Polish Journal of Microbiology
2008, Vol. 57, No 1, 19–26

ORIGINAL PAPER

Regulation of *Yersinia enterocolitica mal* genes by MalT and Mlc proteins

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Received 25 October 2007, revised 29 September 2007, accepted 5 December 2007

Abstract

To show the role of MalT protein in the regulation of *mal* genes, encoding proteins involved in transport and metabolism of maltose/maltodextrins in *Yersinia enterocolitica*, we constructed a *malT* mutant which was characterized by a strong reduction in maltose transport and a loss of MBP protein. We also studied the influence of MalT activity on the production of Yop proteins in *Y. enterocolitica* and found that the level of these virulence factors is not changed in the *malT* mutant. Subsequently, transcriptional fusion *malT*::*lacZYA* was applied to study the activity of *malT* promoter. Monitoring of β-galactosidase activity suggests the influence of catabolic repression on *malT* transcription, since the activity of *malT* promoter was decreased twofold in the presence of glucose. Furthermore, Mlc protein was identified in *Y. enterocolitica* as a factor regulating the transcription of *malT*. We observed a two-fold increase in the level of *malT* transcription in the *mlc* mutant background. Moreover, overproduction of Mlc protein strongly inhibited the activity of *malT* promoter. Thus, the data presented in this study suggest that the level of *mal* gene expression in *Y. enterocolitica* may be regulated by two proteins: MalT, the activator of *mal* transcription and Mlc, the repressor of *malT* expression.

Key words: *Yersinia enterocolitica,* MalT protein, maltose regulon

Introduction

The maltose regulon in *Escherichia coli* is composed of several genes encoding proteins which are involved in the effective use of maltose and maltodextrins as carbon and energy sources. Proteins required for active transport of these sugars are encoded by genes of two divergent operons, *malEFG* and *malKlamBmalM* (Boos and Schuman, 1998). In the first operon, the *malE* gene encodes the periplasmic maltose-binding protein (MBP), whereas the *malF* and *malG* genes encode the inner membrane components of the maltose transport system. In the second operon, the *malK* gene codes for an ATPase energizing maltose transport, which is attached to the inner surface of the cytoplasmic membrane *via* the EAA loop of MalF and MalG. The *lamB* gene codes for the lambda receptor (maltoporin) which facilitates the permeation of maltose/maltodextrins through the outer membrane. The last gene of this operon, *malM*, encodes an envelope protein with an unknown function. Metabolism of maltose/maltodextrins involves three enzymes: amylomaltase, maltodextrin phosphorylase and amylase, which are encoded by the *malQ*, *malP* and *malS* genes, respectively (Schneider *et al*., 1992).

Expression of the maltose regulon in *E. coli* is positively controlled by a specific transcriptional activator, MalT protein (Richet and Raibaud, 1987). MalT belongs to a class of bacterial transcriptional activators of MalT or LAL family. MalT binds and activates its target promoters only in the presence of ATP and a specific internal inducer *i.e.* maltotriose. Maltose added to the growth medium of *E. coli* functions as an external inducer (Ehrmann and Boos,1987; Raibaud and Richet, 1987). The regulation of MalT activity involves at least three negative effector proteins: MalK, the ATPase of the maltose/maltodextrins ABC (ATP-binding cassette) transporter (Schreiber *et al*., 2000; Joly *et al*., 2004), MalY, a protein homologous to βC-S lyases (Schlegel *et al*., 2002), and Aes, which is homologous to acylesterases (Joly *et al*., 2002). Furthermore, *malT* expression is regulated by the cAMP/CAP complex and is, therefore, under catabolite repression control (Chapon, 1982). The *malT* gene transcription is also dependent on the activity of Mlc global regulator.

