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Expression of heterologous genes using the slpA promoter and signal sequence in *Lactobacilli*

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Abstract

A gene coding endo-β,-1, 4 glucanase from A*ctinomyces sp. KNG40* and phytase from *Hansenula polymorpha* were cloned into *Esherichia coli* JM101 by using *E. coli/Lactobacillus* shuttle vector pNZ3004 and pNZ123. The plasmid p3PS(1-4) and p123(1-4) have slpA promoter and slpA signal sequence. So, I constructed expression vectors, p3PS(1-4)Endo, phy and p123(1-4)Endo, phy. These constructed vector was transformed in target host *Lactobacillus gasseri* and *reutri*. These transformed host expressed endoglucanase and phytase as extracellular fraction. In the enzyme activity of the same vector, host *L. gasseri* was higher activity than *L. reuteri*. This indicates that *L. gasseri* recognize promoter and signal sequence very well.

Introduction

Lactobacillus is one of the major genera of the lactic acid bacteria (LAB), a diverse group of economically important microorganisms that have been used in various food and agricultural fermentation processes[2]. In recent years, much attention has been paid to the molecular genetic characterization of traits essential for the industrial use of LAB. Also elucidation of the capacity of LAB to produce and secrete protein has been initiated to improve their use as production hosts for applications in food, feed and health care industries. Favorable properties of LAB as putative protein production hosts include for instance GRAS status, a very low exoprotease activity in some strains and the presence of very few indigenous secretory proteins.

Surface (S) layer are commonly found structures in prokaryotes[3] and may

have considerable application potentials. Since the S-layer proteins represent up 10% - 15% of the total cellular protein of an S-layer-carrying bacterial cell[1], the expression and secretion signal of S-layer protein genes are obvious candidates for the construction of efficient vectors both for intra- and extracellular protein production.

SlpA promoter and the cleavage site of the signal peptide was about 180bp and synthesized by PCR amplification from genomic DNA of *Lactobacillus brevis*. To construct the expression vector this fragment was inserted into our expression vector, pNZ3004 and pNZ123. The structural gene of endoglucanase and phytase, lacking their own translation-initiation codon, were fused between the slpA promoter and MCS. *Lactobacilli* were transformed by electroporation and plated on MRS agar plate with erythromycin and chrolamphenicol. Transformants were screened for endoglucanase producing on the basis of Congo-red test on CMC plate and for phytase producing on the PSS(phytase screening solution) test.

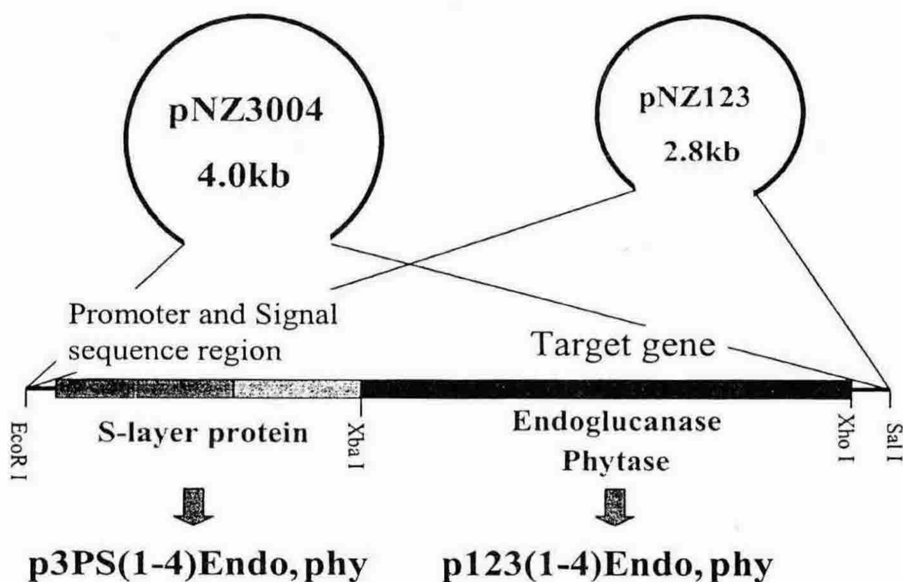
Materials and methods

1. Hosts and vectors
   - *E. coli* : JM101
   - LAB : *Lactobacillus gasseri, reuteri*
   - Vectors : pNZ123 and pNZ3004 (*E. coli* and *Lactobacillus* shuttle vector)
2. Target gene cloning
   - Promoter and signal sequence of S-layer protein (from *L. brevis*)
   - Endoglucanase (from *Actinomyces sp.* isolated in KNG)
   - Phytase (from *Hansenula polymorpha*)
3. Vector construction
   - p3PS(1-4)Endo, p3PS(1-4)phy : based on pNZ3004
   - p123(1-4)Endo, p123(1-4)phy : based on pNZ123
4. Gene manipulation
   - PCR amplification, ligation, transformation ·.
5. Enzyme activity
   - CMCase (Congo red method)
   - Phytase (PSS method)

Results

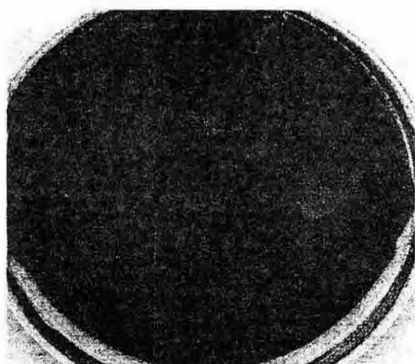
1. vector construction

Promoter and signal sequence region of S-layer proterin was fused with target gene, endoglucanase and phytase and this cassette was ligated in pNZ3004 and pNZ123 vector. So, I constructed expression vector p3PS(1-4)Endo, phy and p123(1-4)Endo, phy.

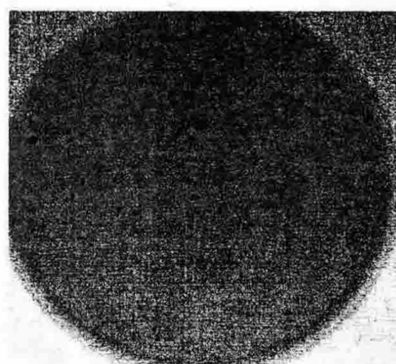


2. Transformation in *Lactobacillus*

From the culture supernatant in transformed *Lactobacillus*, I carried out



1. *E. coli* JM101 / p123(1-4)Endo -Supernatant
2. *L. reuteri* / p3PS(1-4)Endo
3. *L. reuteri* / p123(1-4)Endo
4. *L. gasseri* / p3PS(1-4)Endo



1. Control - *L. reutri*
2. *E. coli* JM101 / p3PS(1-4)phy
3. *L. reuteri* / p3PS(1-4)phy

enzyme activity test. I loaded 10㎕ culture supernatant of each transformants on 1% CMC agar plate.

References

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3. Messner, P. and V. B. Sleytr. "Crystalline bacterial cell-surface layers" (1992). Adv. microb. Physiol. 33 : 213-275