

Multiplex PCR for detecting beer-spoilage bacteria including recently proposed species



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Summary

In 2006, three species of Gram negative beer-spoilage bacteria, *Pectinatus haikarae*, *Megasphaera sueciensis* and *M. paucivorans*, were newly proposed. Previously we reported that three sets of multiplex PCR methods for detecting beer-spoilage *Lactobacillus*, *Pectinatus* and cocci were developed. In this study, we attempted to improve our multiplex PCR methods for *Pectinatus* and cocci by including these newly assigned beer-spoilers.

PCR primers were designed in the 16S rRNA gene by comparing the sequences among the related species. Species-specific primer pair was designed for *P. haikarae*, while common primer pair simultaneously detecting *M. sueciensis* and *M. paucivorans* was designed because of more than 99 % similarity in the 16S rRNA gene sequences between these species.

The primer pair for *P. haikarae* and common primer pair for *M. sueciensis* and *M. paucivorans* were added to the existing primer mix of the corresponding multiplex PCR respectively, and multiplex PCR was conducted to evaluate the performance of modified primer mixes. The sensitivity of new multiplex PCR was comparable to that of simplex PCR and universal primers, the latter of which were used as control of DNA extraction. Moreover, new multiplex PCR methods did not amplify the false positive PCR products from DNA of closely related species and frequently isolated species from the brewery environment, and were found to cover all of the investigated strains belonging to the target species. These results showed that their specificity and reactivity are practically suited to the application in breweries.

In conclusion, we developed new multiplex PCR methods for detecting beer-spoilage bacteria including recently proposed species.

Introduction

We previously developed three sets of multiplex PCR methods for known beer-spoilage bacteria, which can detect six species of *Lactobacillus*, two species of *Pectinatus* and four species of cocci respectively⁽¹⁾. While, in 2006, newly proposed species of Gram negative bacteria, *Pectinatus haikarae*, *Megasphaera sueciensis* and *M. paucivorans* were reported to have beer-spoilage ability⁽²⁾, and could not be detected by our previous multiplex PCR methods. Therefore, in this study, we attempted to improve our previous multiplex PCR methods for *Pectinatus* and cocci to be applicable to these new species, and will report our advanced multiplex PCR methods for beer-spoilage bacteria.

DNA extraction

For evaluating the reactivity and sensitivity, DNA was extracted from serial ten-fold dilutions of a single colony grown on the agar media by Prepman Ultra Sample Preparation Reagent (Applied Biosystems). For the specificity, DNA was extracted from pure culture of each test-strains with Mag Extractor DNA extraction Kit (TOYOBO).

PCR condition

PerfectShot Ex Taq (TaKaRa Bio) 25 µl	94°C 2.5 min.
Primer 0.8µM each	94°C 15 sec. } 30cycles
DNA 5 µl	55°C 15 sec. }
DW up to 50 µl	72°C 30 sec. }
	72°C 3 min.
	4°C ∞