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Mlc (makes large colonies) is a repressor that regulates the expression of several genes involved in sugar metabolism (Bohm and Boos, 2004; Plumbridge, 1998; Kim *et al*., 1999). The activity of Mlc protein is modulated through the interaction with the major glucose transporter, EIICB^{Glc}, in response to external glucose (Tanaka *et al.*, 2000). In the absence of glucose, PTS protein is phosphorylated by PEP (phosphoenolpyruvate). The phospho-form of EIICB^{Glc} does not interact with Mlc which could bind to DNA and repress target genes encoding the components necessary for glucose transport. On the other hand, the transport of glucose results in the dephosphorylation of EIICB^{Glc} and the derepression of Mlc-dependent genes (Seitz *et al*., 2003).

The maltose system and homologs of *mal* genes have been also identified and described in other Gram-negative bacteria (Boos and Schuman, 1998). In the case of *Klebsiella pneumoniae* additional *pulA* gene, encoding pullulanase, appears to be a part of the maltose regulon (Konishi *et al*., 1979).

Very little is known about the maltose system in *Yersinia enterocolitica*, a human enteropathogen. These bacteria secrete several essential virulence factors called Yop proteins using Type III secretion system encoded by the pYV plasmid. Six of these Yop proteins are effectors, which after translocation into the cytoplasm of host cells interfere with the signaling pathways involved in the regulation of the actin cytoskeleton, phagocytosis, apoptosis and the inflammatory response (Navarro *et al*., 2005). The remaining substrates of Type III secretion system regulate synthesis, secretion and facilitate transport of Yop effector proteins (Cornelis *et al*., 1998).

We have previously identified two components involved in the maltose/maltodextrins transport in *Y. enterocolitica*, namely maltoporin (OmpM), analogous to LamB of *E. coli* (Brzostek *et al*., 1993), and periplasmic maltose-binding protein MalE (MBP, maltose binding protein) (Brzostek and Raczkowska, 2001). Moreover, we have found a correlation between the functioning of the maltose system and the level of *Y. enterocolitica* Yop proteins secreted into the growth medium. Maltose mutants generated by transposon mutagenesis were both impaired in the maltose transport and Yops production (Brzostek *et al*., 1993). Inactivation of some *mal* genes by transposon insertion has been reported to affect the production and secretion of main virulence factors in *Vibrio cholerae*. In addition, the intact maltose transport system seems to be crucial for the translocation of these virulence factors across the outer membrane (Lang *et al*., 1994).

In this study, we focused on the role of MalT in the regulation of maltose transport in *Y. enterocolitica*. We also searched for a correlation between the functioning of MalT and the production of Yop proteins. The effect of catabolic repression and the influence of the Mlc global repressor on the transcription of *malT* in *Y. enterocolitica* were investigated.

Experimental

Materials and Methods

Bacterial strains and plasmids. The strains and plasmids used in this study are listed in Table I.

Growth media and culture conditions. *E. coli* strains were grown aerobically in LB medium at 37°C, whereas *Y. enterocolitica* strains were incubated at 25°C in LB medium and minimal medium A (MMA) (Miller, 1972) supplemented with casamino acids (0.5%), glycerol (0.2%) and glucose (0.2%). In other experiments glycerol (0.2%) and maltose (0.2%) were used as the carbon source. For the induction of Yop proteins, the LB-MOX medium was applied (LB supplemented with 20 mM $MgCl_2$ and 20 mM sodium oxalate). When necessary, antibiotics were used at following concentrations: nalidixic acid, 30 μg/ml; chloramphenicol, 12.5 μg/ml; kanamycin, 50 μg/ml.

