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Regulation of genes expression on *Escherichia coli* genome with communications among microbes

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Research aims

Bacteria mostly live in communities composed of many other microorganisms and often in association with higher organism hosts, such as animals, plants, and insects. Individual bacteria must alter their behaviour through interaction between microorganisms or interaction between bacteria and host to maintain their survival in complex natural environments. The chemical interaction between bacterial cells is known to be quorum sensing (QS), by which bacterial population-dependent response is controlled through diffusible molecules produced by individual cells. The QS has been found to be involved not only in interactions between cells of the same bacterial species, but also involved in interactions between cells of different bacterial species or between bacterial cells and their host cells. Here we attempted to identify *Escherichia coli* genomic functions related to communications between *E. coli* cells or communications between cells of *E. coli* and another bacterial species, *Bifidobacterium longum*.

N-acetylymuramic acid (MurNAc), a component of *E. coli* cell wall, can be used as a carbon source by *E. coli* cells.[1)] The *murQP* operon of *E. coli* encodes the enzymes for the degradation of MurNAc. The operon is located next to the *yfeT* gene on the *E. coli* genome, each being transcribed into opposite directions. In this study, YfeT was identified as a regulator for the *murQP* operon.[1,2)] Furthermore, we identified *E. coli* promoters induced only when *E. coli* was cocultured with *B. longum*.

Methods

For measurement of the activity of *yfeT* and *murQ* promoters, we constructed *yfeT-lacZ* and *murQ-lacZ* protein fusions. The *lacZ* fusion genes on the plasmid were inserted into λRS45 phage by recombination. Using the recombinant lambda phages, lysogens of *E. coli* BW25113 (parent strain) and DI0200 (*ΔyfeT*) were constructed: [DI0101 (BW25113, *λmurQ-lacZ*); DI0102 (BW25113, *λyfeT-lacZ*); DI0201 (DI0200, *λmurQ-lacZ*); and DI0202 (DI0200, *λyfeT-lacZ*)]. The lysogens were grown in M9-glycerol medium and then subjected to measurement of β-galactosidase activity.[2)]

Construction of the promoter collection of *E. coli* K-12 W3110 strain is being carried out in PPT (Promoter Project Team) using pGRP as a vector. The PPT collection set contains about 1000 plasmid clones in which each *E. coli* promoter was translationally fused with the *gfp* (green fluorescent protein) reporter gene. Each *E. coli* transformant harboring one of the PPT plasmid clones was grown with and without *B. longum* in ABCM medium in a well on a 96-well plate under anaerobic condition and then the intensity of GFP in each well was measured.

Results

Transcriptional regulation of enzymes for N-acetylymuramic acid degradation in E. coli.

The lysogens, DI0101, DI102, DI0201, and DI0202, were grown in M9-glycerol medium at 37°C. At stationary phase (OD 600 nm = 1.4), cultures were subjected to measurement of β-galactosidase activity. The high-level expression of *murQ-lacZ* and *yfeT-lacZ* was detected only in the *yfeT* deficient mutant (Fig. 1A). Jaeger and Mayer recently reported that YfeT is indeed involved in repression of the *murQ* promoter and proposed to rename it to MurR.[3)] In good agreement with their report, our finding confirmed that YfeT represses transcription of both *murQP* and *yfeT* operons. MurNAc is transported and converted to MurNAc-6-phosphate by MurNAc-specific membrane component (MurP) of MurNAc-specific enzyme IIBC PTS (phosphotransferase system). The addition of MurNAc and MurN induced the expression of both *murQ-lacZ* and *yfeT-lacZ* in wild-type *E. coli* (Fig. 1B), supporting the prediction that MurNAc-6-phosphate is the inducer of YfeT *in vitro*.[3)]

Identification of E. coli promoters induced in co-culture with B. longum.

Each of the approximate 1,000 PPT plasmid clones, each of which contains a different *E. coli* promoter fused to the *gfp* reporter gene, was transformed into *E. coli* DH5α. *E. coli* transformants obtained and *B. longum* were separately grown in ABCM medium at 37°C under anaerobic condition. Then, each of *E. coli* culture (5×10^4 cells) was mixed with *B. longum* culture (4.95×10^6 cells) in fresh ABCM medium and then incubated at 37°C for 20 hours under anaerobic condition. The fluorescence intensity of each culture was measured with FL600 (Bio Tek) and plotted in the scatter plot (Fig. 2). Twenty-eight *E. coli* promoters were induced in co-culture with *B. longum*. Finally four promoters, *lysC*, *macA*, *proX*, and *ygaU*, were confirmed to be induced in a manner dependent on number of *B. longum* cells

