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Characterization of an Oxygen-Dependent Inducible Promoter Systems, the nar Promoter of *Escherichia coli*, and Gram negative host strains

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ABSTRACT

The nar promoter of *Escherichia coli* was known to induce maximally under anaerobic or microaerobic conditions in the presence of nitrate. In this study, the nar promoter was tested to see whether the expression level of a reporter gene which fused lacZ gene at nar promoter's downstream, in the some gram negative host strains(*Agrobacterium*, *Pseudomonas* and *Rhizobium*). A nar promoter system(combination of nar promoter and gram negative strain) was grown under aerobic conditions to absorbance at 600 nm of nearly 2.0 and then, the nar promoter was induced by lowering DO to 1-2% with alternating microaerobic and aerobic condition in the fermentor cultures, using different gram negative hosts. For a wild type nar promoter (pNW61), it was possible to maintain production of β-galactosidase activity per cell(specific β-galactosidase activity) at 14,000, 9600, 45 Miller units in the presence of 1% nitrate. and for a nitrate - independent nar promoter (pNW618) at 12,000, 10,400 and 58 Miller units in the absence of nitrate ion, respectively.

INTRODUCTION

Plasmid-based expression vectors with inducible promoters have been used to ensure expression at the proper time. In the present time, although many expression vectors including lac, trp, tac, λpL, and phage T7 promoter have been developed and used for the industrial production. Each promoter has its own problem in the induction process[1] as an alternative to these promoters, oxygen-dependent inducible promoters. The nar promoter obtained from E.coli, has been characterized for the following advantages[1)2)3)4)] as the nar promoter can be induced by lowering DO concentration in the absence or presence of nitrate ion. The induction properties of the nar promoter cloned into pBR322

plasmid are dependant on the nucleotide sequence of the nar promoter, mutations on the chromosome of the host, and host E.coli strains. when the wild type nar promoter was used, addition of nitrate ion in addition to anaerobic condition was necessary for the maximal induction of the nar promoter.[6]

MATERIALS AND METHODS

The gram negative strains, *Agrobacterium.tumefaciens* LBA 4404, *Pseudomonas.fluorescens* and *Rhizobium.meliloti* MB501 were a wide-type strain. For Plasmids, pMW61 having backbone of pBR322 was constructed by fusing the nar promoter containing amino terminus of the first structural gene of the nar operon, narG, with the lac Z gene and pMW618, a derivative of pMW61, was made by site directed mutagenesis on the -10 and -35 regions of the wild-type nar promoter and pNW61, pNW618 having backbone of pBBR122 to use broad host range vector, a derivative of pMW61, pMW618, respectively, was cloned on the regions of nar promoter and lacZ. The specific β -galactosidase activity, β-galactosidase activity per cell, was measured by the Miller method. Miller units were used to express the specific β-galactosidase activity.

RESULTS & DISCUSSION

To find a nar promoter can be used as an oxygen-dependent inducible promoter, two E.coli mutants having pMW61 or pMW618 and three wild-type gram negative strains were tested with manufacturing of DO level for induction in the absence or presence of 1% nitrate ion from the time of inoculation. The pMW61, pMW618 system of the specific β-galactosidase activity increased sharply and continuously after induction up to OD_{600} = 3.10, 3.35. Each of nar promoter system had their highest expression level of β- galactosidase were approximately 29,700 and 28,400 Miller units, respectively.[Fig. 1] Three gram negative strains with wild-type or modified nar promoter were studied to find their characterization was oxygen-dependent inducible in the absence or presence of nitrate ion in fermentor cultures. For *Agrobacterium* system with pNW61 or pNW618, the specific β-galactosidase activity increased sharply and continuously up to OD600 - 2.36 and 2.57 where it became 14,600 and 13,900 Miller units, respectively, after 2-3 h of induction. This results were similar to E.coli system but their maximal specific β-galactosidase activity was lower than

E.coli's.[Fig. 2] For *Pseudomonas* systems with pNW61 or pNW618, the specific β-galactosidase activity was increased continuously up to OD_{600} = 2.22 and 2.48 after induction so, their systems had the highest 12,900 and 12,400 Miller units, respectively, in the same conditions after 5-8 h of induction. Specially, because they had the high specific β-galactosidase activity before induction their induction ratio was lower than E.col and Agrobaterium's.[Fig. 3] When the nar promoter systems with wild-type *Rhizobium* were tested in the same conditions we saw the results unlike the other systems. their specific β-galactosidase activity never increased after they were induced by lowering DO level at OD_{600} of 2.0.[Fig. 4] The wild-type *Agrobacterium* strain regulated well to nar promoter of E. coli but had low maximal specific β-galactosidase activity than E. coli's. Therefore, it has the capability which regulatory proteins were transacted from its chromosome. For *Pseudomonas*, expression of β-galactosidase was regulated well by DO level but by nitrate ion because NARL, important one of regulatory proteins was not transacted or had low binding force. At last, *Rhizobium* was not regulated and the specific β-galactosidase activity was never increased or decreased by DO level, nitrate ion, maybe, because of depletion of one or all of regulatory protein, which was unknown.

