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Nitrite activates the transcription of the *Pseudomonas aeruginosa* nitrite reductase and cytochrome *c*-551 operon under anaerobic conditions

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The transcription of the *Pseudomonas aeruginosa denAB* operon, which consists of the nitrite reductase and cytochrome *c*-551 genes, is induced under anaerobic conditions. However, under anaerobic non-denitrifying conditions (anaerobic growth on arginine), the promoter activity of the operon was approximately one-fifth of that under anaerobic denitrifying conditions (anaerobic growth in the presence of nitrite or nitrate). This result clearly demonstrates that the presence of nitrite or nitrate activates the transcription of *P. aeruginosa denAB* operon under anaerobic conditions.

Nitrite reductase; Cytochrome *c*-551; Regulation; *Pseudomonas aeruginosa*

1. INTRODUCTION

Nitrite reductase (cytochrome cd_1: EC 1.9.3.2) of *Pseudomonas aeruginosa* [1] catalyzes the reduction of nitrite to nitric oxide [2] and thus plays an important role in anaerobic respiration (denitrification). Coyne et al. have immunochemically shown that the enzyme is synthesized exclusively in denitrifying cells [3]. We have recently proved that the transcription of the nitrite reductase gene (*denA*) is induced only under anaerobic conditions [4]. The gene encoding the physiological electron donor for nitrite reductase, cytochrome *c*-551 (*denB*) which is located 50 bp downstream of *denA* [5] is also co-transcribed as an operon. In this work, we report that nitrate and nitrite, the terminal electron acceptors of denitrification, activate the transcription of the *denAB* operon.

2. MATERIALS AND METHODS

A new effective promoter probe system was developed for this experiment. pHA50 was constructed by ligating a *Bam*HI-*Xho*I fragment carrying *xylE* encoding catechol 2,3-dioxygenase (C23O) from pTS1045 [6] to the *Bam*HI, *Sal*I sites of a broad host range vector, pMFY40 [7] (pHA50, Fig. 1). pHA51 was also constructed to be used as a control vector. To remove the promoter of the tetracycline resistance gene of pMFY40 which is encoded in the *Bam*HI-*Hin*dIII region of pHA50, pHA50 was linearized by *Bam*HI and *Hin*dIII, recessed 3′ termini were filled by the Klenow fragment of *E. coli* DNA polymerase I [8] and re-circularized (pHA51, Fig. 1). A 1.5 kb *Sph*I fragment from *Pseudomonas aeruginosa* chromosomal DNA [4] which carries the promoter, regulatory region and the 5′ part of *denA* was once ligated to the *Sph*I site of pUC19 and then cut down by digesting with *Hin*dIII and *Bam*HI, and substituted for the *Hin*dIII, *Bam*HI fragment of pHA50 (pHA501, Fig. 1). pHA501 is a transcriptional fusion of the *denAB* promoter and *xylE*, thus transcription from the *denAB* promoter can be monitored by measuring C23O activity. pHA51 and pHA501 were transferred to *P. aeruginosa* PAO1161 [9] and the enzyme activities of C23O in cell-free extract of cells grown under various growth conditions were measured (Table I). For growth under denitrifying conditions, $NaNO_3$ (100 mM) or $NaNO_2$ (20 mM) was added to YT broth. Arginine (40 mM) was added to YT broth for the anaerobic, non-denitrifying cultivation, because *P. aeruginosa* can grow without the respiratory electron acceptors such as oxygen or nitrate when arginine is added to the medium [10]. The methods of DNA manipulation, anaerobic cultivation and enzyme assay were described previously [4].

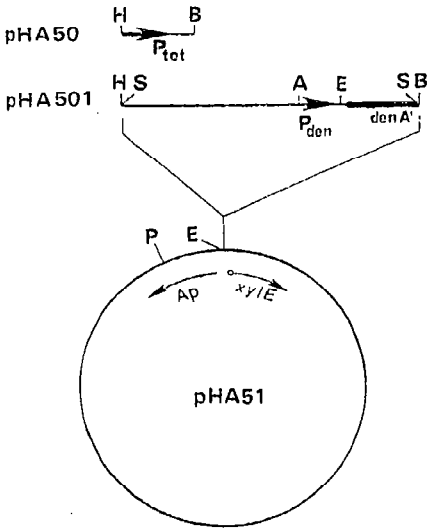

Fig. 1. Plasmids used for the C23O assay. pHA51 was constructed by introducing *xylE* of pTS1045 to pMFY40. pHA501 was constructed by substituting the promoter of *denAB* (P_{den}) for the promoter of tetracycline resistance gene (P_{tet}). As a negative control, pHA51 was constructed by removing P_{tet}. Ap, ampicillin resistance gene; A, *Apa*I; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sph*I.

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Table I

Activity of catechol 2,3-dioxygenase (C23O) in the cell-free extracts of *P. aeruginosa* PAO1161 harbouring pHA51 or pHA501

	C23O activity (U/mg protein)						
	Aerobic			Anaerobic			
	YT	NO_3^- (100 mM)	NO_2^- (20 mM)	NO_3^- (100 mM)	NO_2^- (20 mM)	Arg (40 mM)	NO_2^- + Arg
pHA51	2.04	2.89	2.16	2.71	1.70	2.64	2.26
pHA501	3.94	2.02	3.22	46.92	49.79	10.55	60.40

3. RESULTS AND DISCUSSION

C23O activity was induced only under anaerobic conditions. Under non-denitrifying conditions, however, C23O activity was very low compared to denitrifying conditions. This low activity was not due to the inhibition by arginine, because the activity was restored when both arginine and nitrite were added. This result clearly demonstrates that the presence of nitrate or nitrite activates the transcription of the *denAB* operon. The substrate for nitrite reductase, nitrite, seems to be the activating factor, because under anaerobic conditions, nitrate is reduced to nitrite by nitrate reductase. As described previously [4], transcription from the *denAB* promoter was not induced under aerobic conditions even in the presence of nitrate or nitrite (see also Table I). Thus, the expression of *denAB* is believed to be regulated by two steps, namely, repression by molecular oxygen and induction by nitrite.

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