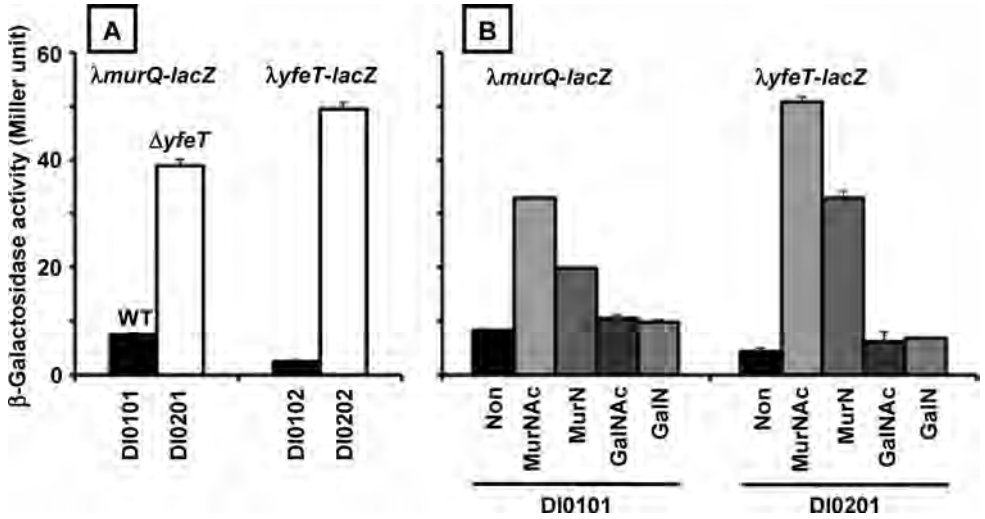

Fig. 1. Repression of *murQ* and *yfeT* expressions by YfeT in an MurNAc-dependent manner. [A] *murQ* (left) and *yfeT* (right) expression in *E. coli* wild-type (black bar) and *ΔyfeT* (white bar). [B] The effect on *murQ* (left) and *yfeT* (right) expressions generated by addition of MurNAc, MurN, GalNAc, and GalN in *E. coli*.

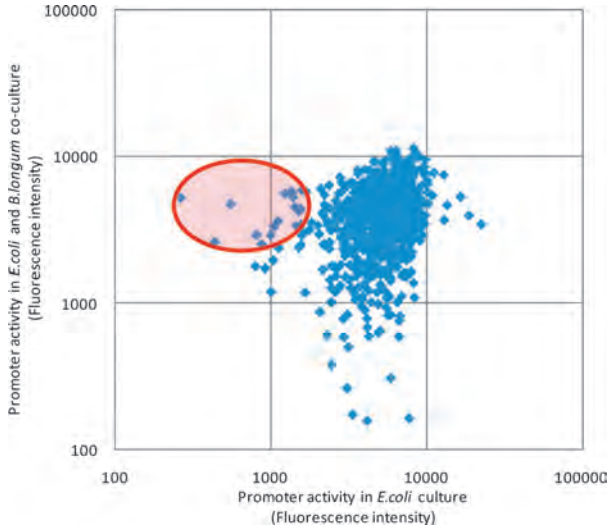

Fig. 2. The expression profile of *E. coli* promoters with and without *B. longum*. The intensity of fluorescence from promoters was measured in culture with (vertical line) and without (horizontal line) *B. longum* and spotted. Red circle indicates the induced promoters in co-culture with *B. longum*.

present in the stationary cultures.

E. coli promoters induced only in the presence of B. longum cells.

One possible mechanism of promoter induction in co-culture with *B. longum* is that small molecule secreted by *B. longum* is a signal triggering gene expression from the promoters, as is the case in QS system. If this is the case, conditioned medium (CM) of *B. longum* must induce the identified *E. coli* promoters even in the absence of *B. longum* living cells. *B. longum* was grown to stationary phase and pelleted by centrifugation. The supernatant was recovered and pH was adjusted to 7.2 with NaOH. The solution was supplemented with LB medium (final conc., 0.25×LB), and then sterilized by filtration through a 0.22 μm filter to generate CM. Each *E. coli* transformant carrying the *lysC*, *macA*, *proX*, or *yagU* promoter fused to the *gfp* gene was grown in CM and the intensity of fluorescence was measured. Among four promoters, only *ygaU* promoter was induced by CMs of *B. longum*. These results suggested that *ygaU* is induced by some factor(s) in *B. longum* CM and the remaining three promoters, *lysC*, *macA*, and *proX*, seem to be induced by an as-yet-unidentified mechanism operating in cell-cell communication between cells of *E. coli* and *B. longum*.

Conclusion

Molecular mechanism for communication between E. coli cells

MurNAc is a component of *E. coli* cell wall. The *E. coli murQP* operon encoding enzymes for MurNAc degradation is induced by MurNAc or MurN, which is derived from dead *E. coli* cells.[4] Thus, the regulatory system for expression of *E. coli murQP* operon could represent a mechanism for communication between cells of *E. coli*, by which *E. coli* living cells can effectively utilize MurNAc molecules derived from dead cells of *E. coli* in natural environments.

Molecular mechanism for communication between E. coli and B. longum cells

Three *E. coli* promoters, *lysC*, *macA*, and *proX*, were induced only in the presence of living cells of *B. longum*, suggesting that a novel molecular mechanism might be operating in communication between *E. coli* and *B. longum* cells.

References

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