# PCR-Based Detection of Genes Responsible for Oxalate Detoxification in Probiotic Microorganisms


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## Introduction

Oxalyl coenzyme A (CoA) decarboxylase (Oxc) is involved in bacterial oxalate metabolism, catalyzing oxalyl-CoA decarboxylation to formyl-CoA, thereby leading to oxalate detoxification. Oxalate is important due to its involvement in kidney stone formation. The intestinal organism *Oxalobacter formigenes*, which uses oxalate as its' sole energy substrate, contains the *oxc* gene. Some probiotic bacteria also consume oxalate, suggesting they may also contain the *oxc* gene. In this study, primers for *oxc* detection in *O. formigenes* (416-bp amplicon), *Lactobacillus acidophilus* NCFM (1,771-bp amplicon) and *Bifidobactrium lactis* Bl-07 (640-bp amplicon) were used to detect the *oxc* gene in different probiotic bacteria. The identification of gene sequences analogous to that of the *oxc* gene of *O. formigenes* in probiotic microorganisms may be a valuable tool, combined with culture-based techniques, in evaluating the potential oxalate-degrading activity of organisms used in commercial probiotics (Fig. 1).

**Figure 1. Commercial probiotics**


## Objectives

(1) To determine if probiotic microorganisms contain genes responsible for detoxification of oxalate similar to the *oxc* gene in *Oxalobacter formigenes*, as well as genes specific to those found in *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bl-07.

(2) To evaluate the specificity of primers to aide in the detection of genes responsible for oxalate detoxification in probiotic microorganisms.

NCFM-fp (5'-CTTGAAATGCAAGATGAAAGCA-3')

NCFM-rp (5'-CTTCAGTCATTATTTATTCTCC-3')

Bl07-fp (5'-GGTCACTGATTTCGCACGTAT-3')

Bl07-rp (5'-GACATCGGCAGGAAGGGAAT-3')

OxF-fp (5'-AATGTAGAGTTGACTGA-3')

OxF-rp (5'-TTGATGCTGTTGATACG-3')

Primer sets used in this study. Detection of *oxc* gene specific to *L. acidophilus* NCFM (NCFM), *B. lactis* Bl-07 (Bl07), and *O. formigenes* (OxF).

## Methods

Seventeen pure cultures were tested for oxalate consumption. Organisms were maintained on 5.5% MRS, 0 mM oxalate and transferred (1.0 mL) to duplicated tubes of 5.5% Difco Lactobacilli MRS medium containing 5 mM oxalate. Loss of oxalate was determined using high performance liquid chromatography (HPLC). Samples were taken at 0, 24, 48, 72, 96, and 120 hours for analysis.

DNA was extracted from the seventeen pure cultures of probiotic microorganisms, *Escherichia coli* (negative control), and *Oxalobacter formigenes* (positive control) using the QIAamp DNA Stool Mini Kit (QIAGEN). DNA amplification was carried out with protocol from Sidhu, et al. (1999) [1]. Products were run on 1.2% agarose gel in TBE buffer and visualized and photographed under UV light.

## Results

¦ *L. acidophilus* NCFM, *L. acidophilus* LA-14, and *B. lactis* Bl-07 showed significant levels of oxalate consumption after 120 h of incubation (Table 1)

☐ All organisms tested showed measurable amounts of oxalate consumption

**Table 1: Oxalate consumption by pure cultures of probiotic microorganisms**

| Pure Culture | Oxalate Consumed (mM)[ab] | Oxalate Consumption (%)[c] |
|---|---|---|
| ***Lactobacillus acidophilus* NCFM** | **3.17** | **100** |
| ***Lactobacillus acidophilus* LA-14** | **2.98** | **98.7** |
| ***Bifidobacterium lactis* BL-07** | **2.50** | **86.5** |
| ***Lactococcus lactis* LL-23** | **0.61** | **20.1** |
| ***Pediococcus acidilactici* P751** | **0.57** | **16.4** |
| ***Lactobacillus casei* LC-11** | **0.49** | **14.2** |
| ***Bifidobacterium lactis* BL-04** | **0.46** | **14.4** |
| ***Bifidobacterium breve* BB-03** | **0.45** | **13.8** |
| ***Bifidobacterium longum* BL-05** | **0.43** | **13.3** |
| ***Lactobacillus helveticus* Lh-138** | **0.43** | **12.6** |
| ***Lactobacillus paracasei* LPC-37** | **0.33** | **10.8** |
| ***Lactobacillus rhamnosus* LR-32** | **0.27** | **8.8** |
| ***Streptococcus thermophilus* ST-21** | **0.22** | **7.4** |
| ***Lactobacillus brevis* LBR-35** | **0.21** | **6.9** |
| ***Lactobacillus plantarum* LP-115** | **0.21** | **7.0** |
| ***Lactobacillus salivarius* LS33** | **0.18** | **6.0** |
| ***Lactobacillus bulgaricus* Lb-64** | **0.16** | **5.4** |