Isolation of *Y. enterocolitica* insertional *malT* mutant. For the construction of MalT-deficient derivative, a 695 bp fragment of the *malT* gene from *Y. enterocolitica* Ye9 (serotype O9) was amplified with Taq polymerase using primers TfX1 (5'-TCGTCTAGACG CCTGACCGGTGAAGATAACG-3' [underlining indicates an additional XbaI site]) and TrS2 (5'-TCCCC CGGGCTCGGTTTGCTGCATCATCGGC-3' [underlining indicates an additional SmaI site]). The obtained fragment was ligated into the commercial pDrive cloning vector (Qiagen) to generate plasmid pDT4. Subsequently, the XbaI/SmaI fragment of *malT* was subcloned into the SmaI/XbaI-digested suicide conjugative plasmid pEP185.2 (Cm^R) (Kinder *et al*., 1993). The resulting plasmid, pET8, was conjugated into *Y. enterocolitica* Ye9N (Nal^R) and transconjugants, *malT* mutants, carrying this construct inserted into their chromosome, were selected on LB medium with chloramphenicol (12.5 μg/ml). Transconjugants were tested on McConkey agar supplemented with maltose (1%).

Construction of *malT'*::*lacZYA* fusion. For the construction of *malT'*::*lacZYA* chromosomal transcriptional fusion, XbaI/SmaI fragment of the *malT* gene from the pDT4 plasmid was cloned into SmaI/XbaI-digested suicide conjugative plasmid, pFUSE (Cm^R) with *ori*R6K, *mob*RP4 and the promoterless *lacZYA* genes (Bäumler *et al*., 1996). The resulting plasmid, pFT2 (Fig. 1), was conjugated into *Y. enterocolitica* Ye9N (Nal^R) and transconjugants carrying this construct inserted into their chromosome were selected. The function of the *malT* promoter driving *lacZYA* expression in selected transconjugants was initially

Table I
Strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Reference or source
Y. enterocolitica O9 strains		
Ye9N	pYV$^+$, Nal^R	Department of Applied Microbiology, Warsaw University
MB3	pYV$^+$, Nal^R, *malT*::pET8	This study
Ye18	pYV$^+$, Nal^R, *malT'*::*lacZYA* (Cm^R)	This study
Ye20	pYV$^+$, Nal^R, *malT'*::*lacZYA* (Cm^R), pBBRM7 (Km^R)	This study
AR2	pYV$^+$, Nal^R, *mlc*::pEPM1, *malT*::*lacZYA* (Cm^r)	This study
E. coli strains		
DH5α	*recA1 endA1 gyrA96 thi-1 hsdR17*($r_k^- m_k^+$) *supE44ΔlacU169* F' (Φ*80dlacZ*M15)	(Sambrook *et al*., 1989)
S17-1 λ*pir*	*recA thi pro hsdR*$^-$ M$^+$ (RP4–2 Tc::Mu-Km::Tn7), λ *pir*	(Simon *et al*., 1983)
Plasmids		
pDrive	Cloning vector, Amp^R, Km^R	Qiagen
pDT4	pDrive with 695 bp fragment of *malT* gene	This study
pEP185.2	Suicide vector, Cm^R	(Kinder *et al*., 1993)
pET8	pEP185.2 with XbaI/SmaI fragment of *malT* gene from pDT4, Cm^R	This study
pFUSE	Suicide vector, derivative of pPEP185.2 with the promoterless *lacZYA* genes, Cm^R	(Bäumler *et al*., 1996)
pFT2	pFUSE with SmaI/XbaI fragment of *malT* from pDT4 to give *malT*::*lacZYA*, Cm^R	This study
pD5Mlc	1283 bp fragment of entire *mlc* gene with *rbs*	This study
pBBR1 MCS-2	Cloning vector, Km^R	(Kovach *et al*., 1995)
pBBRM7	pBBR1 MCS-2 with SmaI/XbaI fragment of *mlc* with rbs from pD5Mlc, Km^R	This study
pRK2013	Helper plasmid, Km^R	(Figurski and Helinski, 1979)
pDM3	pDrive with 633 bp fragment of *mlc*, Km^R	This study
pEP12	Suicide, conjugative vector, Km^R	Department of Applied Microbiology, Warsaw University
pEPM1	pEP12 with EcoRV/XhoI fragment of *mlc* from pDM3, Km^R	This study

confirmed on LB agar plate supplemented with X-gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside, 20 μg/ml) and incubated at 25°C.

