Copper Tolerance of *Listeria monocytogenes* strain DRDC8

Francesca Y Bell, B. Biotech. (Hons.) (Adelaide)

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School of Molecular and Biomedical Science
Faculty of Sciences,
The University of Adelaide
Adelaide, South Australia, Australia

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Figure 4.9: Southern hybridisation analysis of pCT0018::*erm* strain DSE951.

Panel A. *Dra*I digested DNA prepared from *L. monocytogenes* strain DSE951 was probed with a digoxigenin-labelled 645 bp *erm* fragment. Probe DNA hybridised to two DSE951 *Dra*I DNA fragments 3456 bp (**a**) and 924 bp (**b**) in size. Labelled probe also hybridised to *Dra*I DNA fragments from positive control strains DSE201 (1014 bp and 3347 bp) and pKS951 co-integrate (*ca*. 924 bp and 2765 bp), and from plasmid pKS951 (*ca*. 924 bp and 2765 bp). Probe DNA also hybridised to the 1136 bp *Bam*HI *erm* fragment of plasmid pCT800. Labelled probe did not hybridise with DNA from the DRDC8Sm* negative control.

Panel B. *Eco*RV digested DSE951 DNA was probed with a digoxigenin-labelled 645 bp fragment of *erm*. Probe DNA hybridised to an *Eco*RV DSE951 DNA fragment 7273 bp (**c**) in size. Labelled probe also hybridised to *Eco*RV DNA fragments for positive control strains DSE201 (7273 bp) and pKS951 co-integrate (*ca*. 9039 bp), and from plasmid pKS951 (*ca*. 9039 bp). Probe DNA also hybridised to the 1136 bp *Bam*HI *erm* fragment of plasmid pCT800. Labelled probe did not hybridise with DNA from the DRDC8Sm* negative control.

Panel C. Diagrammatic representation of the theoretical *erm* insertion in ORF pCT0018 in strain DSE951. The relative position of all *Dra*I and *Eco*RV restriction sites are indicated and numbered accordingly. The *Bam*HI sites that flank the *erm* insertion are also indicated. The *Dra*I (**a** and **b**) *Eco*RV (**c**) DNA fragments that *erm*-specific probe DNA hybridised to is shown.

