Development of Molecular Technique for Detection of *Bifidobacterium* spp.

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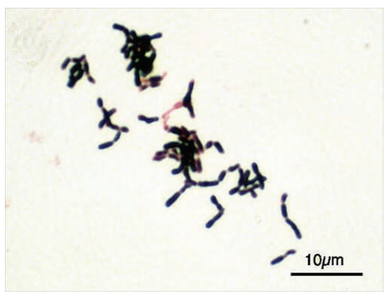
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Abstract

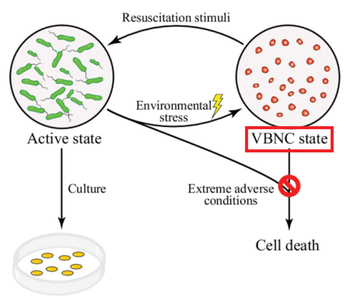
The detection of *Bifidobacterium* in functional foods and probiotic products is important to confirm the presence of the microorganism in accordance with product claims, which affects product credibility and consumer confidence. Therefore, this study aimed to develop a molecular biology method for detection of *Bifidobacterium* spp. *Bifidobacterium*-specific primers were designed based on 109 sequences of 16S rRNA and 103 sequences od *xfp* gene in the NCBI database. Primers were then tested using polymerase chain reaction with reference *Bifidobacterium* species and other 15 strains belonging to the genera *Lactolentiplantibacillus*, *Lacctobacillus*, *Lacticaseibacillus*, *Streptococcus*, *Bacillus*, *Klebsiella*, *Escherichia* and *Staphylococcus*. The results showed that two primer pairs targeting the 16S rRNA and D-Xylulose 5-Phosphate/D-Fructose 6-Phosphate Phosphoketolase (*xfp*) genes were specific to the *Bifidobacterium* genus. Optimal conditions were achieved at annealing temperatures of 55–65°C, with a detection limit of 10^2 CFU/mL in milk. The developed method can be a tool for screening and quality control to confirm the presence of *Bifidobacterium* spp. in functional foods and probiotic products.

Background

- **What is *Bifidobacterium*?: A Gram-positive, Y-shaped, strictly anaerobic probiotic bacterium commonly found in the gut, breast milk, and fermented dairy products.**
- **What is the challenge?: Environmental stress such as temperature, pH, oxygen, and food processing can cause *Bifidobacterium* to enter a viable but non-culturable (VBNC) state.**
- **What is the problem?: Rapid detection is needed because VBNC cells remain alive but cannot be detected by normal culture methods. Current detection approaches include biological methods, which require more time and expertise, and molecular methods such as LAMP and PCR, which are faster and more efficient.**







Objective

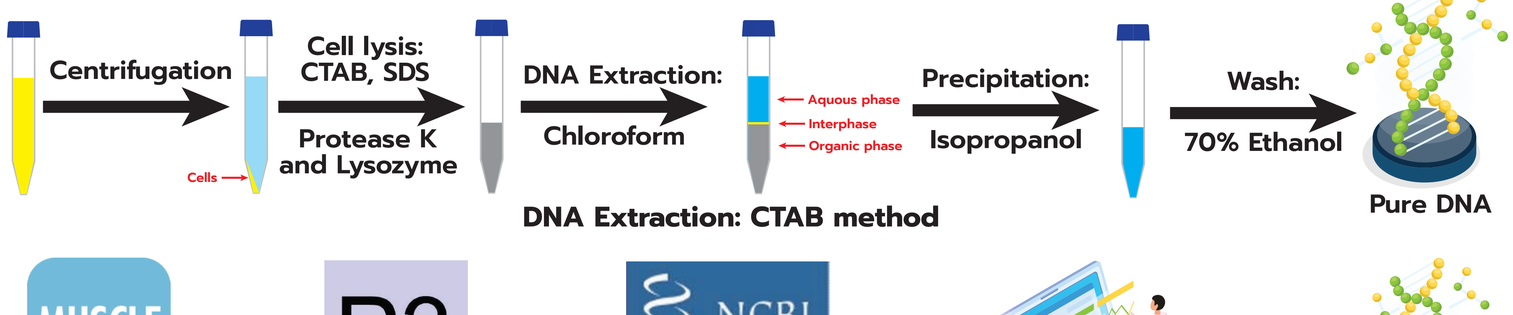
To develop a molecular method for specific detection of *Bifidobacterium* spp.