Cloning of the *mlc* sequence in pBBR1 MCS-2 vector. The 1283 bp PCR product containing entire *mlc* coding sequence with *rbs* was amplified with primers YmlcS1 (5'-TCCCCCGGGCTCGGTGACAGAGAA GGAGC-3' [underlining indicates an additional SmaI site]) and YmlcX2 (5'-TGCTCTAGACCTATCCTTG CAGGAGAGTTTCACC-3'[underlining indicates an additional XbaI site]). The resulting product was ligated into the pDrive cloning vector (pD5Mlc), then subcloned into a medium copy, mobilizable pBBR1 MCS-2 cloning vector (Km^R) (Kovach *et al*., 1995) digested with SmaI and XbaI to create pBBRM7 construct. Then, using the *E. coli* DH5α strain containing the helper plasmid pRK2013 (Figurski and Helinski, 1979), which provides *tra* and *mob* genes, the pBBRM7 plasmid was mobilized into *Y. enterocolitica* strains by triparental mating. Strains with active *malT'*::*lacZYA* transcriptional fusion were selected on LB medium with Nal, Cm and Km.

Isolation of *Y. enterocolitica mlc* mutant. For construction of an Mlc-deficient derivative, a 633 bp fragment of the *mlc* gene was PCR amplified with Taq polymerase using primers MlcE1 (5'-TCCGATATCT GACGGCTATCGCCATTACCATGCA-3'[underlining indicates an additional EcoRV site]) and MlcX2 (5'-TGCCTCGAGTGGCGAATACAAGATCCAATG GCGA-3'[underlining indicates an additional XhoI site]). The obtained product was ligated with the pDrive cloning vector (pDM3), then subcloned into a suicide, conjugative vector, pEP12 (Km^R), digested with EcoRV and XhoI. The resulting plasmid, pEPM1, was integrated into the chromosome of Ye18 strain by a single recombination event between the 633 bp *mlc* sequence in pEPM1 and the corresponding region of *mlc* on the chromosome. Transconjugants were selected on LB plates supplemented with the appropriate antibiotics (Nal, Cm, Km).

β-galactosidase assay. The β-galactosidase activity was assayed at 28°C as described by Miller (1972), using chloroform and 0.1% SDS to disrupt the cells. The enzyme activities, expressed in Miller units, represent the average from assays performed in duplicate in three independent experiments. The variations observed between independent experiments did not exceed 20%.

Isolation of periplasmic proteins. Bacterial periplasmic proteins were prepared according to the method described previously (Brzostek and Raczkowska, 2001). Cultures were grown overnight at 25°C in MMA supplemented with casamino acids (0.5%) and glycerol (0.2%). For induction, maltose (0.2%) was added to the growth medium.

Induction and isolation of Yop proteins. Overnight cultures of *Y. enterocolitica* strains were subcultured in LB-MOX medium at 25°C and incubated to

