

Biochemical study of CymJ, the putative regulator of cyclodextrin metabolism in *Klebsiella oxytoca* M5a1

Tri Yudani Mardining Raras¹, August Boeck²

Abstrak

Untuk melangsungkan metabolisme siklodekstrin, suatu oligoglukosida siklik, *Klebsiella oxytoca* (*K. oxytoca*) memerlukan produk dari sepuluh gen yang tergabung dalam dua operon yang berlawanan arah. Telah dilakukan penelitian mengenai fungsi CymJ, yaitu produk gen yang berada pada posisi paling jauh dari promoter pada salah satu operon *cym*. Overekspresi *cymJ* pada *K. oxytoca* menyebabkan penekanan ekspresi operon *cym*. Akan tetapi represi ini dapat dikembalikan pada kondisi normal dengan penambahan α -cyclodextrin ke dalam medium. Diduga ada hubungan antara CymJ dan CymD karena ketiadaan CymD pada mutan delesi *cymD* mencegah efek represi oleh overekspresi CymJ. Penemuan yang tidak disangka-sangka adalah hambatan pembelahan sel yang berimbas pada terhambatnya pertumbuhan akibat overekspresi *cymJ*. Perubahan morfologik ini disertai dengan peningkatan sensitivitas *K. oxytoca* terhadap ampisilin. (*Med J Indones* 2007; 16:69-77)

Abstract

The products of ten genes clustered in two divergently oriented operons are required for the metabolism of cyclic oligoglucosides, the cyclodextrins, by *Klebsiella oxytoca*. The function of CymJ, the product of the promoter distal gene in one of the operons was studied. Over expression of *cymJ* in *K. oxytoca* led to strong reduction of the expression of the *cym* operons. This repression could be alleviated by addition of high concentration of α -cyclodextrin into the medium. There is a possible relationship between CymJ and CymD since the absence of CymD in a *cymD* deletion mutant prevented the repression effect of CymJ. An intriguing finding was that the presence of CymJ in large amount in the cell caused severe cell division inhibition leading to retardation of growth. This morphological change was paralleled by a significant increase in the susceptibility of *K. oxytoca* to ampicillin. (*Med J Indones* 2007; 16:69-77)

Keywords: *cym* operon, putative regulator, over expression

At least ten genes have been identified up to now to code for proteins which are involved in cyclodextrin metabolism by *Klebsiella oxytoca* (*K. oxytoca*) M5a1. They are designated as *cymA-J* (cyclodextrin metabolism genes).¹⁻³

The biochemical functions of several gene products have been characterised; *cymA* encodes a cyclodextrin specific outer membrane porin;¹ CymE is a periplasmic binding protein and CymF, CymG and CymD are other members of an ABC type transporter specific for cyclodextrins. They are functionally homologous to MalE, MalF, MalG and MalK of *E. coli*, respectively.¹ CymH is a cytoplasmatically located cyclodextrinase.⁴

The functions of several gene products of the *cym* operon, namely CymB, CymC, CymI and CymJ are not known. In this study we concentrated on the analysis of the function of CymJ. The *cymJ* gene is the promoter most distal gene in one of the two *cym* operons. Computer analysis of the CymJ sequence suggested that it is 31 kD in size and that it does not contain a signal peptide.⁵ CymJ lacks significant sequence similarity to any other protein in the data base. It displays only very low sequence similarity to the N-terminal region of YhrO from *Bacillus subtilis*,⁶ and to AF1009 of *Archaeoglobus fulgidus*.⁷ Circumstantial evidence had indicated that CymJ could be a regulator of the *cym* operon. This assumption was based on experiments in which the overexpression of *cymJ* in wild-type cells lead to the repression of the expression of the *cym* operon.¹ In this study we report more detailed information concerning the function of CymJ in the *cym* metabolism system.

¹ Laboratory of Biochemistry-Molecular Biology, Faculty of Medical Science, Brawijaya University, Malang, Indonesia

² Department of Biology I, Ludwig-Maximilians University, Munich, Germany

METHODS

To characterize CymJ several biochemical and physiological approaches were conducted i.e construction of *cymJ* overexpression system, antibiotic susceptibility study of *cymJ* overexpression mutant, determination of cellular localization of CymJ, biochemical, morphological, and physiological studies upon overexpression of *cymJ* both in homologous and heterologous system as well as in *cymJ* deletion mutant.

