Journal of Microbiology, Immunology and Infection (2017) xx, 1–10

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

A "Ct contrast"-based strain-specific real-time quantitative PCR system for *Lactobacilllus paracasei* subsp. *paracasei* NTU 101

Chih-Hui Lin [a], Tsung-Wei Shih [b], Tzu-Ming Pan [c,*]

[a] *Department of Life Science, National Taitung University, Taiwan*
[b] *SunWay Biotech Ltd., Taiwan*
[c] *Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Taiwan*

Received 21 October 2015; received in revised form 18 June 2016; accepted 24 May 2017
Available online ■ ■ ■

KEYWORDS
RAPD;
Strain-specific;
qPCR;
Lactobacillus;
Probiotics

Abstract *Background/Purposes:* Routine cell number determination for specific *Lactobacillus* strain by cultivation requires at least 4–7 days. Thus rapid and specific cell number determine methods such as strain-specific quantitative PCR (qPCR) are valuable. However, qPCR method is vulnerable to difficult PCR target such as dimer/secondary structure forming sequence.
Methods: In this study, a two-component, "Ct contrast" approach was applied to strain-specific qPCR system following the development of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (NTU 101) strain-specific PCR with random amplification of polymorphic DNA (RAPD)-derived strain-specific sequences.
Results: The quantitative range of the NTU 101 strain-specific qPCR system was 3.0×10^1 to 3.0×10^5 copies for pure cultures, and 3.0×10^2 to 3.0×10^5 copies for multi-strain or unknown food samples. The results of spike in test and real sample testing suggested that non-specific weak background signals did not compromise test specificity, and demonstrated the potential of the NTU 101 strain-specific qPCR system in food samples.
Conclusion: The two-component, "Ct contrast" approach is useful for qPCR discrimination when no ideal PCR target is available or the variance of the target site is unpredictable. The Ct contrast approach might provide a simple and robust solution for other challenging qPCR targets.

* Corresponding author. Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei, Taiwan. Fax: +886 2 3366 3838.
E-mail address: tmpan@ntu.edu.tw (T.-M. Pan).

http://dx.doi.org/10.1016/j.jmii.2017.05.002

Introduction

Lactobacillus strains are well-known lactic acid bacteria (LAB) that are used in foods and as probiotics, and there are many reports of their beneficial effects on human and animal health.[1–3] DNA/PCR-based methods such as random amplification of polymorphic DNA (RAPD), pulse-field gel electrophoresis (PFGE), and ribotyping have been widely used for bacterial strain discrimination. However, PCR methods that target the 16S rRNA gene or the intergenic spacer region between the 16S and 23S genes are not applicable at strain level because of the highly conserved *Lactobacillus* rRNA gene sequences. RAPD markers that derived from highly polymorphic genomic sequences usually also represent a potential strain-specific sequence region. Thus, the concept of RAPD-derived strain-specific sequence was developed and successfully applied to strain-specific detection of LAB and lactobacilli.[4–6]

Real-time quantitative PCR (qPCR) is the current method of choice for detecting and quantifying cell numbers. Several allele-specific methods such as allele-specific blocker PCR (ASB-PCR) have been successfully developed to detect minor sequence variations such as single nucleotide polymorphisms (SNPs).[7] Introduction of allele-specific qPCR strategy might help to establish strain-specific qPCR method that only minor sequence variation available.

Lactobacilllus paracasei subsp. *paracasei* NTU 101 (NTU 101; DSMZ 28047) was originally isolated from an infant,[8] and is a LAB used in yogurt and probiotic products. It benefits human health in several ways, including hypocholesterolemic,[9] anti-atherosclerotic,[10] anti-hypertensive,[11] anti-osteoporosis,[12] and immunomodulating effects,[13,14] as well as preventing infection,[15] gastric mucosal lesions,[16] and fat accumulation.[17]

In this study, we established RAPD-derived NTU 101-specific PCR to detect NTU 101. Unfortunately, the NTU 101-specific sequence was difficult for routine qPCR assay due to its dimer/secondary structure forming trend. Thus a novel strategy which based on the "Ct contrast" of two qPCR assays to discriminate the background qPCR signal from close relatives of NTU 101 was developed for strain-specific qPCR detection of NTU 101.

Methods

Lactobacillus strains and cultivation

Thirty-four strains/species of *Lactobacillus casei* group lactobacilli (Table 1) and 70 strains/species of non-*L. casei* group lactobacilli (Table 2) were purchased from Bioresource Collection and Research Center (BCRC; Hsinchu, Taiwan). NTU 101 was provided by Professor Tzu-Ming Pan (Department of Biochemical Science and Technology, National Taiwan University).

DNA extraction, quantification, and sequence analysis

Lactobacilli DNA were extracted using a QIAamp® DNA mini kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. Geneious R6-1 software (Biomatters Ltd., Auckland, New Zealand) was used for all DNA sequence analyses includes primer design, sequence assembly, and alignment.

Strain-specific PCR

The NTU 101-specific primers A3-5F3 (5′-CGCCGAACGCGACTTACATC-3′) and A3-5R3 (5′-GGCAAATTTAAACTTGCCTTCAACG-3′) were designed corresponding to the unique

Table 1 The *L. casei* group lactobacilli used in this study.

Microorganism	Strain ID/BCRC serial number
Lactobacillus paracasei subsp. *paracasei*	NTU 101
Lactobacillus casei	BCRC 10358
Lactobacillus casei	BCRC 10697T
Lactobacillus casei	BCRC 11197
Lactobacillus casei	BCRC 12272
Lactobacillus casei	BCRC 14025
Lactobacillus casei	BCRC 16093
Lactobacillus casei	BCRC 16094
Lactobacillus casei	BCRC 17001
Lactobacillus casei	BCRC 17004
Lactobacillus casei	BCRC 17487
Lactobacillus paracasei subsp. *paracasei*	BCRC 12188
Lactobacillus paracasei subsp. *paracasei*	BCRC 12248T
Lactobacillus paracasei subsp. *paracasei*	BCRC 14001
Lactobacillus paracasei subsp. *paracasei*	BCRC 14023
Lactobacillus paracasei subsp. *paracasei*	BCRC 16100
Lactobacillus paracasei subsp. *paracasei*	BCRC 17002
Lactobacillus paracasei subsp. *paracasei*	BCRC 17483
Lactobacillus paracasei subsp. *paracasei*	BCRC 17484
Lactobacillus paracasei subsp. *tolerans*	BCRC 17485
Lactobacillus paracasei subsp. *paracasei*	BCRC 17488
Lactobacillus paracasei subsp. *paracasei*	BCRC 17489
Lactobacillus paracasei	BCRC 80062
Lactobacillus zeae	BCRC 17647T
Lactobacillus zeae	BCRC 17942T
Lactobacillus zeae	BCRC 80156
Lactobacillus rhamnosus	BCRC 10940T
Lactobacillus rhamnosus	BCRC 11673
Lactobacillus rhamnosus	BCRC 12249
Lactobacillus rhamnosus	BCRC 14027
Lactobacillus rhamnosus	BCRC 16095
Lactobacillus rhamnosus	BCRC 17006
Lactobacillus rhamnosus	BCRC 17007
Lactobacillus rhamnosus	BCRC 80065

Please cite this article in press as: Lin C-H, et al., A "Ct contrast"-based strain-specific real-time quantitative PCR system for *Lactobacilllus paracasei* subsp. *paracasei* NTU 101, Journal of Microbiology, Immunology and Infection (2017), http://dx.doi.org/10.1016/j.jmii.2017.05.002