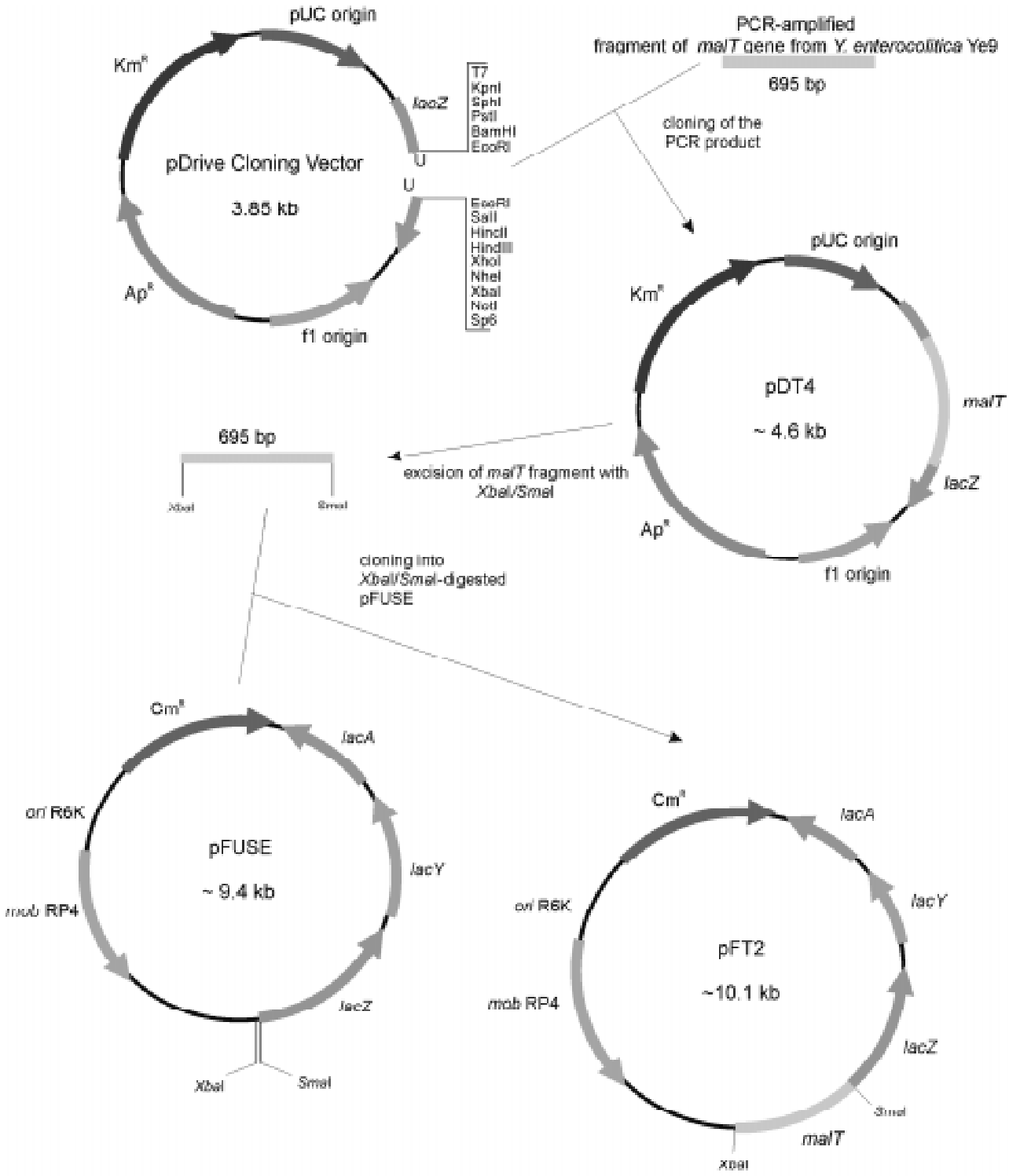

Fig. 1. Construction of the suicide conjugative plasmid carrying a fragment of *malT* to create a chromosomal transcriptional fusion.

OD_{600} 0.3. Induction of Yops was carried out at 37°C for 3 h. Secreted proteins were precipitated at 4°C overnight with 5% TCA and centrifuged at 10 000 × g for 20 min at 4°C. The pellet of Yop proteins was washed with 70% cold acetone and suspended in the electrophoresis sample buffer.

SDS-PAGE. Electrophoresis of periplasmic proteins and secreted Yop proteins isolated from *Y. enterocolitica* strains was done on 10% SDS-polyacrylamide gels according to Sambrook *et al*. (1989).

Transport of maltose. Bacteria were grown in minimal medium A (MMA) supplemented with casamino acids (0.5%), glycerol (0.2%) (uninduced cells) or glycerol (0.2%) and maltose (0.2%) (induced cells). Cells from the exponential phase of growth were washed and resuspended in phosphate-buffered saline

to the value of $OD_{578} = 0.1$. At time zero, [^{14}C] maltose (specific activity 540 mCi/mmol, 20 GBq/mmol; final concentration 3×10^{-2} μM) was added. Samples were withdrawn after 20, 40, 60, 120, 180 s and filtered through membrane filters. The radioactivity of the dried filters was determined.