Media and growth conditions

The rich medium used for routine growth of cells was Luria Broth (LB).⁷ When necessary, antibiotics were added to a final concentration of 100 µg/ml (ampicillin) and 50 µg/ml (kanamycin).⁸ In the case of *K. oxytoca*

M5a1, the concentration of ampicillin had to be increased to 800 µg/ml because of the expression of a chromosomally encoded β-lactamase.⁹ "Werkman" minimal medium was prepared as described previously¹⁰ and contained 1% (w/v) carbon source (glycerol, if not otherwise indicated).

Strains, plasmids and oligonucleotides

The bacterial strains used through this work are listed in Table 1, and Table 2 shows relevant plasmids used. During the experiment employing *K. oxytoca* deletion mutants, the presence of the deletion in the chromosome was confirmed via PCR tests with primers that are listed in table 2.

Table 1. Bacterial strains

Strain	Genotype or relevant property	Reference
<i>K. oxytoca</i>		
M5a1	Wild type	[11]
CYMA	M5a1, <i>cymA</i>	[3]
CYMCD	M5a1, <i>cymCD</i>	[5]
CYME	M5a1, <i>cymE</i>	[12]
REGB	M5a1, <i>lac</i> , <i>scrA</i> :: (<i>'uidA-(cymE'-cymA)'</i> - <i>lacZ</i>)	[5]
<i>E. coli</i> K12		
K38	HfrC <i>phoA4 pit-10 tonA22 ompF627 relA1 λ⁺</i>	[13]

Table 2. Plasmids and oligonucleotides used in this work

Plasmid	Genotype	Reference
Vectors		
pUC18/19	<i>bla lacZα</i>	[14]
pUC19-k	<i>kan lacZα</i>	This work
pT7-5	<i>bla pT7</i>	[15]
pT7-CYMJ	<i>bla cymJ</i>	This work
pGP1-2	<i>kan cI857 T7Gene1</i>	[15]
Recombinant plasmids		
pCYMJ	pUC18, <i>plac-cymJ</i>	[5]
pCymJ-k	pUC18, <i>plac-cymJ</i> , <i>kan</i>	This work
Oligonucleotides		
Designation	Location	Sequence (5'-3')
Universal	pUC19	GTAAAACGACGGCCAGT
Reverse	pUC19	AGCGGATAACAATTTACACAGGA
GM9	5' in <i>cymC</i>	GGCCGAATCGGTCCACGC
GM10	5' in <i>cymA</i>	GGTAACGGTTGGGCTACTGG
MC6	3' in <i>cymA</i>	CGCCACCACTACGATCAG
MC14	5' in <i>cymB</i>	TAGTATCACAGGCCTGGG
MC16	5' in <i>cymCD</i>	GTCGGGGAATAAAATAG
GM17	3' in <i>cymCD</i>	GTGATGAGGTGAGCTACG
MC7	5' in <i>cymE</i>	CCCATTTCCGGAATAGAT
MC15	3' in <i>cymE</i>	CAGCCAGGTAAGATTTAC

General DNA manipulation techniques

Unless otherwise mentioned, the standard DNA procedures, such as restriction endonuclease digestions, cloning and transformation procedures, were performed as described by Sambrook and Ausubel.^{8,16} Due to the excess of slime polysaccharides produced, *K. oxytoca* had to be transformed via electroporation. Plasmids were either prepared following the procedure given by Holmes and Quigley¹⁷ or with the aid of the Qiagen Kit (Qiagen GmbH, Hilden, Germany) as described by the manufacturers.

For the polymerase chain reaction (PCR), Goldstar DNA polymerase was used employing the conditions suggested by the manufacturers (Roche Biochemicals, Penzberg, Germany). Polymerase chain reaction

experiments were performed with a Biometra personal cyclor (Göttingen, Germany). Recovery of DNA-fragments from agarose was carried out by the 'low melting agarose' technique¹⁸ or using the Qiagen Kit.

Construction of *cymJ* over-expression plasmid

To construct the plasmid for the over-expression of the *cymJ* gene, the pT7-5 vector was digested with PstI and EcoRI. The *cymJ* fragment was generated from plasmid pCYMJ by also digesting with PstI and EcoRI. Ligation of the two fragments resulted in pT7CYMJ (Figure 1). This plasmid was then transferred into *E. coli* strain K38. To ensure that the plasmid was maintained in the cells during the experiment, PCR test using reverse and universal primers (Table 2) was performed before and after the experiment.