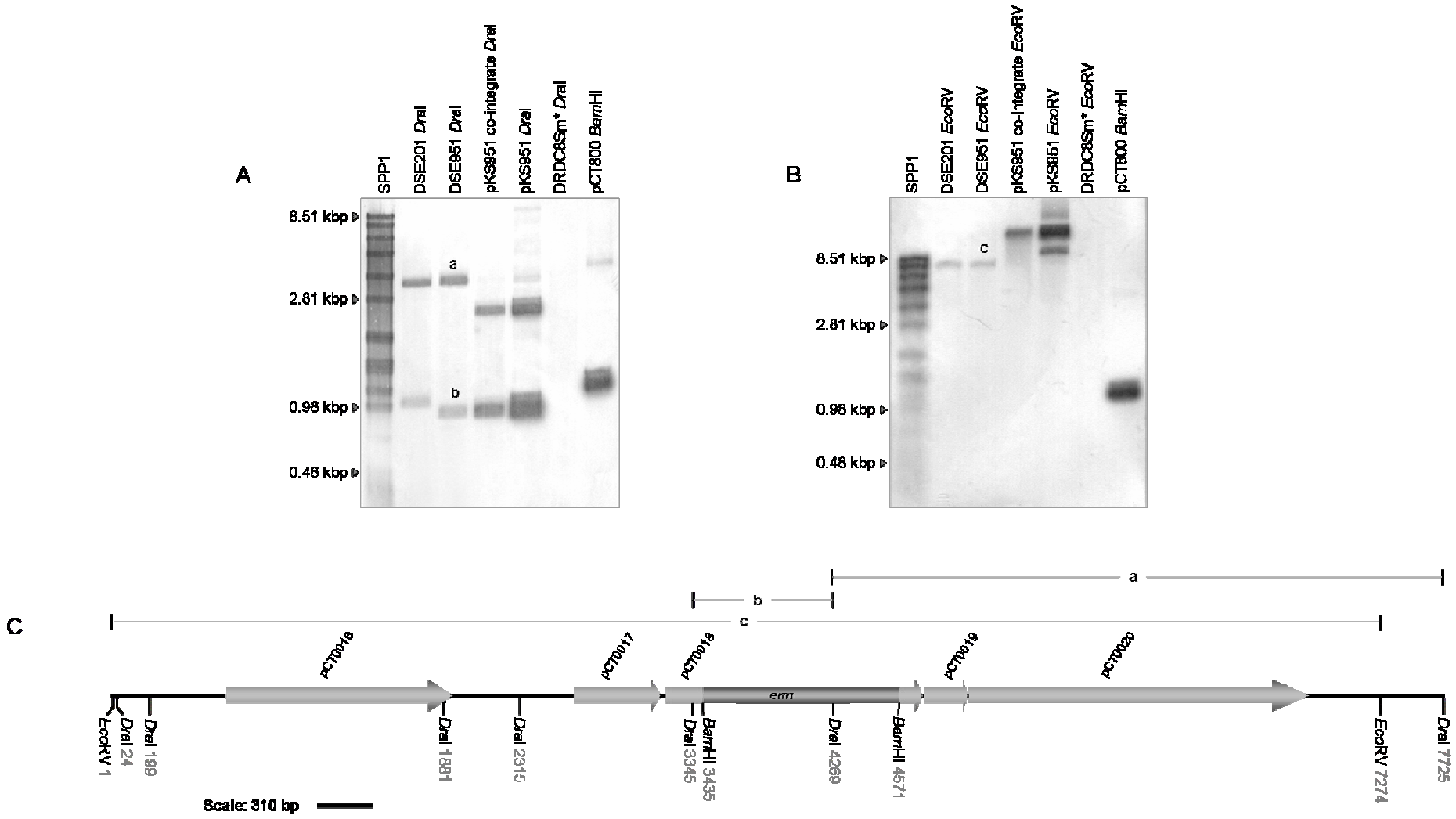
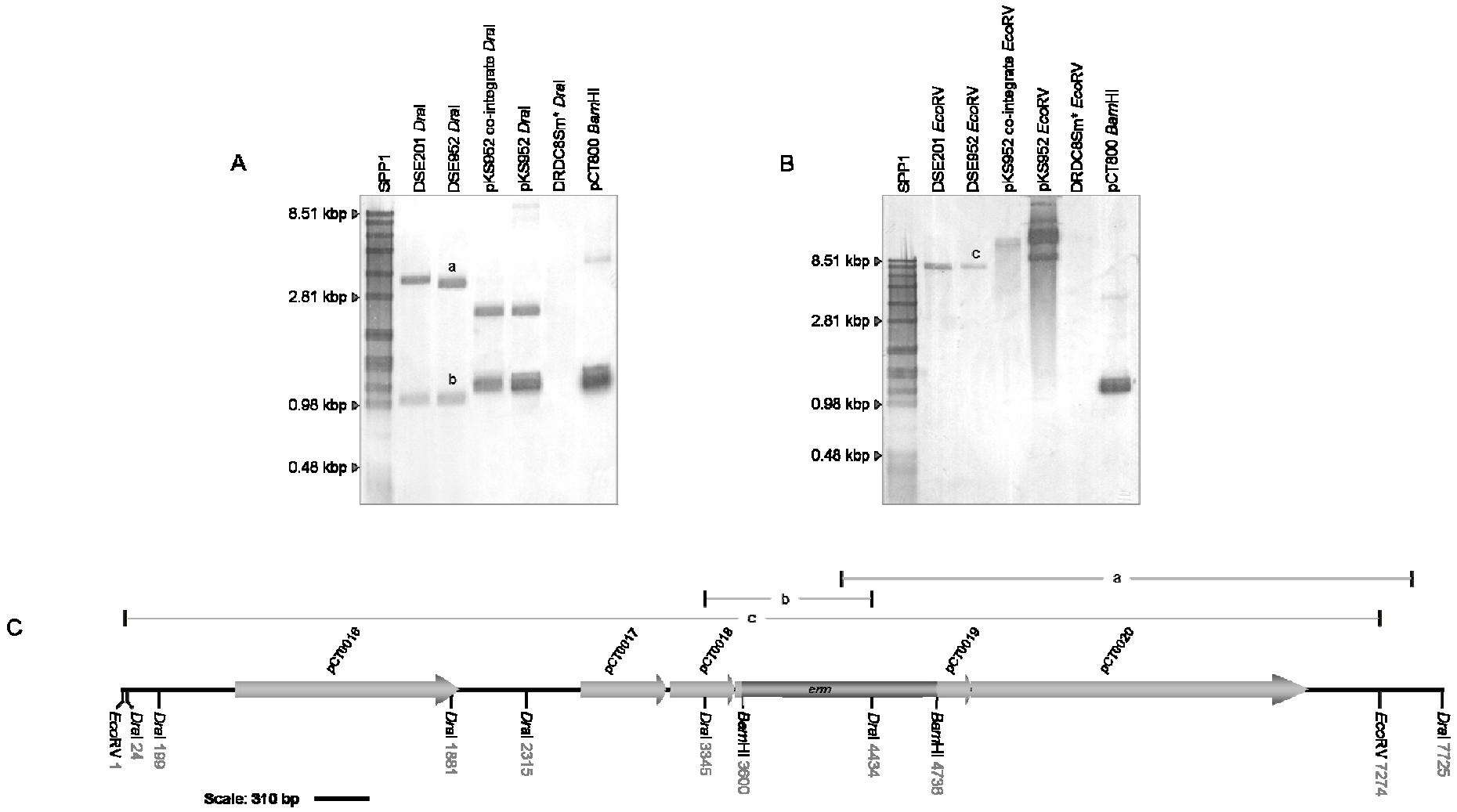

A
SPP1
DSE201 DraI
DSE951 DraI
pKS951 co-integrate DraI
pKS951 DraI
DRDC8Sm* DraI
pCT800 BamHI
8.51 kbp
2.81 kbp
0.98 kbp
0.48 kbp
a
b
B
SPP1
DSE201 EcoRV
DSE951 EcoRV
pKS951 co-integrate EcoRV
pKS851 EcoRV
DRDC8Sm* EcoRV
pCT800 BamHI
8.51 kbp
2.81 kbp
0.98 kbp
0.48 kbp
c
C
a
b
c
pCT0016
pCT0017
pCT0018
ermA
pCT0019
pCT0020
EcoRV 1
DraI 24
DraI 199
DraI 1881
DraI 2315
DraI 3345
BamHI 3435
DraI 4269
BamHI 4571
EcoRV 7274
DraI 7725
Scale: 310 bp

Figure 4.10: Southern hybridisation analysis of pCT0019::*erm* strain DSE952.

Panel A. *Dra*I digested DNA prepared from *L. monocytogenes* strain DSE952 was probed with a digoxigenin-labelled 645 bp *erm* fragment. Probe DNA hybridised to two DSE952 *Dra*I DNA fragments 3291 (**a**) and 1089 bp (**b**) in size. Labelled probe also hybridised to *Dra*I DNA fragments from positive control strains DSE201 (1014 bp and 3347 bp) and pKS952 co-integrate (*ca*. 1089 bp and 2599 bp), and from plasmid pKS952 (*ca*. 1089 bp and 2599 bp). Probe DNA also hybridised to the 1136 bp *Bam*HI *erm* fragment of plasmid pCT800. Labelled probe did not hybridise with DNA from the DRDC8Sm* negative control.