Sequencing of the *mlc* gene from *Y. enterocolitica* O9. The fragment of DNA containing entire *mlc* coding sequence with *rbs* subcloned in pD5Mlc vector was sequenced using standard primers for SP6 promoter and T7 promoter on an ABI PRISM® 377 DNA Sequencer (GMI, Inc) (DNA Sequencing and Oligonucleotide Synthesis Laboratory, IBB PAN, Warsaw). Alignment procedures were carried out using EMBL software.

Results and Discussion

The MalT protein in *E. coli* is a transcriptional activator essential for *mal* gene expression. In this study, we investigated the role of the MalT protein in the functioning of the maltose system in *Y. enterocolitica*. Thus, we constructed *Y. enterocolitica malT* insertional mutants, one of which (named MB3) was chosen for further analysis. The lack of functional MalT resulted in the loss of the ability to ferment maltose, so *malT* mutants formed white colonies on MacConkey plates supplemented with maltose, in contrast to the wild type strain (red colonies). Construction of *malT* mutants was confirmed by PCR and Southern analysis (data not shown).

In order to demonstrate that MalT regulates *mal* genes expression in *Y. enterocolitica,* the production of a periplasmic MBP protein (MalE), a component of the maltose transport system, was examined in Ye9N (wild type) and MB3 (*malT* strain), growing in MMA in the presence or absence of maltose as an external inducer. Electrophoretic analysis (SDS-PAGE) of periplasmic proteins of Ye9N strain indicated the presence of MBP at a position corresponding to the apparent molecular mass of about 40 kDa in the sample obtained from cells grown under conditions inducing the maltose system. In uninduced cells, MBP was not present. The lack of MalT protein in MB3 strain resulted probably in the inhibition of *malE* expression, because we could not detect any band corresponding to the MBP protein in both inducible and uninducible growth conditions (data not shown). These findings are in agreement with our previous studies, which showed that in *Y. enterocolitica* the synthesis of both MBP and LamB proteins as well as the transport of maltose into the cells are induced by maltose, necessary for MalT activation. We also demonstrated that rabbit antibodies raised against the MBP of *E. coli* cross-react with the analogous protein from Ye9 and that the MBP of *Y. enterocolitica* restores the maltose transport activities in the *E. coli malE* mutant (Brzostek and Raczkowska, 2001).

For additional characterization of *Y. enterocolitica malT* mutant, the rate of maltose transport was determined (Fig. 2). The uptake of maltose into Ye9N cells increased about twenty-eight-fold in strain growing in the presence of maltose, compared with the medium supplemented with glycerol alone. The mutant lacking MalT protein (MB3 strain) was drastically impaired in transport, irrespective of the growth medium (maltose induction). These results show that the