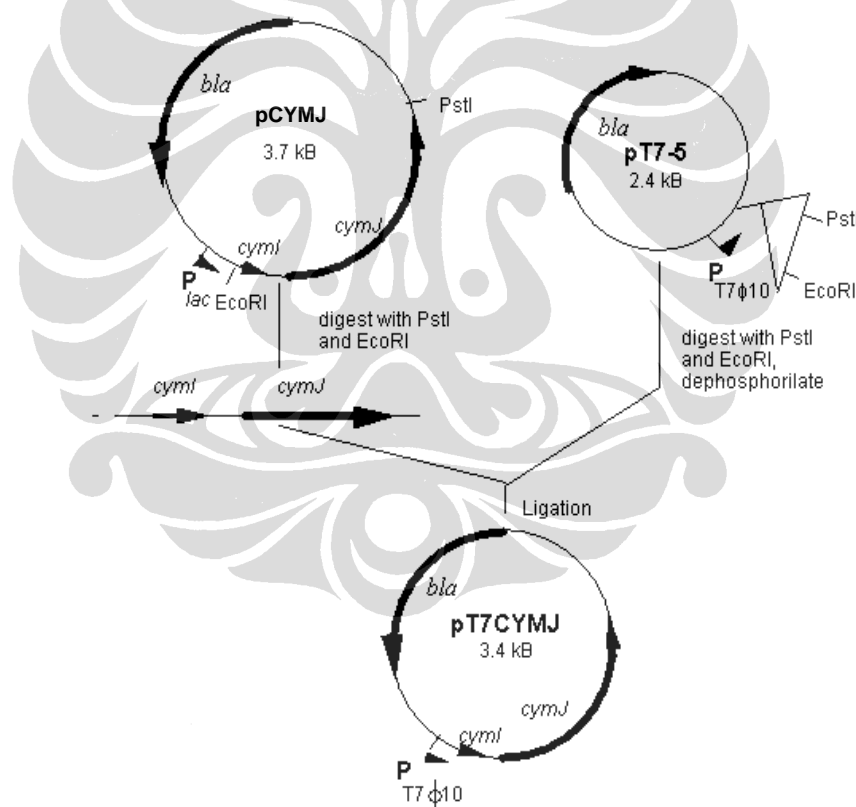


Figure 1. Construction of pT7CYMJ. pCYMJ and pT7-5 were digested with EcoRI and PstI. The fragment of *cymI-cymJ* was ligated into linearised pT7-5.

Cellular localisation of CymJ protein

The over-expression of *cymJ* for cellular localisation studies was performed employing the system described by Tabor and Richardson.¹⁵ For that purpose, *E. coli* strain K38/pGP1-2 was transformed either with plasmid pT7CYMJ or pT7-5. Twenty ml cultures containing the transformants were grown aerobically at 30°C in LB medium containing 100 µg/ml ampicillin and 50 µg/ml kanamycin to an OD₆₀₀ of 0.7. The cells were harvested by centrifugation at 6000 x g, washed twice with minimal medium and re-suspended in 1 ml minimal medium supplemented with 0.4% glucose, thiamine (2 µg/ml) and 18 amino acids (100 µg/ml) except cysteine and methionine. The suspension was used to inoculate 24 ml of the same medium which was then further incubated for one hour at the same temperature. To induce the synthesis of T7-polymerase, the culture was shifted to 42 °C for 20 min. The inhibition of the synthesis of the host RNA-polymerase was achieved by addition of rifampicin (300 µg/ml) and the culture was incubated for additional 20 min. After transfer of the culture to 30 °C and incubation for 10 min, 10 µCi/ml of [³⁵S]-methionine (NEN Life Science Products, Bad Homburg, Germany) were added and incubation was continued for 15 min. The labelling was terminated by addition of unlabelled methionine to 1 mg/ml. After 5 min cells were collected by centrifugation and stored at -80 °C.

Cells were thawed, suspended in 50 mM potassium phosphate buffer pH 7.0 and broken by sonification three times for 20 seconds set at 40. To separate the soluble protein from the insoluble material, the samples were centrifuged at 8000 x g for 10 min followed by centrifugation at 30.000 x g for 30 min. The separation of cytosolic proteins and membrane proteins was performed by centrifugation at 100.000 x g. The membrane associated proteins were released using sarkosyl¹⁹ or Triton X-100 (Sigma, Deisenhofen, Germany). The labelled protein was separated on 12.5 % SDS-polyacrylamide gels and the gel was autoradiographed. The localisation of CymJ was analysed using a phosphoimager.

Biochemical study of the *cym* gene expression

In this experiment *cymJ* was cloned into pCYMJ under the control of *lac* promotor and was overexpressed in *K. oxytoca* REGB. This strain carries a chromosomally integrated *cym* intergenic region flanked by the

reporter genes *lacZ* and *uidA*. The transcription can be followed by measuring β-galactosidase and glucuronidase activities.⁵

Determination of beta-galactosidase activity

The β-galactosidase activity of *lacZ* fusion strains was determined in permeabilised cells.⁵ Cultures were grown in LB medium until they reached an OD₆₀₀ of 0.7, then 1.25 ml were taken and used to inoculate 8.75 ml prewarmed minimal medium supplemented with α-cyclodextrin (final concentration 0.5%). The cultures were further incubated for one hour and the β-galactosidase activity was measured.