[a]Oxalate consumed within 120 h
[b]Numbers are results of duplicate tubes
[c]Percentage of soluble oxalate consumed within 120 h

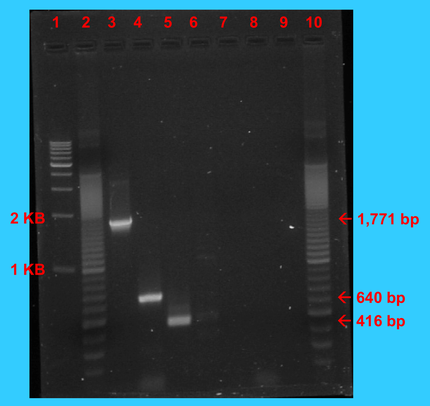


Figure 3. PCR gel with positive and negative controls. Positive controls show the presence of amplicons for the *oxc* gene specific for *O. formigenes* (416-bp amplicon), *B. lactis* Bl-07 (640-bp amplicon), and *L. acidophilus* NCFM (1,771-bp amplicon). Lane 1: 1 KB DNA ladder. Lane 2: 100 bp DNA ladder. Lane 3: *L. acidophilus* NCFM, NCFM primer. Lane 4: *B. lactis* Bl-07, Bl07 primer. Lane 5: *O. formigenes*, OxF primer. Lane 6: *E. coli*, OxF primer. Lane 7: NCFM primer, no template DNA. Lane 8: Bl07 primer, no template DNA. Lane 9: OxF primer, no template DNA. Lane 10: 100 bp DNA ladder.

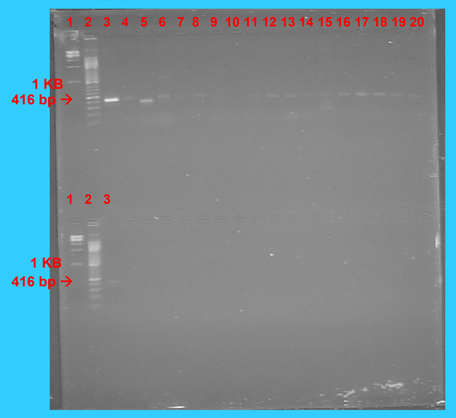


Figure 4. PCR gel with OxF primer for detection of the *oxc* gene specific to *Oxalobacter formigenes* (416-bp amplicon). Row 1. Lane 1: 1 KB DNA ladder. Lane 2: 100 bp DNA ladder. Lane 3: *Oxalobacter formigenes*. Lane 4: *Lactobacillus acidophilus* NCFM. Lane 5: *Bifidobacterium lactis* Bl-07. Lane 6: *Escherichia coli*. Lane 7: *Streptococcus thermophilus* ST-21. Lane 8: *Lactobacillus salivarius* LS33. Lane 9: *Lactobacillus plantarum* LP-115. Lane 10: *Lactobacillus bulgaricus* Lb-64. Lane 11: *Lactobacillus casei* LC-11. Lane 12: *Pediococcus acidilactici* P751. Lane 13: *Lactobacillus helveticus* Lh-138. Lane 14: *Bifidobacterium lactis* Bl-04. Lane 15: *Lactococcus lactis* LL-23. Lane 16: *Lactobacillus brevis* LBR-35. Lane 17: *Lactobacillus rhamnosus* LR-32. Lane 18: *Bifidobacterium longum* BL-05. Lane 19: *Bifidobacterium breve* BB-03. Lane 20: *Lactobacillus paracasei* LPC-37. Row 2. Lane 1: 1 KB DNA ladder. Lane 2: 100 bp DNA ladder. Lane 3: *Lactobacillus acidophilus* LA-14.

