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Escherichia coli BL21(DE3) chromosome contains a group II capsular gene cluster☆

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Abstract

During our study of *de novo* synthesis of *Escherichia coli* K1 capsular polysaccharides, we found that *E. coli* BL21(DE3) has a capsular gene cluster, similar to those of group II capsular *E. coli* strains. Analysis of the nucleotide sequence of the *E. coli* BL21(DE3) gene cluster showed homologues to all group II regions 1 and 3 genes and the presence of an IS1 element in one of the region 2 ORFs, which likely prevents capsule expression. Complementation analysis showed that region 1 and 3 genes encode functional proteins that are sufficient for the export of newly synthesized polysaccharide. The gene products of Bl21(DE3) *kpsC* and *kpsS* supported *in vitro de novo* synthesis of K1 polysaccharide when co-expressed with K1 NeuE and NeuS. Sequence homology between BL21(DE3) region 2 open reading frames and capsule-related genes in other bacteria such as *Haemophilus influenzae* serotype b, suggests that the encapsulated ancestor of BL21(DE3) may have produced a ribose/ribitol-phosphate containing polysaccharide.

Keywords: *E. coli* B; Capsule gene cluster; Polysaccharide

1. Introduction

Escherichia coli has been shown to produce more than 80 chemically and serologically distinct capsular polysaccharides (Ørskov and Ørskov, 1992). These capsular polysaccharides have been classified according to chemical structure and genetic properties of the strains into three (Jann and Jann, 1992) or four (Whitfield and Roberts, 1999) groups. A small group, classified as group II, contains most of the capsular types associated with invasive *E. coli* disease (Cross et al., 1984; Roberts, 1996). *E. coli* group II capsular polysaccharides have chemical structures similar to those of *Haemophilus influenzae* and *Neisseria meningitidis* capsular polysaccharides (Roberts, 1996). The gene clusters responsible for the production of group II capsules are organized in three regions. Region 2 is capsule specific, while regions 1 and 3 are genetically conserved and functionally interchangeable among group II encapsulated strains (Roberts et al., 1986).

E. coli K-12 and B strains and their derivatives are commonly used for genetic manipulations and are not encapsulated. *E. coli* BL21(DE3) is commonly used for the high-yield expression of recombinant proteins. This strain is a descendant of *E. coli* B, and was designed by Studier and Moffatt (Studier and Moffatt, 1986) as a host for the pET expression vectors containing the T7 promoter. In this study we show that the *E. coli* BL21(DE3) chromosome contains a gene cluster characteristic of group II encapsulated strains. There are many bacterial strains, encapsulated and acapsular, which contain homologues to various *E. coli* group II capsule genes. It is possible that these proteins have similar functions, or use the same substrates as their *E. coli* counterparts. Therefore, caution in interpretation of the results should be advised when *E. coli* B derivatives are used as host strains in capsule synthesis studies.

Abbreviations: CMP-NeuNAc, Cytidine-5'-monophospho-*N*-acetylneuraminic acid; CTAB, Cetyltrimethylammonium bromide; DEPC, Diethylpyrocarbonate; NeuNAc, *N*-acetylneuraminic or sialic acid; μl, Microliter; NMR, Nuclear magnetic resonance; PCR, Polymerase chain reaction.

☆ Nucleotide sequence accession number. Sequence of BL21(DE3) gene cluster was assembled with CAP3 (Contig Assembly Program) (Chao et al., 1999) and deposited at GenBank under accession number DQ242482.

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2. Materials and methods

2.1. Bacterial strains, media and growth conditions

The strains used in this study are described in Table 1. Bacteria were grown at 37 °C on Luria–Bertani (LB) agar plates or broth, or M9 medium (see below), supplemented with suitable antibiotics as needed. We used kanamycin (50 μg/ml), in experiments with pCR XL TOPO based vectors, or chloramphenicol (20 μg/ml), in experiments with pACYC Duet-1 based vectors, or ampicillin (50 μg/ml), in experiments with pSR426 (Table 2). In co-expression experiments in which two antibiotics were necessary, we reduced the concentration of each individual antibiotic by 25%.

2.2. Recombinant DNA procedures

Total BL21(DE3) chromosomal DNA was prepared by the CTAB method (without additional CsCl purification) as described in Ellington (1988).

All primers used in this study are presented in Table 2. Primers were custom synthesized by the CBER core facility.

