Taq (5 PRIME)
- HotStarTaq Master Mix Kit (QIAGEN)
- MyTaq (Bioline)
- MyTaq HS (Bioline)
- OneTaq (New England BioLabs)
- Pfu (Fermentas)
- Phusion High-Fidelity (New England BioLabs)
- PicoMaxx High Fidelity PCR System (Agilent Technologies)
- TaKaRa Ex Taq HS (TaKaRa Bio)
- TITANIUM Taq (Clontech)
- Tth (Roche)
- VeriQuest Probe qPCR Master Mix (Affymetrix)

These were evaluated on a dilution series of purified *Salmonella* ser. Typhimurium DNA analyzed by a validated standardized real-time PCR assay[2] using the accompanying PCR buffers for each polymerase.

The 5 best performing polymerases/kits were further evaluated using minced meat samples diluted in BPW 1:10 and enriched for 18 h at 37°C followed by artificial contaminated with *Salmonella* ser. Typhimurium. DNA extraction was performed on the samples followed by real-time PCR.

References

1. Hedman et al (2012) Pre-PCR processing strategies. In: PCR Technologies, current innovations CRC Press, 3rd Ed.
2. Löfström et al (2009) BMC Microbiol, 9(1):85.

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