Morphological studies on CymJ overexpression

To observe any morphological changes caused by the overexpression of *cymJ*, cultures were grown to an OD₆₀₀ of 0.2 at 37°C with vigorous shaking in minimal medium containing 0.4 % glucose. The over-expression of *cymJ* was then induced by addition of 0.5 mM IPTG; after 3 h α-cyclodextrin was added at the final concentration of 0.5 % (w/v). At intervals of one hour the growth of the culture was monitored spectrophotometrically and the cell morphology was studied by microscopy. Cells were visualized and photographed using a Zeiss-Axioplan microscope, using phase contrast conditions. Estimation of the difference in an average cell length between strains REGB/pCYMJ and REGB/pUC19 was achieved by measuring cell length in photographs printed at the same magnification.

For the study of the overexpression of *cymJ* in *cym* deletion mutant, plasmid pCYMJ was transferred into *K. oxytoca* CYMA, *K. oxytoca* CYME and *K. oxytoca* CYMCD. These transformants were then grown in the same medium as above and under the same condition and treated exactly the same way as the wild type.

RESULTS

Antibiotic susceptibility of various *K. oxytoca* is shown in Table 3.

Cellular localisation of CymJ

The autoradiograph of the SDS-PAGE (Figure 2) shows that the labelled CymJ is present predominantly in the soluble fraction (lane 2). The result does not indicate whether CymJ is located in the cytoplasm or

periplasm, however, in a few preparations it was found out that some CymJ protein also co-sedimented with membrane fractions (data not shown).

Table 3. Antibiotic susceptibility of *K. oxytoca* REGB/pCYMJ transformants compared to *K. oxytoca* M5a1 and *K. oxytoca* REGB/pUC19

Strain	MIC in LB	MIC in Minimal Medium + 1 % Glycerol
	Amp (µg/ml)	Amp (µg/ml)
<i>K. oxytoca</i> M5a1	800	800
<i>K. oxytoca</i> REGB/CYMJ	800	200
<i>K. oxytoca</i> REGB/pUC19	800	800

Amp : Ampicillin

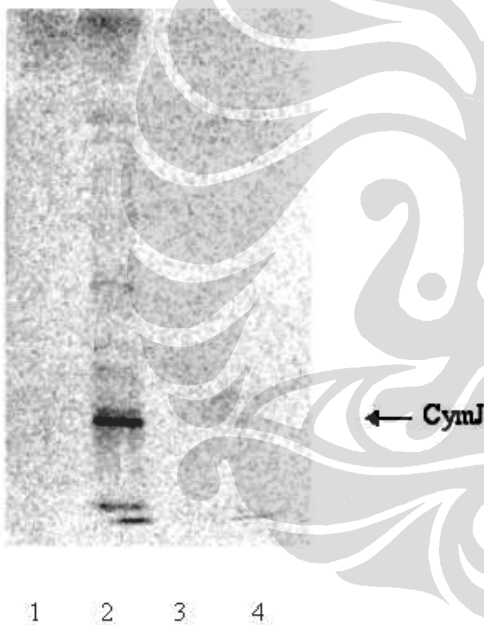


Figure 2. Autoradiograph of an SDS gel in which soluble(s) and pellet (P) fractions of ³⁵S-methionine-labelled cells were separated. Lane 1: S100 of K38/pGP1-2/pT7-5, Lane 2: S100 of K38/pGP1-2/pT7CYMJ, Lane 3: P100 of K38/pGP1-2/pT7-5, Lane 4: P100 of K38/pGP1-2/pT7CYMJ

CymJ is a putative repressor of *cym* gene expression

It was speculated previously⁵ that CymJ could be a candidate for cyclodextrin-specific repressor, so it was plausible to assume that cyclodextrins may relieve the repression. However, it is shown by the result in

Figure 3, that one hour after induction of the *cym* operon using 500 µM α-cyclodextrin the activity of the β-galactosidase was even lower than that measured from strain carrying the pUC19 vector indicating that the α-cyclodextrin could not alleviate the repression mediated by CymJ.