Panel B. *Eco*RV digested DSE952 DNA was probed with a digoxigenin-labelled 645 bp fragment of *erm*. Probe DNA hybridised to an *Eco*RV DSE952 DNA fragment 7273 bp (**c**) in size. Labelled probe also hybridised to *Eco*RV DNA fragments for positive control strains DSE201 (7273 bp) and pKS952 co-integrate (*ca*. 9039 bp), and from plasmid pKS952 (*ca*. 9039 bp). Probe DNA also hybridised to the 1136 bp *Bam*HI *erm* fragment of plasmid pCT800. Labelled probe did not hybridise with DNA from the DRDC8Sm* negative control.

Panel C. Diagrammatic representation of the theoretical *erm* insertion in ORF pCT0019 in strain DSE952. The relative position of all *Dra*I and *Eco*RV restriction sites are indicated and numbered accordingly. The *Bam*HI sites that flank the *erm* insertion are also indicated. The *Dra*I (**a** and **b**) *Eco*RV (**c**) DNA fragments that *erm*-specific probe DNA hybridised to is shown.


A
SPP1
DSE201 DraI
DSE952 DraI
pKS952 co-integrate DraI
pKS952 DraI
DRDC8Sm* DraI
pCT800 BamHI
8.51 kbp
2.81 kbp
0.98 kbp
0.48 kbp
a
b
B
SPP1
DSE201 EcoRV
DSE952 EcoRV
pKS952 co-integrate EcoRV
pKS952 EcoRV
DRDC8Sm* EcoRV
pCT800 BamHI
8.51 kbp
2.81 kbp
0.98 kbp
0.48 kbp
c
C
a
b
c
pCT0016
pCT0017
pCT0018
ermB
pCT0019
pCT0020
EcoRV 1
DraI 24
DraI 189
DraI 1881
DraI 2315
DraI 3345
BamHI 3600
DraI 4434
BamHI 4738
EcoRV 7274
DraI 7725
Scale: 310 bp

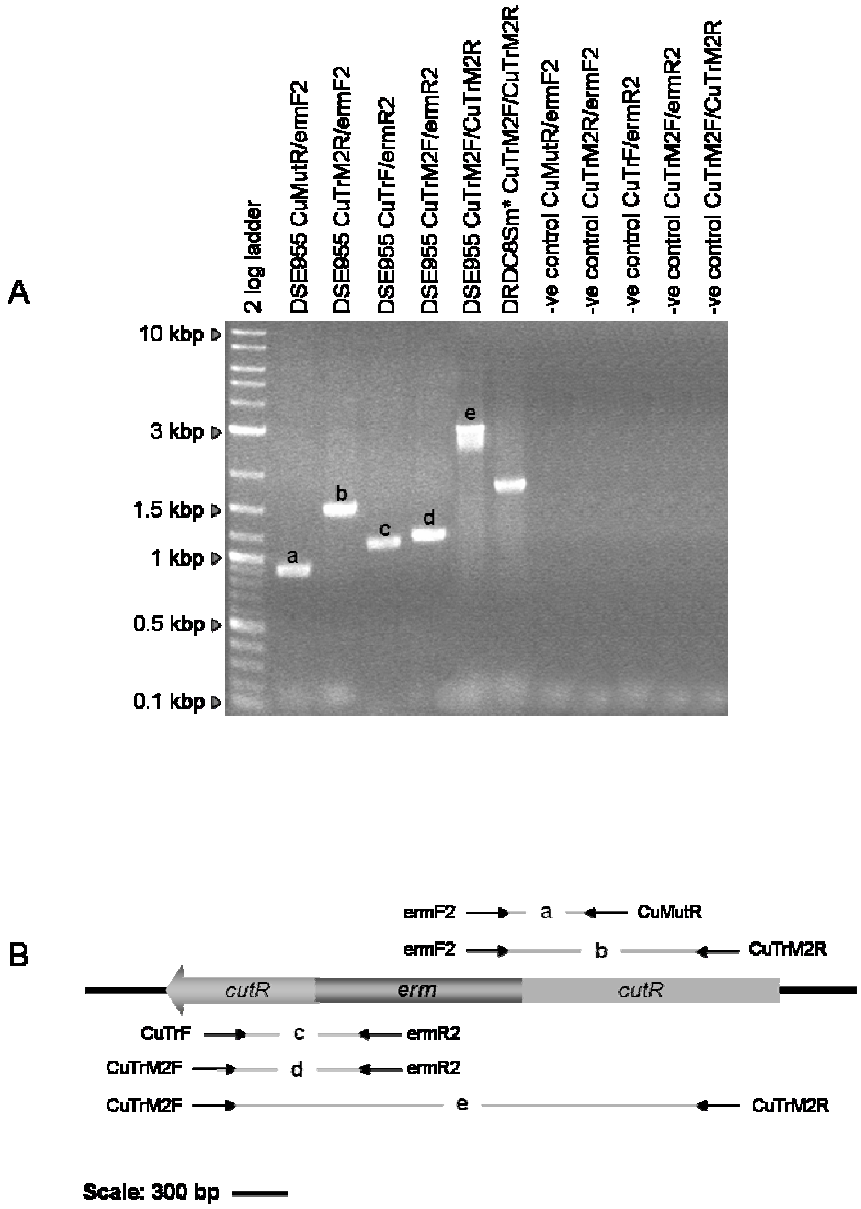


Figure 4.11: PCR analysis of the putative *cutR*::*erm* mutant strain DSE955.

Panel A. Products **a** (916 bp), **b** (1502 bp), **c** (1108 bp), **d** (1155 bp), and **e** (3019 bp) were amplified by PCR from DNA extracted from DSE955 using the CuMutR/ermF2, CuTrM2R/ermF2, CuTrF/ermR2, CuTrM2F/ermR2, and CuTrM2F/CuTrM2R oligonucleotide pairs, respectively. A 1883 bp product was amplified from the positive control strain DRDC8Sm* using the CuTrM2F/CuTrM2R oligonucleotide pair. Amplicons were not produced from the no DNA negative (-ve) controls.

Panel B. Diagrammatic representation of the theoretical *erm* insertion in *cutR* in strain DSE955. The relative position of oligonucleotide pairs specifically designed to amplify corresponding DNA fragments (products **a** - **e**) within this region are shown. The respective nucleotide sequence for each oligonucleotide is listed in Table 2.7.