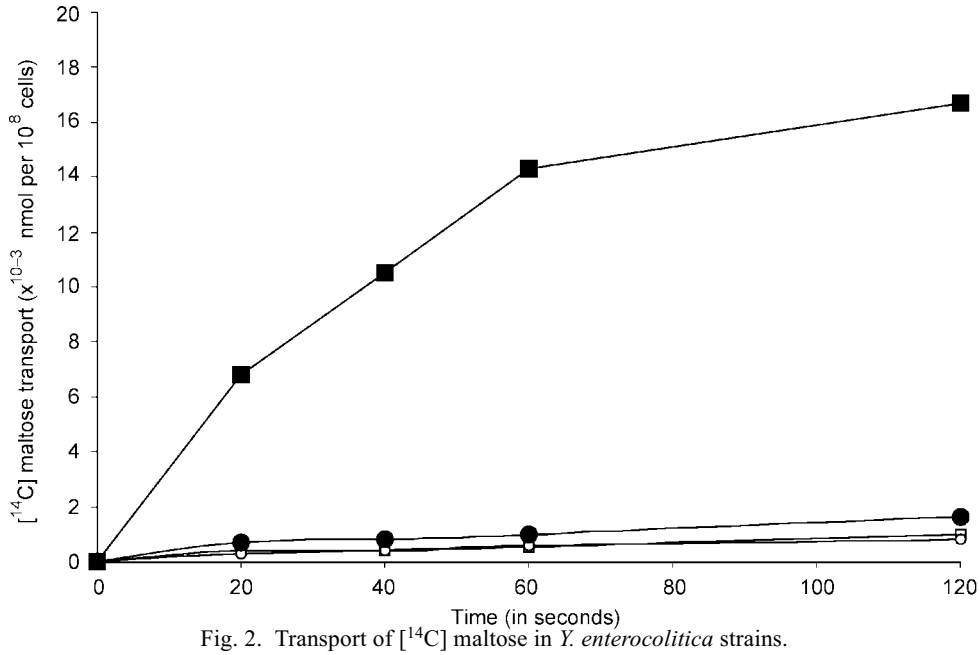

Fig. 2. Transport of [^{14}C] maltose in *Y. enterocolitica* strains.

Bacteria were grown in MMA supplemented with glycerol (0.2%) or glycerol (0.2%) and maltose (0.2%) as a inducer. Ye9N strain, induced (■) and uninduced cells (□); MB3 strain, induced (●) and uninduced cells (○). Data shown are means of duplicate experiments.

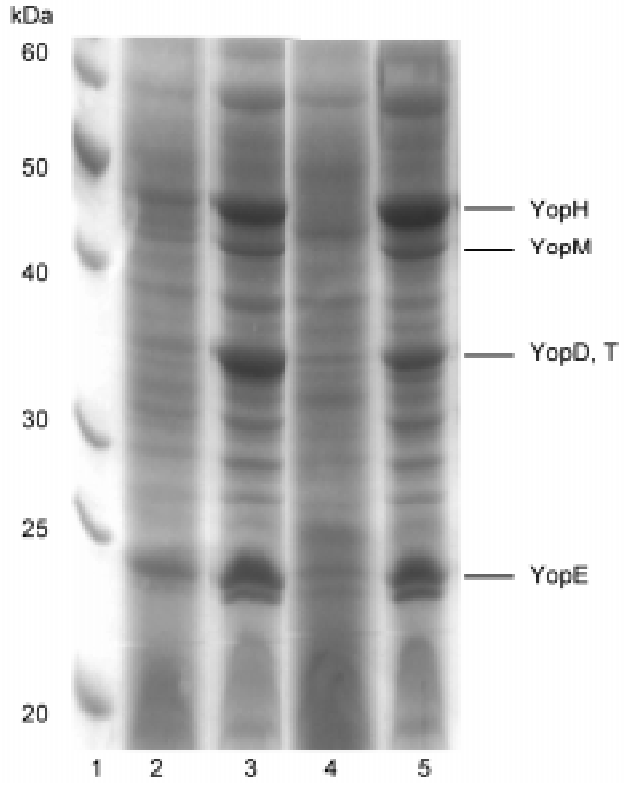

Fig. 3. SDS-PAGE of Yop proteins isolated from *Y. enterocolitica* strains.

Yop proteins were induced 3 h at 37°C in LB medium (MOX version) and precipitated with 5% TCA from the supernatant of a culture. Lane 1 – Protein Molecular Weight Marker (MBI Fermentas); lane 2 – Ye9N (25°C); lane 3 – Ye9N (37°C); lane 4 – MB3 (25°C); lane 5 – MB3 (37°C). The position of same Yop proteins is indicated.

activity of MalT in *Y. enterocolitica* depends on the presence of the external inducer *i.e.* maltose and that this protein regulates the expression of *mal* genes playing a similar role as in *E. coli*.