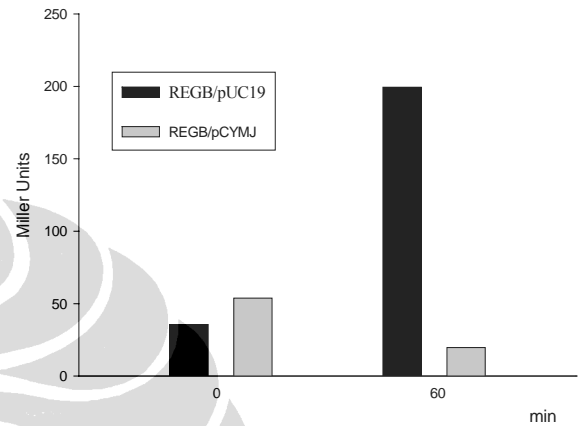


Figure 3. Addition of 500 µM α-cyclodextrin could not alleviate the repression effect of CymJ in LB medium.

In order to avoid any possible interference which may come from compounds of the rich medium, minimal media was used in the next experiment. Intriguingly, the repression of the *cym* genes could be alleviated as is shown by the increase of the β-galactosidase activity of cells carrying overexpressed *cymJ* after addition of α- cyclodextrin. After 30 min. it reached almost 95% of the value measured for cells carrying pUC19 (Fig. 4).

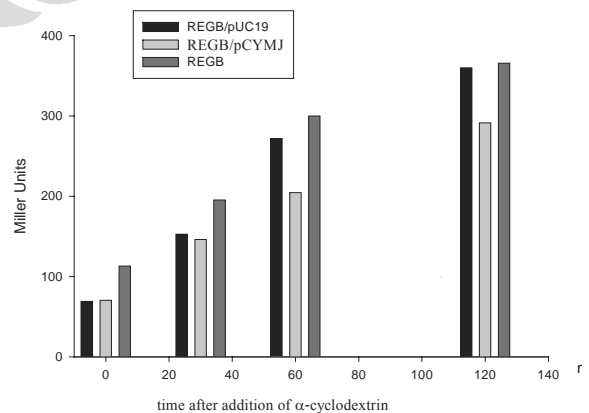


Figure 4. Release of the repression effect exerted by CymJ in minimal medium by the addition of 500 µM α-cyclodextrin.

Inhibition of cell septation upon overexpression of *cymJ*

K. oxytoca REGB/pCYMJ showed wrinkled colonies on LB plates containing 100 µg/ml ampicillin. Observation of the cells under the microscope demonstrated that they formed long filaments. In liquid minimal medium containing 0.4% glucose, the cells showed a similar phenotype as those grown on plates. The overexpression of *cymJ* resulted in cells that grew slower and varied significantly in their length when compared with *K. oxytoca* REGB cells carrying the vector (compare Fig. 5A.i and 5B.i); they became very long, around 10 to 20 times longer than wild type cells particularly after the *lac* promoter had been activated with IPTG (Fig. 5B.ii).

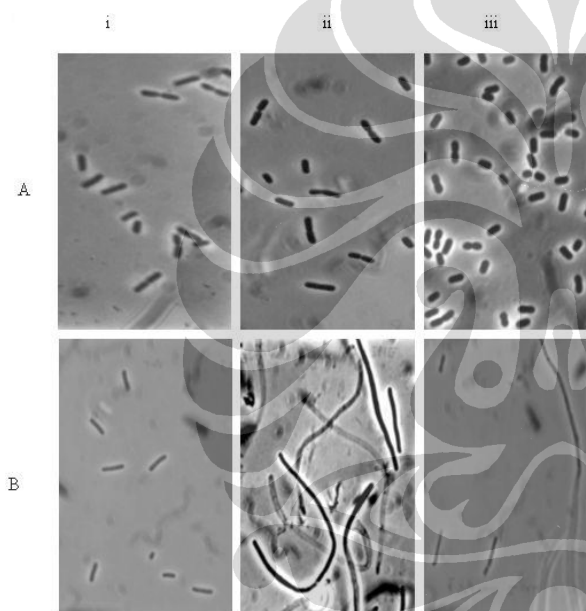


Figure 5. Morphology of *K. oxytoca* REGB carrying overexpressed *cymJ*. Cells in their mid-exponential growth phase were imaged with a Zeiss-Axiophoto phase-contrast microscope, photographed, and printed at the same magnification. A(i) *K. oxytoca* REGB/pUC19 grown in minimal medium supplemented with 0,4% glucose and 100 µg/ml ampicillin, (ii) 2h after induction with IPTG, (iii) 2 h after addition of 0,5% α -cyclodextrin.

B(i) Strain *K. oxytoca* REGB/pCYMJ, (ii) 2h after induction with IPTG, (iii) 2 h after addition of 0,5% α -cyclodextrin.