Materials and Methods

1. Culture Collection

Target microorganism (*Bifidobacterium*)	Non-target microorganism		
	Lactic acid bacteria (LAB)	Spore forming bacteria	Pathogenic bacteria
B. animalis *B. longum*	*Lactiplantibacillus* *Lactobacillus* *Lacticaseibacillus* *Lactococcus* *Streptococcus*	*Bacillus cereus* *Bacillus subtilis* *Bacillus coagulans*	*Klebsiella pneumoniae* *Escherichia coli* *Staphylococcus aureus*
2 isolates	11 isolates	4 isolates	3 isolates

2. DNA Extraction



DNA Extraction: CTAB method

3. Primer Design and *In Silico* Analysis

16S rRNA NCBI dataset
- 109 sequences of *Bifidobacterium*
- 27,561 sequences of non-target microorganisms

***xfp* gene from NCBI**
- 103 sequences of *Bifidobacterium*
- 47 sequences of non-target microorganisms

→ MUSCLE: Multiple Sequence Alignment: MSA → P3: Generate primer candidates → NCBI BLAST: *In-silico* specificity screening via Online/Local BLAST → Ranking and Section → Primer test

4. PCR Evaluation

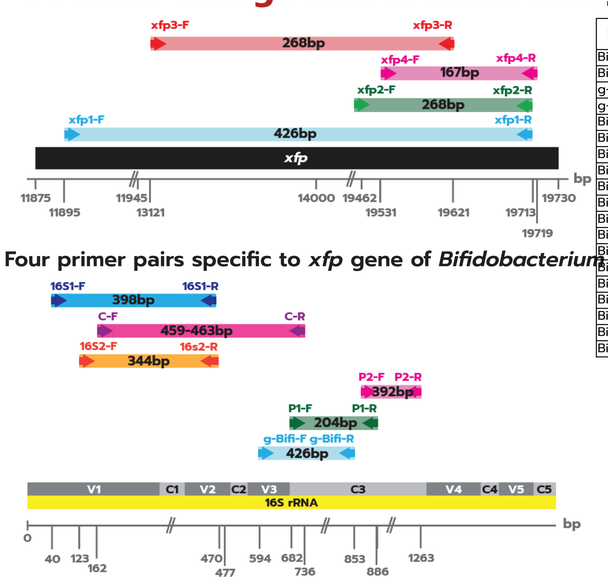
Primer Specificity Test → Single PCR → Duplex PCR

5. Validation

Limit of Detection (LOD) → Sample Test

Results and Discussion

1. Primer Design and *In silico* analysis



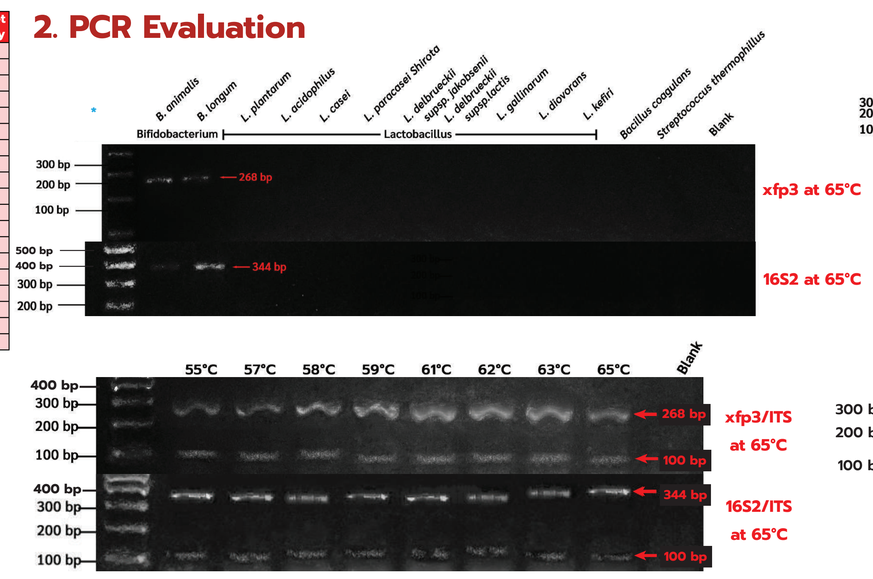
Four primer pairs specific to *xfp* gene of *Bifidobacterium*