Figure 4.12: Southern hybridisation analysis of *cutR*::*erm* strain DSE955.

Panel A. *Dra*I digested DNA prepared from *L. monocytogenes* strain DSE955 was probed with a digoxigenin-labelled 645 bp *erm* fragment. Probe DNA hybridised to two DSE955 *Dra*I DNA fragments 1266 bp (**a**) and 1085 bp (**b**) in size. Labelled probe hybridised to *Dra*I DNA fragments from positive control strains DSE201 (1014 bp and 3347 bp) and pKS955 co-integrate (*ca*. 1266 bp and 1862 bp), and plasmid pKS955 (*ca*. 1266 bp and 1862 bp). Probe DNA also hybridised to the 1136 bp *Bam*HI *erm* fragment of plasmid pCT800. Labelled probe did not hybridise with DNA from the DRDC8Sm* negative control.

Panel B. *Eco*RV digested DSE955 DNA was probed with a digoxigenin-labelled 645 bp fragment of *erm*. Probe DNA hybridised to an *Eco*RV DSE955 DNA fragment 7134 bp (**c**) in size. Labelled probe hybridised to *Eco*RV DNA fragments for positive control strains DSE201 7273 bp and pKS955 co-integrate (*ca*. 8866 bp), and plasmid pKS955 (*ca*. 8866 bp). Probe DNA also hybridised to the 1136 bp *Bam*HI *erm* fragment of plasmid pCT800. Labelled probe did not hybridise with DNA from the DRDC8Sm* negative control.

Panel C. Diagrammatic representation of the theoretical *erm* insertion in *cutR* in strain DSE955. The relative position of all *Dra*I and *Eco*RV restriction sites are indicated and numbered accordingly. The *Bam*HI sites that flank the *erm* insertion are also indicated. The *Dra*I (**a** and **b**) *Eco*RV (**c**) DNA fragments that *erm*-specific probe DNA hybridised to is shown.

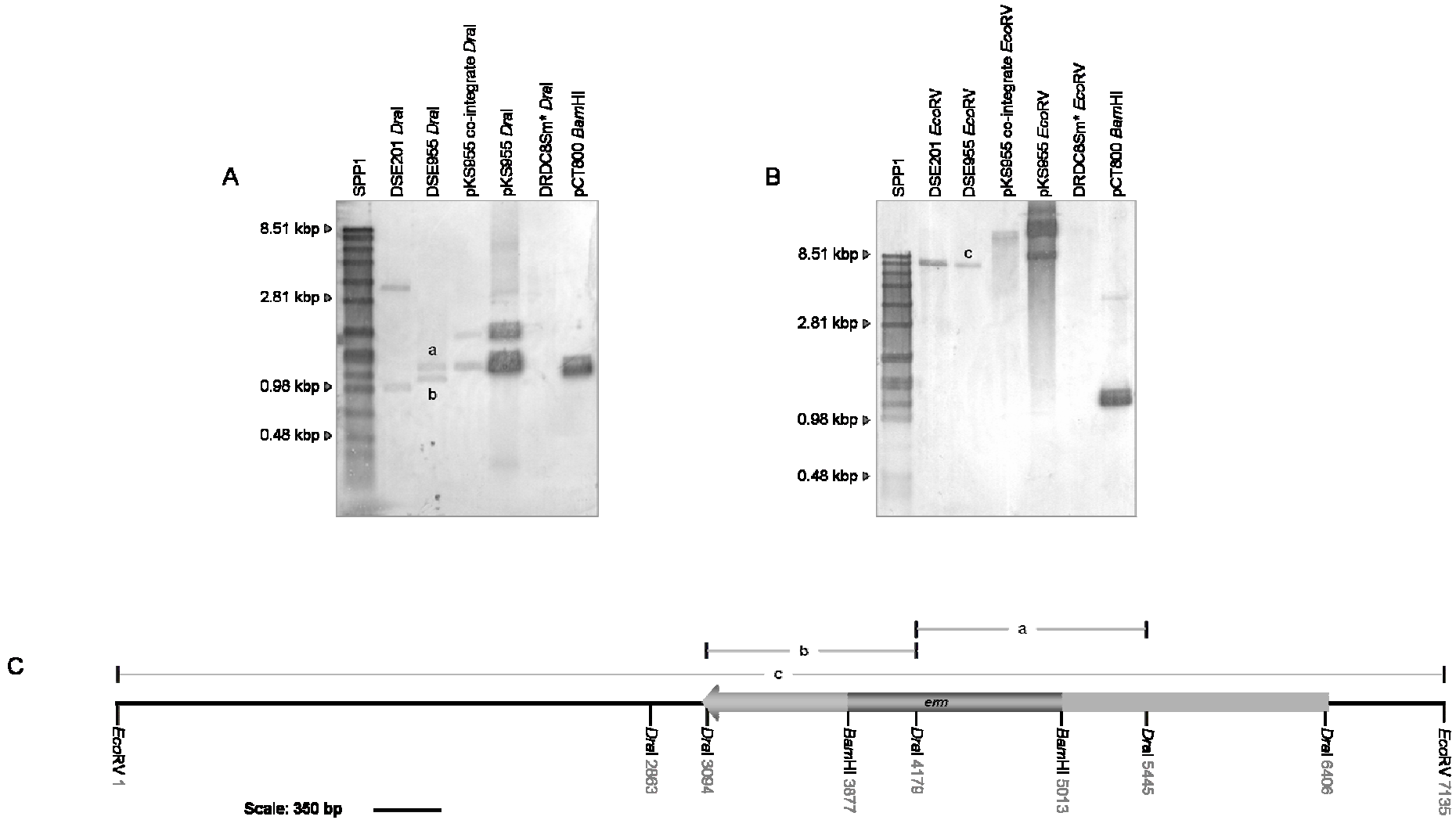
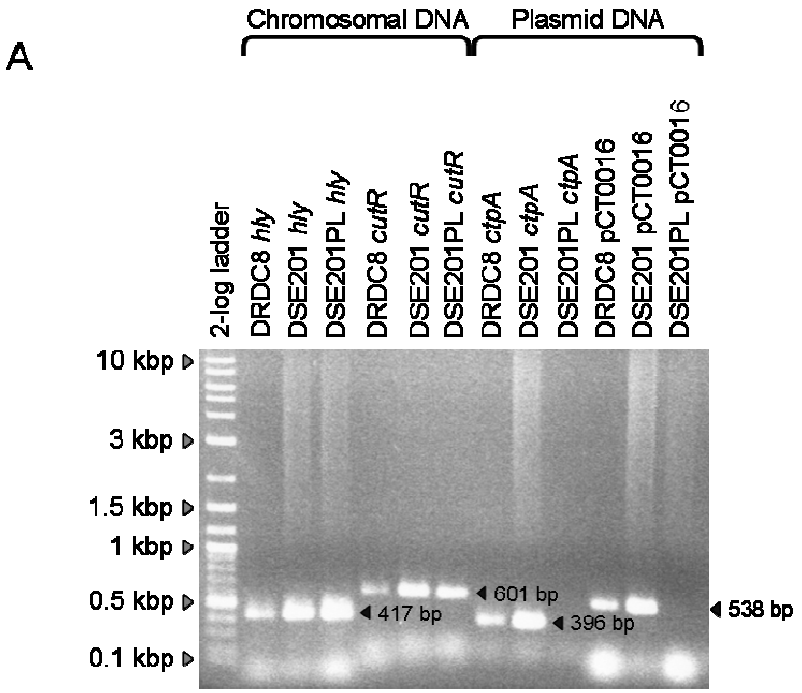
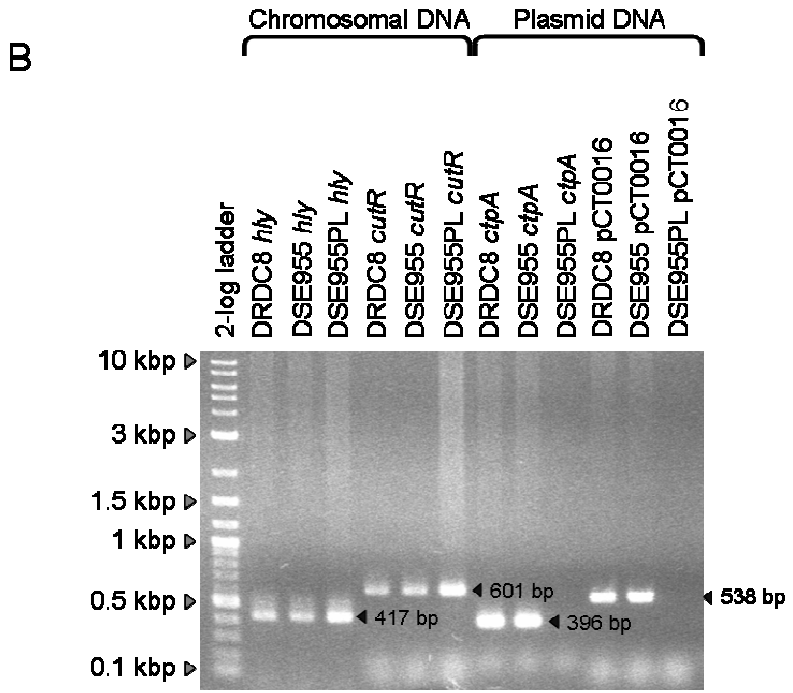