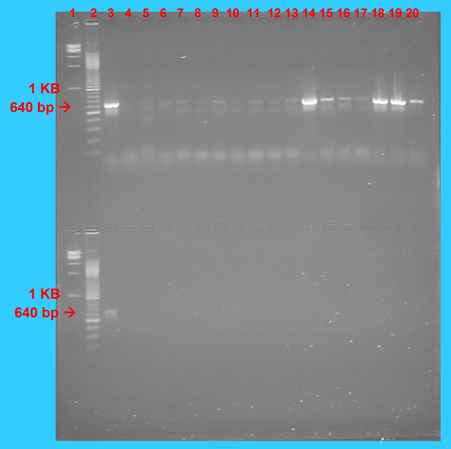


Figure 5. PCR gel with Bl07 primer for detection of the *oxc* gene specific to *Bifidobacterium lactis* Bl-07 (640-bp amplicon). Row 1. Lane 1: 1 KB DNA ladder. Lane 2: 100 bp DNA ladder. Lane 3: *Bifidobacterium lactis* Bl-07. Lane 4: *Lactobacillus acidophilus* NCFM. Lane 5: *Oxalobacter formigenes*. Lane 6: *Escherichia coli*. Lane 7: *Streptococcus thermophilus* ST-21. Lane 8: *Lactobacillus salivarius* LS33. Lane 9: *Lactobacillus plantarum* LP-115. Lane 10: *Lactobacillus bulgaricus* Lb-64. Lane 11: *Lactobacillus casei* LC-11. Lane 12: *Pediococcus acidilactici* P751. Lane 13: *Lactobacillus helveticus* Lh-138. Lane 14: *Bifidobacterium lactis* Bl-04. Lane 15: *Lactococcus lactis* LL-23. Lane 16: *Lactobacillus brevis* LBR-35. Lane 17: *Lactobacillus rhamnosus* LR-32. Lane 18: *Bifidobacterium longum* BL-05. Lane 19: *Bifidobacterium breve* BB-03. Lane 20: *Lactobacillus paracasei* LPC-37. Row 2. Lane 1: 1 KB DNA ladder. Lane 2: 100 bp DNA ladder. Lane 3: *Lactobacillus acidophilus* LA-14.

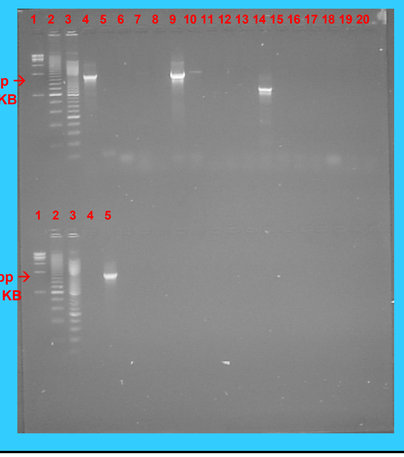


Figure 6. PCR gel with NCFM primer for detection of the *oxc* gene specific for *Lactobacillus acidophilus* NCFM. Row 1. Lane 1: 1 KB DNA ladder. Lane 2: 100 bp DNA ladder. Lane 3: 100 bp DNA ladder. Lane 4: *Lactobacillus acidophilus* NCFM. Lane 5: *Bifidobacterium lactis* Bl-07. Lane 6: *Oxalobacter formigenes*. Lane 7: *Escherichia coli*. Lane 8: *Streptococcus thermophilus* ST-21. Lane 9: *Lactobacillus salivarius* LS33. Lane 10: *Lactobacillus plantarum* LP-115. Lane 11: *Lactobacillus bulgaricus* Lb-64. Lane 12: *Lactobacillus casei* LC-11. Lane 13: *Pediococcus acidilactici* P751. Lane 14: *Lactobacillus helveticus* Lh-138. Lane 15: *Bifidobacterium lactis* Bl-04. Lane 16: *Lactococcus lactis* LL-23. Lane 17: *Lactobacillus brevis* LBR-35. Lane 18: *Lactobacillus rhamnosus* LR-32. Lane 19: *Bifidobacterium longum* BL-05. Lane 20: *Bifidobacterium breve* BB-03. Row 2. Lane 1: 1 KB DNA ladder. Lane 2: 200 bp DNA ladder. Lane 3: 100 bp DNA ladder. Lane 4: *Lactobacillus paracasei* LPC-37. Lane 5: *Lactobacillus acidophilus* LA-14.

| | OxF | NCFM | Bl-07 |
|---|---|---|---|
| *ctobacillus acidophilus* NCFM | - | + | - |
| *ctobacillus acidophilus* LA-14 | - | + | - |
| *Bifidobacterium lactis* Bl-07 | - | - | + |
| *Lactococcus lactis* LL-23 | - | - | - |
| *ediococcus acidilactici* P751 | - | - | - |
| *Lactobacillus casei* LC-11 | - | - | - |
| *Bifidobacterium lactis* BL-04 | - | - | + |
| *Bifidobacterium breve* BB-03 | - | - | + |
| *ifidobacterium longum* BL-05 | - | - | + |
| *ctobacillus helveticus* Lh-138 | - | - | - |
| *ctobacillus paracasei* LPC-37 | - | - | - |
| *ctobacillus rhamnosus* LR-32 | - | - | - |
| *ptococcus thermophilus* ST-21 | - | - | - |
| *Lactobacillus brevis* LBR-35 | - | - | - |
| *ctobacillus plantarum* LP-115 | - | - | - |
| *actobacillus salivarius* LS33 | - | + | - |
| *ctobacillus bulgaricus* Lb-64 | - | - | - |
| *Oxalobacter formigenes* | + | - | - |
| *Escherichia coli* | - | - | - |

[a]*oxc* gene specific for *Oxalobacter formigenes*
[b]*oxc* gene specific for *Lactobacillus acidophilus* NCFM
[c]*oxc* gene specific for *Bifidobacterium lactis* Bl-07

## Summary


**Literature cited**

[1] Sidhu, H., R. P. Holmes, M. J. Allison, and A. B. Peck. 1999. Direct Quantification of the Enteric Bacterium *Oxalobacter formigenes* in Human Fecal Samples by Quantitative Competitive-Template PCR. Journal of Clinical Microbiology 37:1503-1509.



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