The filamentous cells which tended to clump and aggregate did not show any constriction. Moreover, most of the elongated cells were anucleated and only a minority contained a single nucleoid (10%). The most interesting finding was that the filamentous cells gradually reverted to the normal form, when α -cyclodextrin was

added. One hour after the addition of α -cyclodextrin, the filamentous cells began to form a constriction and started to divide again (Fig. 5B.iii). Finally three hours after the induction of the *cym* operon all the cells were completely reverted to normal morphology. We ensured that the cells under observation were not contaminants, since samples that were streaked on LB plate containing glucose grew homogenously without contaminants on plates. In addition, PCR test of the culture that was performed three hours after addition of α -cyclodextrin showed that the cells still carried the corresponding plasmid.

Overexpression of *cymJ* in *K. oxytoca cym*-deletion mutant

Microscopic observation of the results of overexpression of *cymJ* in different *K. oxytoca cym* deletion mutants, i.e. *K. oxytoca* CYMA, *K. oxytoca* CYME and *K. oxytoca* CYMCD showed different phenomenon. *Klebsiella oxytoca* CYMA/pCYMJ cells showed the same phenotype as the wild type. After addition of α -cyclodextrin, the filamented cells reverted into normal cells. This means that the deletion of *cymA* does not affect the activity of the *cymJ* product (data not shown).

Klebsiella oxytoca CYME/pCYMJ exhibited a similar phenotype as wild-type cells until they reached the exponential phase; however, in contrast to *K. oxytoca* CYMA/pCYMJ, the addition of α -cyclodextrin failed to stimulate the conversion of elongated cells into normally sized cells. Some of them even underwent lysis (Fig. 6). In contrast, *K. oxytoca* CYMCD/pCYMJ did not exhibit a significant phenotypic change, the transformant grew much better than *K. oxytoca*/pCYMJ (Fig. 6). It must be emphasised that the growth curve of the *K. oxytoca* REGB, *K. oxytoca* CYMA as well as *K. oxytoca* CYME overexpressing *cymJ* was obtained by measuring the optical density of the cells under the condition in which the cells were in filamentous form. Therefore, the growth curve presented in Figure 6 does not reflect the growth of cells with a normal morphology.

Overexpression of *cymJ* from a plasmid carrying a kanamycin resistance gene (pCymJ-k)

In the homologous system

The overexpression of *cymJ* that was carried out in *K. oxytoca* REGB/pCymJ-k in kanamycin containing medium did not indicate any growth even when the

amount of glucose was increased up to 1.2% or when different concentrations of α -cyclodextrin were added. However in LB medium supplemented with 0.4% glucose *K. oxytoca* REGB/pCymJ-k formed the elongated cells, which were also observed with the *K. oxytoca* REGB/pCYMJ cells in ampicillin containing medium. However, the filamented cells were shorter and contained evenly spaced nucleoids.

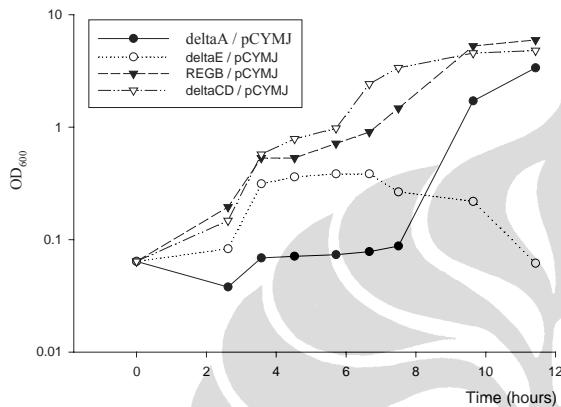


Figure 6. Growth of *K. oxytoca* *cym* mutants overexpressing *cymJ*. Cells were grown on minimal medium supplemented with 0.4% glucose and 100 μ g/ml ampicillin (50 μ g/ml for *K. oxytoca* CYME). First arrow represents the time when IPTG was added, the second arrow indicates the time when α -cyclodextrin was added. *deltaA/pCYMJ*=*K. oxytoca* CYMA/pCYMJ *deltaCD/pCYMJ*=*K. oxytoca* CYMCD/pCYMJ, *deltaE/pCYMJ*=*K. oxytoca* CYME/pCYMJ.

In the heterologous system

The overexpression of *cymJ* in a heterologous system was performed using *E. coli* strain MC4100 which was transformed with pCymJ-k. During the lag phase, the MC4100/pCymJ-k cells showed the same filamentous form as *K. oxytoca* REGB/pCYMJ grown in the presence of ampicillin and became longer after the promoter was induced with IPTG. However, the addition of α -cyclodextrin did not lead to the reversion, in contrary, the cells underwent lysis. The complementation with pCymI carrying all genes involved in the cyclodextrin metabolism system also could not revert the defect.