A
SPP1
DSE201 DraI
DSE955 DraI
pKS955 co-integrate DraI
pKS955 DraI
DRDC8Sm* DraI
pCT800 BamHI
8.51 kbp
2.81 kbp
0.98 kbp
0.48 kbp
a
b
B
SPP1
DSE201 EcoRV
DSE955 EcoRV
pKS955 co-integrate EcoRV
pKS955 EcoRV
DRDC8Sm* EcoRV
pCT800 BamHI
8.51 kbp
2.81 kbp
0.98 kbp
0.48 kbp
c
C
a
b
c
em
EcoRV 1
DraI 2863
DraI 3094
BamHI 3877
DraI 4179
BamHI 5013
DraI 5445
DraI 6406
EcoRV 7135
Scale: 350 bp

Figure 4.13: PCR analysis of plasmid-cured strains DSE201PL and DSE955PL.

Panel A. Amplicons indicative of chromosomal DNA markers, *hly* (417 bp) and *cutR* (601 bp), were produced from DSE201PL DNA using the p234/p319 and CuTrF/CuTrR oligonucleotide pair, respectively. *hly* and *cutR* amplicons were also produced from DNA prepared from the positive control strains DRDC8 and DSE201. Amplicons indicative of plasmid DNA markers, *ctpA* (396 bp) and pCT0016 (538 bp), were also produced from DRDC8 and DSE201 DNA using the FB001/LM2004 and FB1864/S12210R oligonucleotide pairs, respectively. Amplicons were not produced from DSE201PL DNA using the FB001/LM2004 and FB1864/S12210R oligonucleotide pairs.

Panel B. Amplicons indicative of chromosomal DNA markers, *hly* (417 bp) and *cutR* (601 bp), were produced from DSE955PL DNA using the p234/p319 and CuTrF/CuTrR oligonucleotide pair, respectively. *hly* and *cutR* amplicons were also produced from DNA prepared from the positive control strains DRDC8 and DSE201. Amplicons indicative of plasmid DNA markers, *ctpA* (396 bp) and pCT0016 (538 bp), were also produced from DRDC8 and DSE201 DNA using the FB001/LM2004 and FB1864/S12210R oligonucleotide pairs, respectively. Amplicons were not produced from DSE955PL DNA using the FB001/LM2004 and FB1864/S12210R oligonucleotide pairs.


