
Detection of *Brucella* From Cheese by Polymerase Chain Reaction Test

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ABSTRACT

Brucellosis is an endemic zoonosis in Syria, affecting large numbers of animals and an increasing number of humans. *Brucella* is an intracellular pathogen capable of infecting animals and humans. The aim of this study was to develop a polymerase chain reaction (PCR) protocol for the detection of *Brucella melitensis* in artificially contaminated cheese. In order to determine the detection limits tests were performed by inoculating bovine milk samples with different dilutions of bacteria cells (10^1 - 10^7 CFU/ml). The assay was able to detect as low as 10^1 CFU/ml of initial dilution 48 hours after cheese inoculation, including 24 hours of pre-enrichment by using a filter 0.45 μ m for DNA extraction protocol. In conclusion, this new technique was faster and more reliable to detect the presence of *B. melitensis* in artificially contaminated cheese, comparing with conventional microbiological methods.

Key words: *Brucella melitensis* 16M, Cheese, Polymerase chains reaction (PCR).

Brucellaceae

(Moreno *et al.*, 2002) *Pasteurella* *Bordetella*
G+C DNA-DNA

(Moreno *et al.*, 2002)

CO₂) (

() () :
()
(Ficht, 2010)

0.7-0.5

1.5-0.5

(OIE, 2004)

CO₂
WHO,)

(2004

(Boschioli *et al.*, 2001)

(FAO, 1998)

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()
(Corbel, 1997)

(Georgios *et al.*, 2005)

:

(Corbel, 1997)

Mukherjee *et al.*,) PCR DNA .(2007

.Ziehl-Neelsen Stamp ()
Chlamydia psittaci *Brucella ovis* :
(Marianelli *et al.*, 2008) *Coxiella burnetii*

(Al-Mariri and Haj-Mahmoud, 2010)
DNA PCR

(OIE, 2000; Lin *et al.*, 2011)
PCR

DanK L7/L12 IS6501 IS711 :
(DelVecchio *et al.*, 2002) Omp2s 16sRNA BCSP31

:(Alton *et al.*, 1988)

2YT : -1
5 10 10 :
: 37
5000 B 25000 (100)
(5) (20)
100000) 2YT
°56 (15
%5
)
(

2YT 7.5 μ l 100
 37
 100 10 2YT
 37 2YT
 /CFU $10^7 - 10^1$
) O:9 (*Yersinia enterocolitica*)
 : **DNA** -3
 1 () 1 1
 . μ m 0.45
 μ l 200 0.5
 (Triton X-100, 10 mM Tris, 1 mM EDTA%0.25)
 10 μ l 10 °95
 .PCR
 :
 4000 rpm 5 •
 30 TE 567 μ l 4 •
 .20 mg/ml proteinase K 3 μ l 10% SDS μ l •
 .1000 rpm 37
 80 μ l 5ml NaCl 100 μ l •
 65 10 .CTAB/NaCl •
 .1000 rpm
 (24:1) chloroform/isoamyl alcohol 780 μ l •
 14500 rpm 5
 phenol/chloroform/isoamyl alcohol •
 14500 rpm 5 (25:24:1)

0.6 DNA 6-4 •
 14500 rpm 5
 .DNA
 14500 5 %70 1 •
) Concentrator rpm (Eppendorf)
 TE 25 µl •
 .100 ng/µl (Nano Drop)
.PCR -4
 (BCSP31)
 31 223
 DNA PCR
 .(1)

(1)

BCSP31 -F	5'- ACGCAGTCAGACGTTGCCTAT -3'	21	223 bp
BCSP31 -R	5- TCCAGCGCACCATCTTTCAGCCTC -3	24	

(2) 25µl

(3)

.PCR (2)

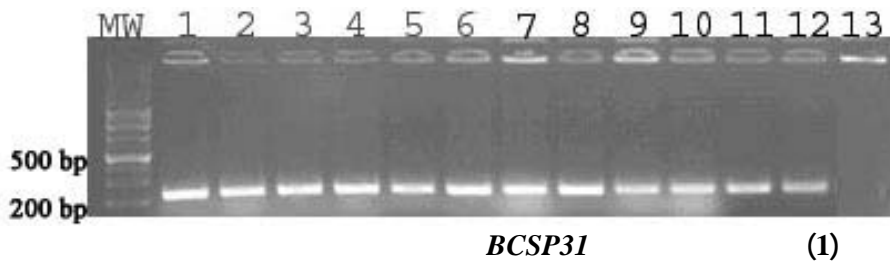
Materials	Final conc. µl	25 µl PCR
Genomic DNA	200-500 ng	2 µl DNA (100ng)
Primer 1 -10 µM	20 µM	2 µl
Primer 2-10 µM	20 µM	
dNTPs 20 mM	0.4 mM	0.5 µl
Buffer 10X	1X	2.5 µl
MgSO ₄ 50 mM	3 mM	1.5 µl
Taq 5U	2 U	0.2 µl
H ₂ O	----	16.3 µl

.PCR (3)

		Temperature	Time
35 Cycle	Initial denaturation	95°C	5 mins
	Denaturation	95°C	1 min
	Annealing	55°C	45 sec
	Extension	72°C	1.5 min
	Final Extension	72°C	10 mins

(W/V) %1.2 PCR
 1 70V 1X TAE

CFU/ml 10^7-10^1 (1)
 1 .(6-5) BCSP31
 0.45 (6-1)
 10µl PCR
 (8) (12-9)
 PCR .BCSP31
 CFU 10^1 /ml



:1 (4-1) :6-1
 :4 10^5 CFU/ml :3 10^4 CFU/ml :2 10^3 CFU/ml
 :6 10^5 CFU/ml :5 (6-5) 10^7 CFU/ml
 10^7 CFU/ml

:7
 0.45
 :8
 (.)
 :12-9
 :9
 .10¹ CFU/ml
 .10⁷ CFU/ml :10
 :10⁷ CFU/ml :12 10¹ CFU/ml :11
 10⁷ CFU/ml :13
 :MW .O:9
 .(Fermentase)

.PCR

BCSP31
Serpe

OMP2

PCR .(Serpe *et al.*, 1999)

%100

%100 %96.7 (2009) Di Giannatale

PCR

Serpe *et al.*, 1999;) %100-%98

Leal-Klevezas *et al.*, 1995, Al-Mariri and Haj-Mahmoud, 2010;

PCR .(Tantillo *et al.*, 2003

Vizcaino *et al.*, 2000; Amin *et al.*, 2001;)

.(Leyla *et al.*, 2003; O'Leary *et al.*, 2006; Mukherjee *et al.*, 2007

Khan *et al.*, 2001;)

.(Ongör *et al.*, 2006; El Miedany *et al.*, 2003

Tantillo *et al.*, 2001; Al Mariri)

.(and Haj Mahmoudm 2010; Bricker, 2002

Cortez *et al.*,)

Sreevatsan *et al.*,)

(Guarino *et al.*, 2000)

(2001

.(2000

PCR

Serpe *et al.*, 1999; Tantillo *et al.*, 2001; 2003; Ongör)

(*et al.*, 2006

).(2008)

.(Garin-Bastuji, 1993)

(...)

(2008)

(Georgios *et al.*, 2005)

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.(Al Mariri and Haj Mahmoudm 2010)

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