DISCUSSION

The regulation of the *cym* operon

The mechanism of the regulation of the *cym* operon in *K. oxytoca* appears to be a subject to both positive and

negative control. However, the regulator remains unidentified. There have been speculations that CymJ could be a candidate for a repressor, since the overexpression of *cymJ* lead to the repression of the *cym* operon.⁵

It has been demonstrated that the expression of the *cym* operon is induced by cyclodextrins, α -cyclodextrin being the best inducer.^{1,5} The repression exerted by *cymJ* can be released by the addition of 500 μ M of α -cyclodextrin. The difference between the results obtained in rich and minimal media, however, indicates that other regulatory regimes exist which override the cyclodextrin-specific control circuit.

The fact that the components of the cyclodextrin transport system show high sequence similarity to those from the maltose uptake system leads to the speculation that the regulation of the *cym* operon could resemble that of the *maltose* regulon. The *mal* genes are regulated by the activator MalT. The expression of the *mal* genes is induced by the binding of the inducer, maltotriose, to MalT and requires ATP.

It is clear that CymJ is involved in the regulation of the *cym* operon. Overexpression of *cymJ* in trans leads to inhibition of the expression of the *cym* operon genes which could be released through an addition of high concentrations of α -cyclodextrin. One possible explanation may be that there is a kind of competition between CymJ and α -cyclodextrin upon binding to the putative regulator. Overexpression of *cymJ* in the cell would then effectively influence the competition. This possibility has been evident in the case of the maltose regulon. MalY represses the maltose operon by binding to the transcription activator MalT, which is activated by maltotriose; accordingly MalY competes directly with the inducer.²⁰ Furthermore, it is known that the inducer plays an important role in the activation of the maltose operon.²⁰

It was an unexpected result that CymD is involved in the activation of CymJ in the regulation of *cym* operon. Since the absence of a functional *cymD* product prevents the CymJ effect. Therefore, CymJ may require the product of CymD. CymD displays high sequence similarity to MalK. MalK is a bifunctional protein, acts as part of a transporter in the presence of maltose and in the absent of maltose binds to MalT, which leads to its inactivation.²¹ The function of CymD as an ATPase has not yet been proven experimentally, however, the high similarity of its amino acid sequence to MalK and the fact that it

can complement the function of MalK strongly supports this suggestion.¹ There is also a lack of information on the function of CymD as a repressor. It is only known that in a heterologous system in *E. coli* deletion of *cymD* rendered the cells unable to grow in the presence of α -cyclodextrin.⁵ Moreover, the overexpression of *cymD* in the *K. oxytoca* REGB dramatically repressed the expression of *cym* operon (data not shown), which is consistent with the previous results.⁵

Under physiological conditions employing normal stoichiometries the components are well balanced; when *cymJ* is overexpressed, however, they are no longer in equilibrium and the system is fully repressed.

We also assume that at excessive overproduction of CymJ may elicit a SOS response with multiple physiological consequences like the binding of Sula to FtsZ. Furthermore, a functional defect in FtsZ could lead to the failure of DNA to segregate.²² However, these occur temporarily due to a competition between the product of the *cymJ* gene and an effect of α -cyclodextrin that could alleviate the inhibitory effect of CymJ on cell septation. However the role of CymE with respect to the presence of abnormal amount of the CymJ remains unanswered, although the inability of the cells to revert to the normal morphology suggests that CymE or its function is required for the conversion step to proceed.

An interesting effect of the overexpression of *cymJ* was the markedly increased susceptibility to β -lactam antibiotics. The overexpression of *cymJ* in a strain with kanamycin resistance background could exclude the argument that the inhibition on the cell septation was caused by ampicillin; rather, it is a consequence of overproduction of *cymJ*. *Klebsiella oxytoca* strains generally display moderate intrinsic ampicillin resistance due to the synthesis of the chromosomally encoded OXY-1 and OXY-2 class A β -lactamases⁹ and consequently the organism grows flourishly in the presence of 1 mg/ml ampicillin.

The reduction of the resistance against antibiotics could be due to several factors. One of them may reside in the inhibition of export of β -lactamase precursor to the periplasm.

In conclusion, Overexpression of *cymJ* in *K. oxytoca* led to strong reduction in the expression of the *cym* operons. This repression could be alleviated by addition of high concentration of α -cyclodextrin into the medium. There is a possible relationship between

CymJ and CymD since the absence of CymD in a *cymD* deletion mutant prevented the repression effect of CymJ. An intriguing finding was that the presence of CymJ in large amount in the cell caused severe cell division inhibition leading to retardation of growth. This morphological change was paralleled by a significant increase in the susceptibility of *K. oxytoca* to ampicillin.