A
Chromosomal DNA
Plasmid DNA
2-log ladder
DRDC8 hly
DSE201 hly
DSE201PL hly
DRDC8 cutR
DSE201 cutR
DSE201PL cutR
DRDC8 ctpA
DSE201 ctpA
DSE201PL ctpA
DRDC8 pCT0016
DSE201 pCT0016
DSE201PL pCT0016
10 kbp
3 kbp
1.5 kbp
1 kbp
0.5 kbp
0.1 kbp
601 bp
417 bp
396 bp
538 bp


B
Chromosomal DNA
Plasmid DNA
2-log ladder
DRDC8 hly
DSE955 hly
DSE955PL hly
DRDC8 cutR
DSE955 cutR
DSE955PL cutR
DRDC8 ctpA
DSE955 ctpA
DSE955PL ctpA
DRDC8 pCT0016
DSE955 pCT0016
DSE955PL pCT0016
10 kbp
3 kbp
1.5 kbp
1 kbp
0.5 kbp
0.1 kbp
601 bp
417 bp
396 bp
538 bp

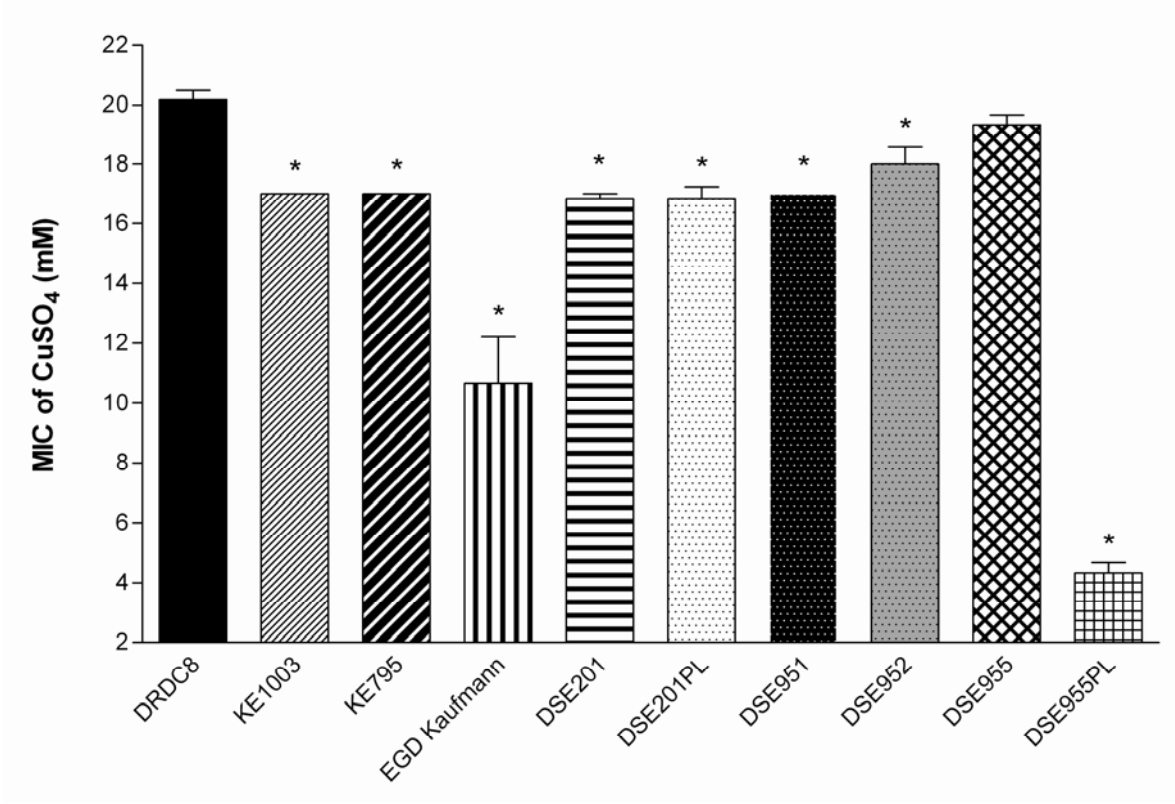


Figure 4.14: MIC of $CuSO_4$ for *L. monocytogenes* strains.

Comparison of the minimal inhibitory concentration (MIC) value of $CuSO_4$ for mutant strains DSE201, DSE951, DSE952 and DSE955, plasmid-cured strains DSE201PL and DSE955PL, and wild type strains DRDC8, KE1003, KE795 and EGD Kaufmann. The MIC value was measured as the lowest concentration that inhibited visible growth of *L. monocytogenes* following incubation on BHI media supplemented with $CuSO_4$ at 37ºC for 48 hours. Results shown are the means of three to six independent observations per experimental group. Error bars represent the standard error of the mean. * Significantly different from strain DRDC8 ($P < 0.05$).