Five primer pairs specific 16S rRNA gene of *Bifidobacterium*
*from previous study

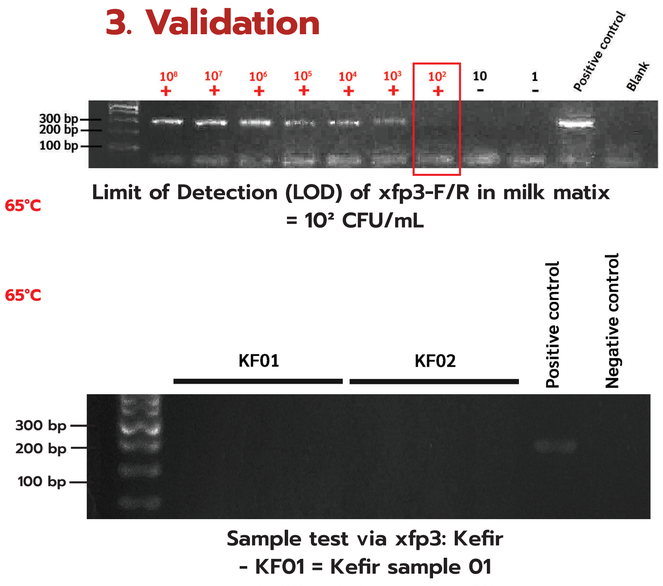
PrimerName	Target Coverage	OffTarget Coverage	Target %Identity	OffTarget %Identity
BifidoC-F	0.00	0.00	0.00	0.00
BifidoC-R	4.77	0.04	100.00	100.00
g-Bifi-F	5.51	0.99	100.00	100.00
g-Bifi-R	4.58	0.01	100.00	100.00
BifidoP1-F	4.77	1.23	100.00	100.00
BifidoP1-R	3.84	0.46	100.00	100.00
BifidoP2-F	4.29	1.00	100.00	100.00
BifidoP2-R	4.58	0.84	100.00	100.00
Bifido16s1-F	0.64	0.00	100.00	95.24
Bifido16s1-R	0.10	0.00	95.24	0.00
Bifido16s2-F	5.89	0.00	100.00	100.00
Bifido16s2-R	2.78	0.00	100.00	100.00
Bifidoxfp1-F	1.76	0.00	100.00	0.00
Bifidoxfp1/2-R	0.61	0.00	100.00	95.00
Bifidoxfp2-F	0.13	0.00	100.00	0.00
Bifidoxfp3-F	2.85	0.00	100.00	100.00
Bifidoxfp3-R	0.35	0.00	100.00	0.00
Bifidoxfp4-F	0.64	0.00	100.00	100.00
Bifidoxfp4-R	0.58	0.00	100.00	0.00

Nine primer pairs were tested → Two primer pairs were found specificity → xfp3-F/R, 16S2-F/R

2. PCR Evaluation



3. Validation



Limit of Detection (LOD) of xfp3-F/R in milk matix = 10^2 CFU/mL

Sample test via xfp3: Kefir
- KF01 = Kefir sample 01
- KF02 = Kefir sample 02

Both Sample were Negative

Conclusion

- **The developed primers target *xfp* and 16S rRNA gene had potentials to be suitable tool detection of Bifidobacterium spp. in foods and probiotic products.**

Acknowledgements

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