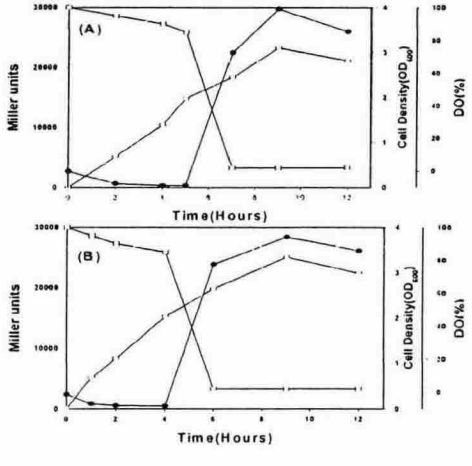

Figure 1. Effect of mutant host E.coli strains on the induction of nar promoter in batch fermentor cultures. (pMW61/RK5265)(A), (pMW618/W3110narL⁻)(B), OD600(○), DO level(□) and the specific β-galactosidase activity(●).

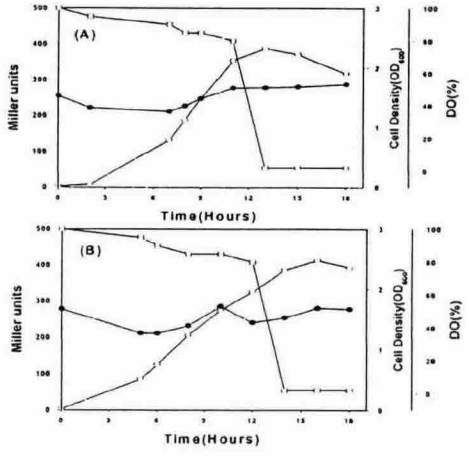

Figure 4. Effect of Wild-type *Rhizobium* host strain on the induction of nar promoter in batch fermentor cultures. (pNW61)(A), (pNW618)(B), OD600(○), DO level(□) and the specific β-galactosidase activity(●).

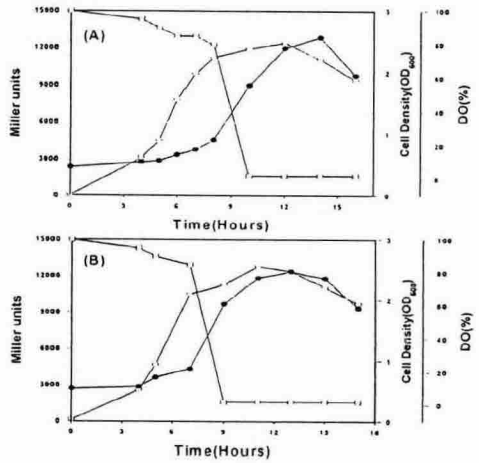

Figure 3. Effect of Wild-type *Pseudomonas* host strain on the induction of nar promoter in batch fermentor cultures. (pNW61)(A), (pNW618)(B), OD600(○), DO level(□) and the specific β-galactosidase activity(●).

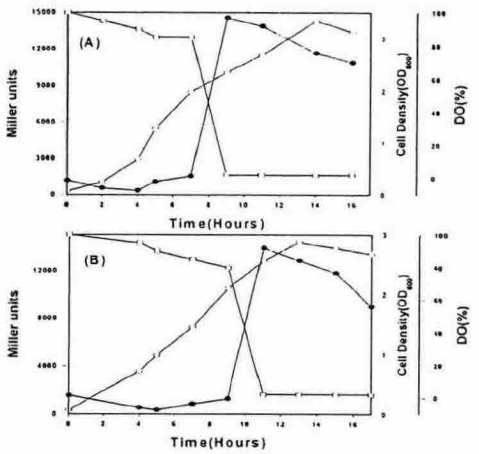

Figure 2. Effect of Wild-type *Agrobacterium* host strain on the induction of nar promoter in batch fermentor cultures. (pNW61)(A), (pNW618)(B), OD600(○), DO level(□) and the specific β-galactosidase activity(●).

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