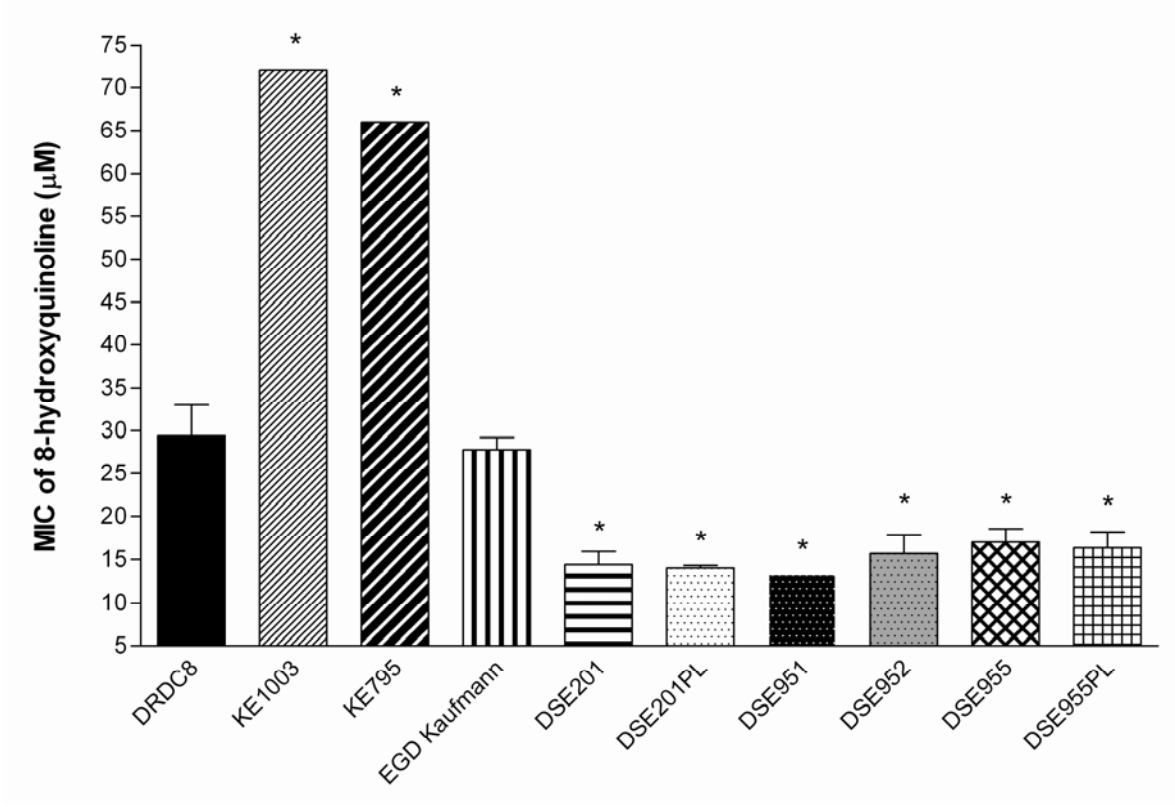


Figure 4.15: MIC of 8-hydroxyquinoline for *L. monocytogenes* strains.

Comparison of the minimal inhibitory concentration (MIC) value for mutant strains DSE201, DSE951, DSE952 and DSE955, plasmid-cured strains DSE201PL and DSE955PL, and wild type strains DRDC8, KE1003, KE795 and EGD Kaufmann. The MIC value was measured as the lowest concentration that inhibited visible growth of *L. monocytogenes* following incubation on BHI media supplemented with 8-hydroxyquinoline at 37ºC for 48 hours. Results shown are the means of three to six independent observations per experimental group. Error bars represent the standard error of the mean. * Significantly different from strain DRDC8 ($P < 0.05$).