We used a Primus 96 PCR-system (MWG Biotech, Inc) for DNA amplification reactions as described previously (Andreishcheva and Vann, 2006). BL21(DE3) region 3 and overlapping fragments of region 1 were amplified with puReTaq Ready-to-Go PCR Beads (Amersham) according to the manufacturer's instructions, PCR fragments were purified with a QIAquick Gel Extraction kit (Qiagen) and cloned into pCR XL TOPO (Invitrogen). We used BL21(DE3) chromosomal DNA as a template in these reactions. The description of six constructs, comprising BL21(DE3) *kpsFE*, *kpsDU*, *kpsCS*, *kpsMT*, *kpsS* 3′-terminus, and *kpsT* 3′-terminus (pWN664, pWN665, pWN666, pWN669, pWN667, and pWN670 correspondingly) is provided in Table 2. Relative positions of the primers used for amplification of these fragments are shown in Fig. 1. Plasmid DNA was purified with BioRad DNA purification kits. All sequencing reactions were performed by the CBER core facility.

The 6705 bp region between the BL21(DE3) *kpsT* 3′-terminus and the *kpsS* 3′-terminus was amplified with Expand High Fidelity Enzyme Mix (Roche) according to manufacturer's instructions. This PCR fragment was purified with QIAquick Gel Extraction kit (Qiagen), and sequenced directly, without preliminary cloning into the plasmid.

Whole cells were screened for the presence of gene sequences by PCR. Individual colonies were suspended in 10 μl of DEPC treated H_2O, and 1 μl aliquots of these suspensions were used per reaction. We used puReTaq Ready-to-Go PCR Beads (Amersham) according to manufacturer's instructions, for screening reactions.

Table 1
Bacterial strains used in this study

Strain	Origin and genotype	Source
BL21 (DE3)	B; F^- *ompT* *hsd*S_B(r_B^- m_B^-) *gal dcm* (DE3)	Invitrogen
HMS 174 (DE3)	K-12; F^- *recA1* *hsdR* (r_{K12}^- m_{K12}^+) (DE3) (Rif^R)	Novagen
MS108	K5; *ΔkfiA* (Str^r)	Kind gift of Dr. Roberts (Stevens et al., 1997)
B	Wild type	ATCC# 11303, deposited by S.E. Luria (Delbrück and Luria, 1942)
EV36	K-12/K1 hybrid strain harboring chromosomal K1 gene cluster; $argA^+$ kps^+ rha^+	Kind gift of Dr. Vimr (Vimr and Troy, 1985)

2.3. Preparation of the membranes for sialyltransferase assays

Membrane fractions were prepared as described in Andreishcheva and Vann (2006).

2.4. Sialyltransferase assays

Sialyltransferase assays were performed as previously described (Andreishcheva and Vann, 2006). Briefly, 0.25 nmol ^{14}C-CMP-NeuNAc (0.15 μCi), membrane preparation (0.5–0.7 mg protein), and 4 μg of colominic acid were mixed in buffer A (50 mM Tris–HCl, pH 8.0, 25 mM $MgCl_2$), to a final volume of 50 μl. The mixture was incubated at 37 °C for 1 h, the reaction was terminated by spotting 30 μl aliquots on Whatman 3M paper, and developed as described (McGowen et al., 2001). Incorporation of the radiolabel into chromatographically immobile product was normalized per mg of membrane protein. Polysaccharide production *de novo* was measured by replacing the colominic acid with a buffer solution.

2.5. Determination of protein concentration

Protein concentrations of the membrane preparations were determined with BCA Protein Assay Reagent (Pierce).

2.6. Recombinant endo-N-acetylneuraminidase

Endoneuraminidase was expressed and purified as described (Andreishcheva and Vann, 2006).

2.7. Isolation of polysaccharide synthesized in vivo

BL21(DE3):pSR426 cells were grown at 37 °C on M9 medium (M9 salts (Sigma Chemical Co.) (11.34 g/l), casamino acids (12 g/l), glucose (10 g/l), and 1% low molecular weight fraction (LMW) of yeast extract (v/v)), supplemented with ampicillin (50 μg/ml), until the $A_{600\ nm}$=0.5–0.6 and induced with 1 mM IPTG for 3 h. The cells were removed by centrifugation, and the polysialic acid was purified from the cleared cultural supernatant as described (Vann and Freese, 1994). The LMW fraction of yeast extract was prepared as described (Vann and Freese, 1994).

2.8. Electrophoresis of polysaccharide synthesized in vivo

Polyacrylamide gels were prepared as described by Pelkonen et al. (Pelkonen et al., 1988). Samples for electrophoresis were