Results

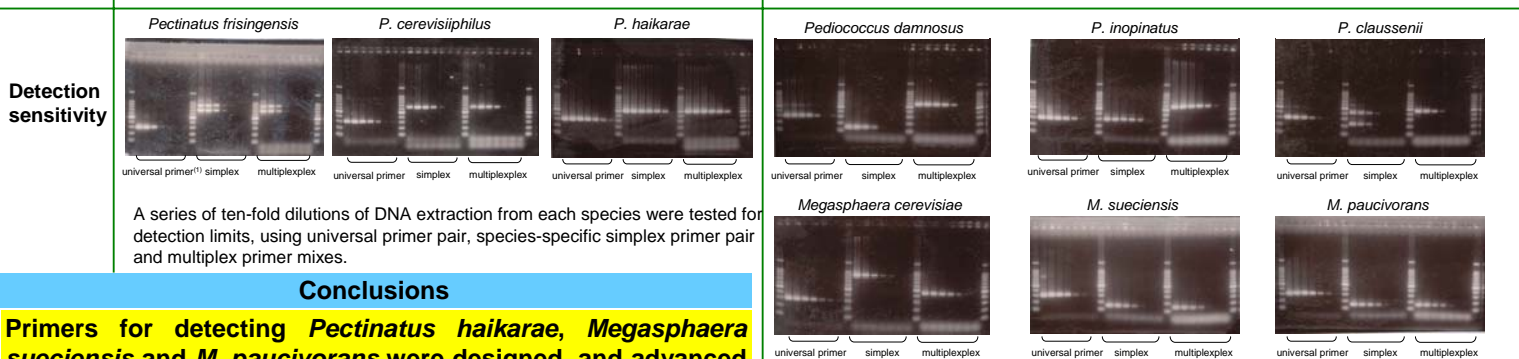
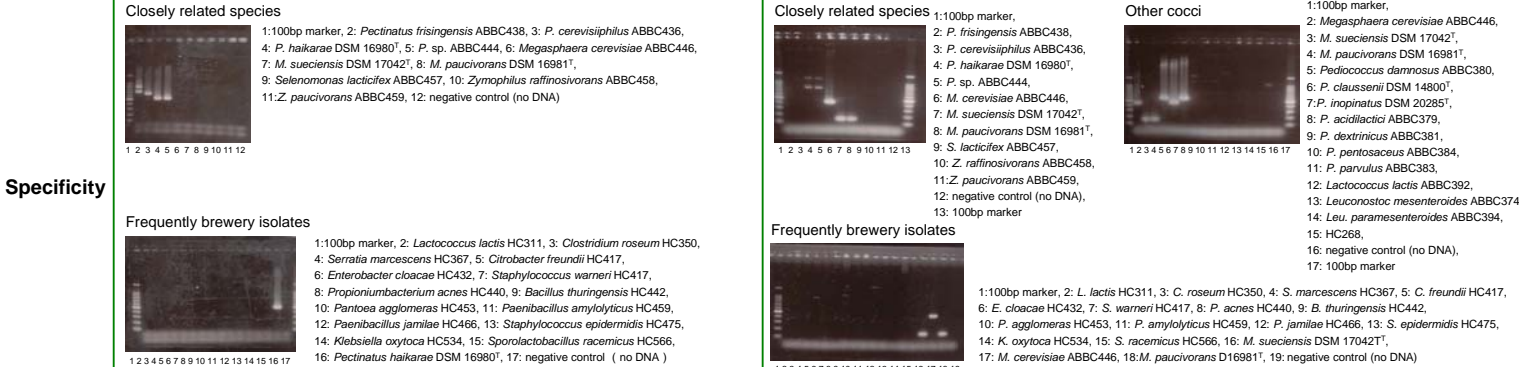
Advanced *Pectinatus* multiplex PCR method

Target species	Primers	Direction	Sequence (5'–3')	Target DNA	Reference
<i>P. cerevisiiphilus</i>	16C-F	Forward	CGTATGCAGAGATGCATATT	16S rDNA	3
	IC-R	Reverse	CACTCTTACAAGTATCTAC	ITS region	3
<i>P. frisingensis</i>	16F-F	Forward	CGTATCCAGAGATGGATATT	16S rDNA	3
	IF-R	Reverse	CCATCCTCTTGAATAATCTC	ITS region	3
<i>P. haikarae</i>	Phf1	Forward	AATACCCGAATGTTGAAGAG	16S rDNA	this study
	Phr2	Reverse	CTCTCCTGCACTCAAGACAT	16S rDNA	this study

Advanced coccal multiplex PCR method

Target species	Primers	Direction	Sequence (5'–3')	Target DNA	Reference
<i>P. damonosus</i> & <i>P. inopinatus</i>	PIDF1	Forward	ACCGAATACGATCTAAAG	16S rDNA	1
	PIDR8	Reverse	TTAAGACCGACTTACCGA	16S rDNA	1
<i>P. clausenii</i>	PCLAF3	Forward	TGTGAGAGTAACGTCTCATG	16S rDNA	1
	PCLAR3	Reverse	ACGCCTAATCTCTTTGGTTA	16S rDNA	1
<i>M. cerevisiae</i>	Mc-f4	Forward	CATTTCCGTTAAAGAATCA	16S rDNA	1
	Mc-r4	Reverse	GGTAAATACCGTCACTGGG	16S rDNA	1
<i>M. sueciensis</i> & <i>M. paucivorans</i>	Msp-f	Forward	TATGGCCAATACCATAGAT	16S rDNA	this study
	Msp-r	Reverse	CACITTTAAGACAGACTTGA	16S rDNA	this study

Reactivity All investigated strains for the reactivity, 54 strains of *Pectinatus* species and 26 strains of beer-spoilage *Pediococcus* and *Megasphaera* species, were detected with advanced *Pectinatus* and coccal multiplex PCR method respectively, and no false-negative results were observed.



Conclusions

Primers for detecting *Pectinatus haikarae*, *Megasphaera sueciensis* and *M. paucivorans* were designed, and advanced multiplex PCR methods for three species of *Pectinatus* species and six species of beer-spoilage cocci were developed.

Reference

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