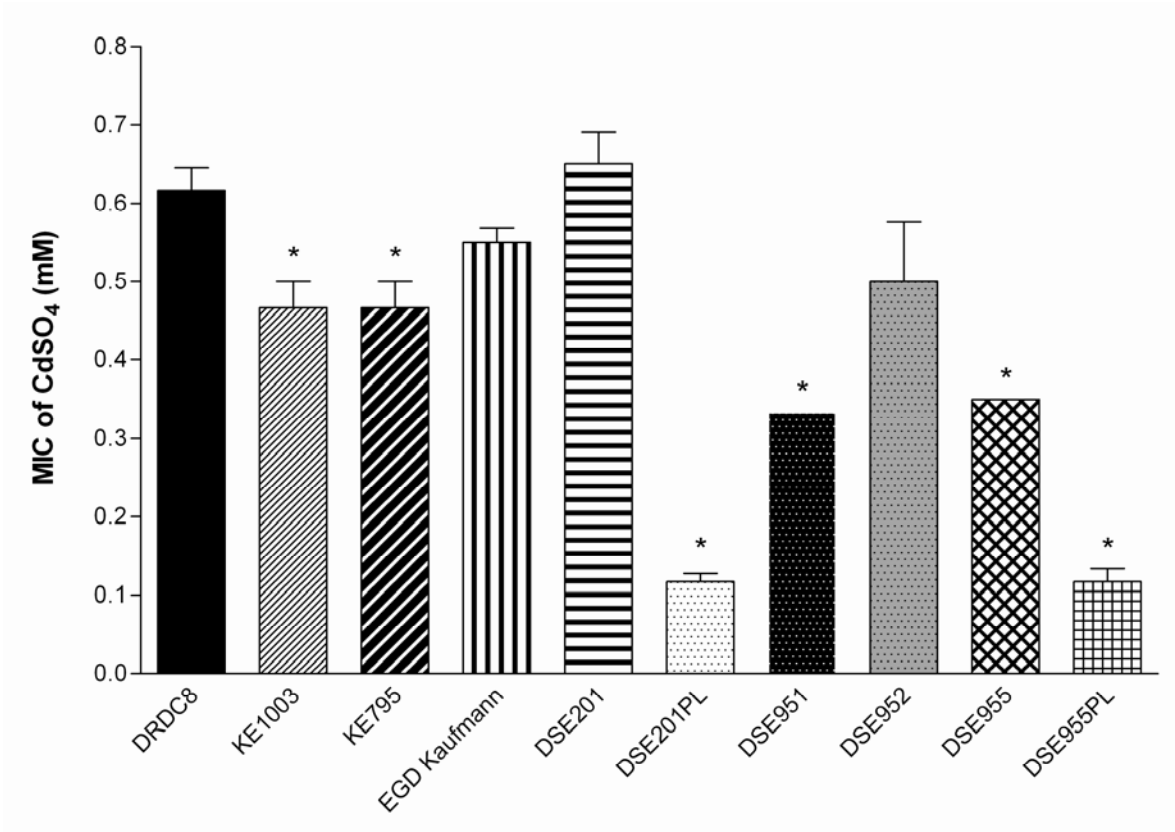


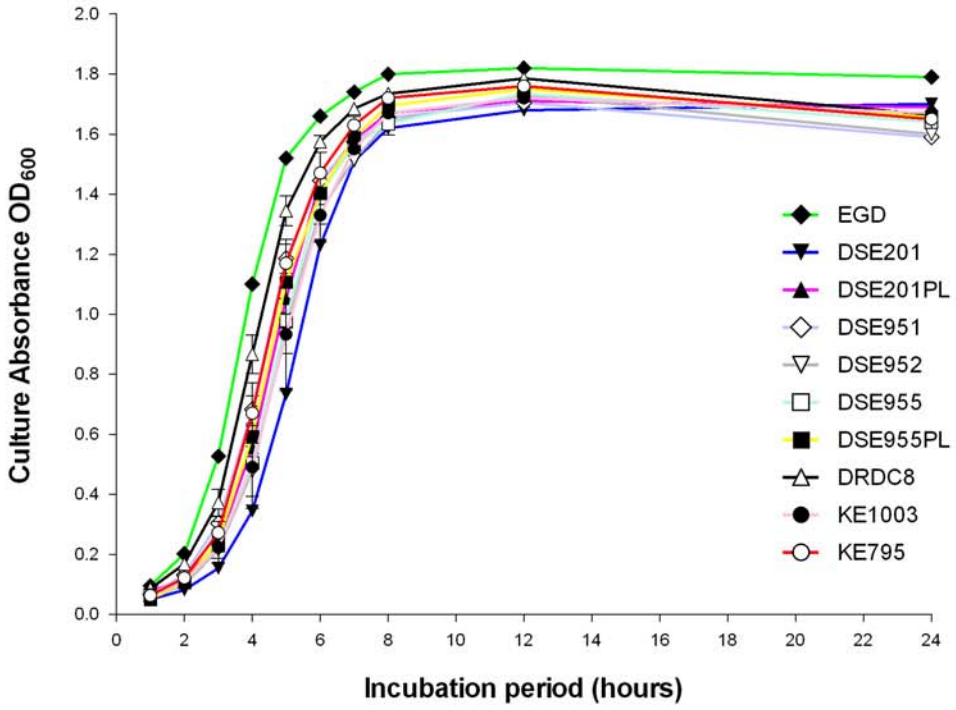
Figure 4.16: MIC of $CdSO_4$ for *L. monocytogenes* strains.

Comparison of the minimal inhibitory concentration (MIC) value for mutant strains DSE201, DSE951, DSE952 and DSE955, plasmid-cured strains DSE201PL and DSE955PL, and wild type strains DRDC8, KE1003, KE795 and EGD Kaufmann. The MIC value was measured as the lowest concentration that inhibited visible growth of *L. monocytogenes* following incubation on BHI media supplemented with $CdSO_4$ at 37ºC for 48 hours. Results shown are the means of three independent observations per experimental group. Error bars represent the standard error of the mean. * Significantly different from strain DRDC8 ($P < 0.05$).

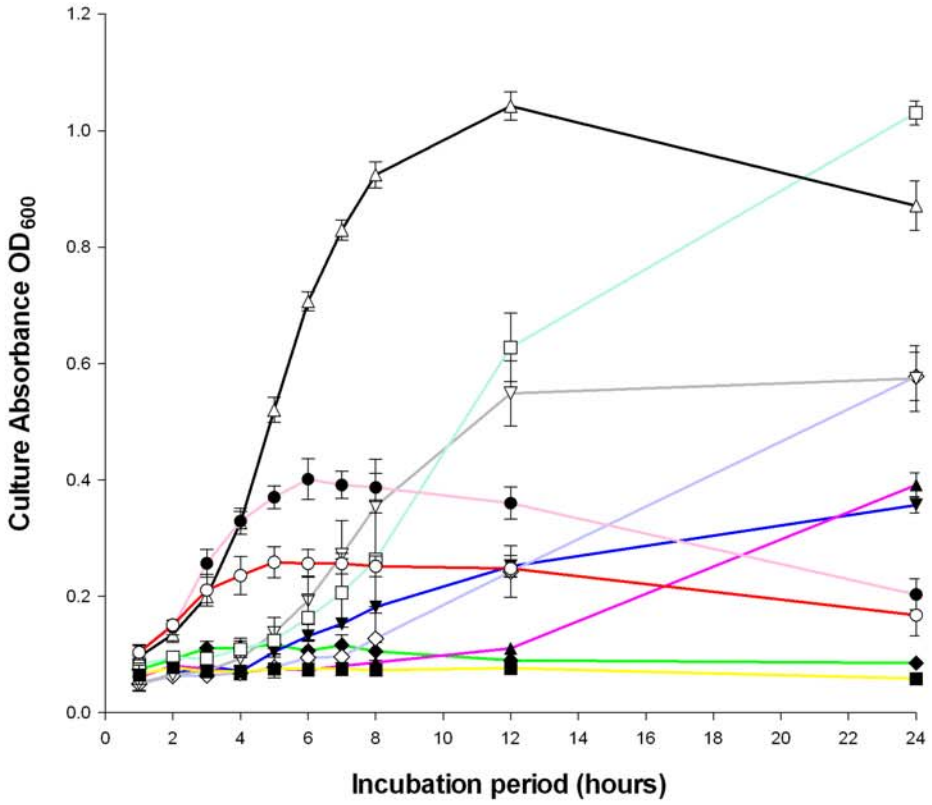
Figure 4.17: Growth of different *L. monocytogenes* strains in 14mM $CuSO_4$.

Culture absorbance of mutant strains DSE201, DSE951, DSE952, DSE955 and plasmid-cured strains DSE201PL and DSE955PL, compared to *L. monocytogenes* strains DRDC8, KE795, KE1003 and EGD Kaufmann (referred to as EGD in legend) in BHI broth (Panel A) and in BHI broth supplemented with 14 mM $CuSO_4$ (Panel B). The OD_{600} reading of each culture was measured over a 24-hour period. The results shown represent the means of four to eight independent observations per experimental group. Error bars represent the standard error about the mean. The legend shown in Panel A also applies to Panel B.

A


Culture Absorbance OD600
Incubation period (hours)
EGD
DSE201
DSE201PL
DSE951
DSE952
DSE955
DSE955PL
DRDC8
KE1003
KE795

B


Culture Absorbance OD600
Incubation period (hours)