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Improvement of Probiotic Activity of Bifidobacteria Through Genetic Engineering

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Bifidobacterium spp. are nonpathogenic, gram-positive and anaerobic bacteria which inhabit the intestinal tract of humans and animals [1]. In breast-fed infants, bifidobacteria comprise more than 90 % of the gut bacterial population [2]. *Bifidobacterium* spp. are used in commercial fermented dairy products and have been suggested to exert health promoting effects on the host by maintaining intestinal microflora balances, improving lactose tolerance, reducing serum cholesterol levels, increasing synthesis of vitamins, and aiding the immune enhancement and anticarcinogenic activity for the host [3]. These beneficial effects of *Bifidobacterium* are strain-related. Therefore continued efforts to improve strain characteristics are warranted.

In these respect, development of vector system for *Bifidobacterium* is very important not only for the strain improvement but also because *Bifidobacterium* is most promising in serving as a delivery system for the useful gene products, such as vaccine or anticarcinogenic polypeptides, into human intestinal tract.

For developing vector system, we have characterized several bifidobacterial plasmids at genetic level [4-7] and developed several shuttle vectors between *E.coli* and *Bifidobacterium* using them [Fig. 1, 9]. Also, we have cloned and sequenced several metabolic genes and strong promoters from *Bifidobacterium,*

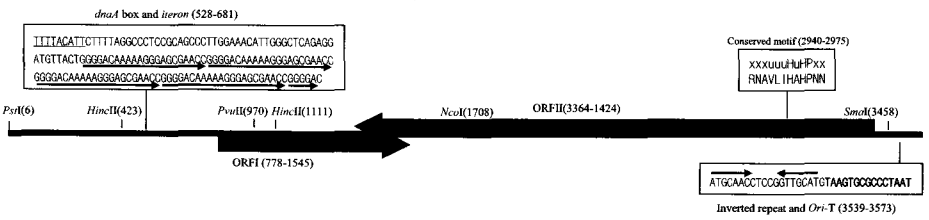

Fig. 1. Schematic presentation of nucleotide sequence of pMG1. Each ORF is indicated as filled arrows. The nucleotide sequence of putative *dnaA* box and iteron region (528-681) and *oriT* region (3539-3573) are shown. The amino acid sequence of highly conserved motif (2940-2975) in ORF II is indicated as a box (u-hydrophobic residue, x-nonconsensus residue). The nucleotide sequence is available in the NCBI and GenBank database under accession number of AY210701.

which are good candidates for gene expression controller and food grade selection marker [8, 13]. Using this information, we have constructed several expression vectors and successfully expressed foreign genes in *Bifidobacterium* such as amylase from *Bifidobacterium* [13], cholesterol oxidase from *Streptomyces* [14], pediocin from *Pediococcus* [11], endostatin from mouse, phytase from *E.coli* [Fig. 2, 10], glutamate decarboxylase from Rice [12] etc.

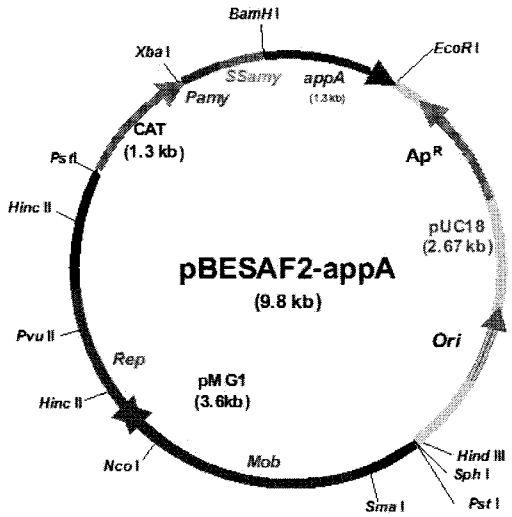

Fig. 2. Genetic map of the expression vector pBESAF2-appA. Pamy, promoter of *amyB;* SSamy, 72 N-terminal amino acids of *amyB,* the first 44 amino acids represent the secretion signal peptide sequence and the remainder encompass the mature N-terminus of amylase; *appA,* phytase gene from *E.coli* MC4100; ApR and CAT, ampicillin and chloramphenicol resistance genes, respectively; Ori, origin of replication of *E.coli;* Rep and Mob, replication initiation and plasmid mobilization determinant of *Bifidobacterium,* respectively; pMG1, plasmid originated from *B. longum* MG1.

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