Acknowledgment

This work was supported by a grant from DAAD (German Academic Exchange Service).

REFERENCES

1. Fiedler G, Pajatsch M, Boeck A. Genetics of a novel starch utilization pathway present in *Klebsiella oxytoca*. J Mol Biol. 1996; 256: 279-91.
2. Pajatsch M, Boeck A, Boos W. Enzymatic preparation of radiolabelled linear maltodextrins and cyclodextrins of high specific activity from ¹⁴C maltose using amyloamylase, cyclodextrin glucosyltransferase and cyclodextrinase. Carbohydr Res. 1998; 307: 375-9
3. Pajatsch M, Andersen C, Methes A, Boeck A, Benz R, Engelhardt H. Properties of a cyclodextrin specific, unusual porin from *Klebsiella oxytoca*. J Biol Chem. 1999; 274 (35):25159-66
4. Feederle R, Pajatsch M, Kremmer E, Boeck A. Metabolism of cyclodextrins by *Klebsiella oxytoca* M5a1: purification and characterisation of a cytoplasmically located cyclodextrinase. Arch Microbiol. 1996;165: 206-12
5. Pajatsch M. Cyclodextrin-Metabolism in *K oxytoca* M5a1. [Dissertation]. Munich: Ludwig-Maximilians University; 1999.
6. Sorokin A, Bolotin A, Purnelle B, Hilbert H, Lauber J, Dusterhoft A, et al. Sequence of the *Bacillus subtilis* genome region in the vicinity of the lev operon reveals two new extracytoplasmic function RNA polymerase sigma factor SigV and SigZ. Microbiology 1997;143: 2939-43.
7. Miller JH. A short course in bacterial genetics. Cold Spring Harbor: Cold Spring Harbor Laboratory; 1992.
8. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory; 1989.
9. Fournier B, Roy PH, Lagrange PH, Philippon A. Beta-lactamase gene promoters of 71 clinical strains of *Klebsiella oxytoca*. Antimicrob Agents Chemother. 1996; 40: 454-9
10. Frankael DG, Neidhard FC. Use of chloramphenicol to study control of RNA synthesis in bacteria. Biochem Biophys Acta. 1961; 53: 96-100
11. Bender H. Cyclodextrin glucanotransferase from *Klebsiella pneumoniae*. 1. Formation, purification and properties of the enzyme from *Klebsiella pneumoniae* M 5 a1 (author's transl). Arch Microbiol. 1977;111: 271-82.

12. Pajatsch M, Gehart M, Peist R, Horlacher R, Boos W, Boeck A. The periplasmic cyclodextrin binding protein CymE from *Klebsiella oxytoca* and its role in maltodextrin and cyclodextrin transport. *J Bacteriol.* 1998;180: 2630-5.
13. Lyons LB, Zinder ND. The genetic map of the filamentous bacteriophage ϕ 1. *Virulogy.* 1972; 49: 45-60.
14. Yanisch-Perron C, Vierle J, Messing J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene.* 1985; 33: 103-19.
15. Tabor S, Richardson CC. A bacteriophage T7RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc Natl Acad Sci USA.* 1985; 82: 1074-8.
16. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidmann JG, Smith JA, et al. *Current protocols in molecular biology.* New York: J Wiley and Sons; 1997.
17. Holmes DS, Quigley M. A rapid boiling method for the preparation of bacterial plasmids. *Anal Biochem.* 1981;114: 193-7.
18. Wieslander L. A simple method to recover intact high molecular weight RNA and DNA after electrophoretic separation in low gelling temperature agarose gels. *Anal Biochem.* 1979; 98: 308-9.
19. Filip C, Fletcher G, Wulff JL, Earhart CF. Solubilization of the cytoplasmic membrane of *Escherichia coli* by the ionic detergent sodium-lauryl sarcosinate. *J Bacteriol.* 1973; 115:717-22.
20. Boos W, Shuman H. Maltose/maltodextrin system in *Escherichia coli*: transport, metabolism, and regulation. *Microbiol and Mol Biol Rev.* 1998; 62: 204-29.
21. Lippincott J, Traxler B. MalFGK complex assembly and transport and regulatory characteristic of MalK insertion mutants. *J Bacteriol.* 1997; 179: 1337-43.
22. Erickson HP. Atomic structures of tubulin and FtsZ. *Trends Cell Biol.* 1998; 8(4):133-7.