In addition, we analyzed the *malT* mutant of *Y. enterocolitica* paying special attention to the virulence phenotype *i.e.* Yops production. Such studies were prompted by the results of our earlier observations indicating that transposon mutants impaired in the maltose transport activity demonstrate a reduced level of Yop proteins (Brzostek *et al*., 1995). To verify the hypothesis that the maltose regulon affects Yops production/secretion, the profile of Yop proteins was analyzed by SDS-PAGE. Ye9 strain synthesized and secreted the set of Yop proteins at 37°C in the absence of calcium ions in the growth medium (Fig. 3, lane 3), but not at 25°C (Fig. 3, lane 2). The loss of MalT did not affect Yop production, the protein profile of the *malT* mutant was identical to the profile of the wild type strain (Fig. 3, lane 4 and 5).

An additional but significant aim of our study was to investigate factors controlling *malT* expression in *Y. enterocolitica*. It is known that the *malT* transcription in *E. coli* is regulated by the cAMP/CAP complex and is subject to catabolite repression (Chapon, 1982). In order to monitor the role of the presence of glucose in the medium in *Y. enterocolitica malT* expression, we applied Ye18 strain, carrying chromosomal transcriptional fusion of *malT'*::*lacZYA*. To create this fusion the suicide conjugative plasmid pFT2 was constructed (Fig. 1). The *malT'*::*lacZYA* fusion located on the chromosome was confirmed by PCR with a pair of primers: MalT1 (5'-TGTCTAGACAAT CGCCAGGACGTCTTC-3'), designed for the nucleotide sequence of the chromosomal part of *malT*, and LacZ2 (5'-AGTCTCAATCTGCACTACAA-3') for *lacZ* sequence present on the pFUSE plasmid. The activity of the *malT* promoter was examined in strains growing in MMA medium supplemented with glycerol or glycerol and glucose. We observed that the level of transcription of the *malT*::*lacZYA* fusion was reduced above two-fold in the presence of glucose in the growth medium compared with the glycerol alone (Table II). These results suggest that *malT* transcription in *Y. enterocolitica* is sensitive to catabolite repression.

Considering that Mlc, known as a global repressor of several genes encoding proteins required for sugar utilization in *E. coli*, may influence *malT* transcription in *Y. enterocolitica*, we studied Mlc activity in *Yersinia* cells. Firstly, *mlc* gene was identified and the sequence analysis indicated that this gene encodes a protein of 406 amino acid residues (Fig. 4). The comparison of the amino acid sequence of Mlc of *Y. enterocolitica* with Mlc from *E. coli* K12 revealed a high level of similarity (82%). Amino acid sequence alignment of the Mlc *Y. enterocolitica* O9 (Ye9) with *Y. enterocolitica* serotype O8 (Ye8) and *Y. pestis* (Yp) revealed 99% and 93% similarity, respectively.

To analyze Mlc properties we measured the effect of *mlc* mutation on *malT* transcription. *Y. enterocolitica mlc* mutant was constructed in a strain carrying a transcriptional fusion *malT'*::*lacZYA*. The level of *malT* transcription in the *mlc* mutant derivative (AR2 strain) was approximately two-fold-higher than in Ye18 strain (Mlc^+), indicating that Mlc negatively regulates *malT* expression at the transcriptional level (Table II). In addition, the *malT* expression in AR2 strain decreased when glucose was added into the growth medium.

Table II
Expression of *malT'*::*lacZYA* transcriptional fusion in *Y. enterocolitica* strains

Strains	*malT'*::*lacZYA* expression[a]	
	Glycerol	Glucose
Ye18 (Mlc^+)	650 ± 15	280 ± 12
Ye20 (Ye18/pBBRM7)	100 ± 6	85 ± 8
AR2 (Mlc^-)	1100 ± 96	580 ± 10

[a] – Cells were grown in MMA medium supplemented with 0.2% glycerol or 0.2% glycerol and 0.2% glucose. Culture samples were taken at the OD_{600} of 0.6, and β-galactosidase activities in Miller units were determined. Each value is the average of three independent experiments ± SD.

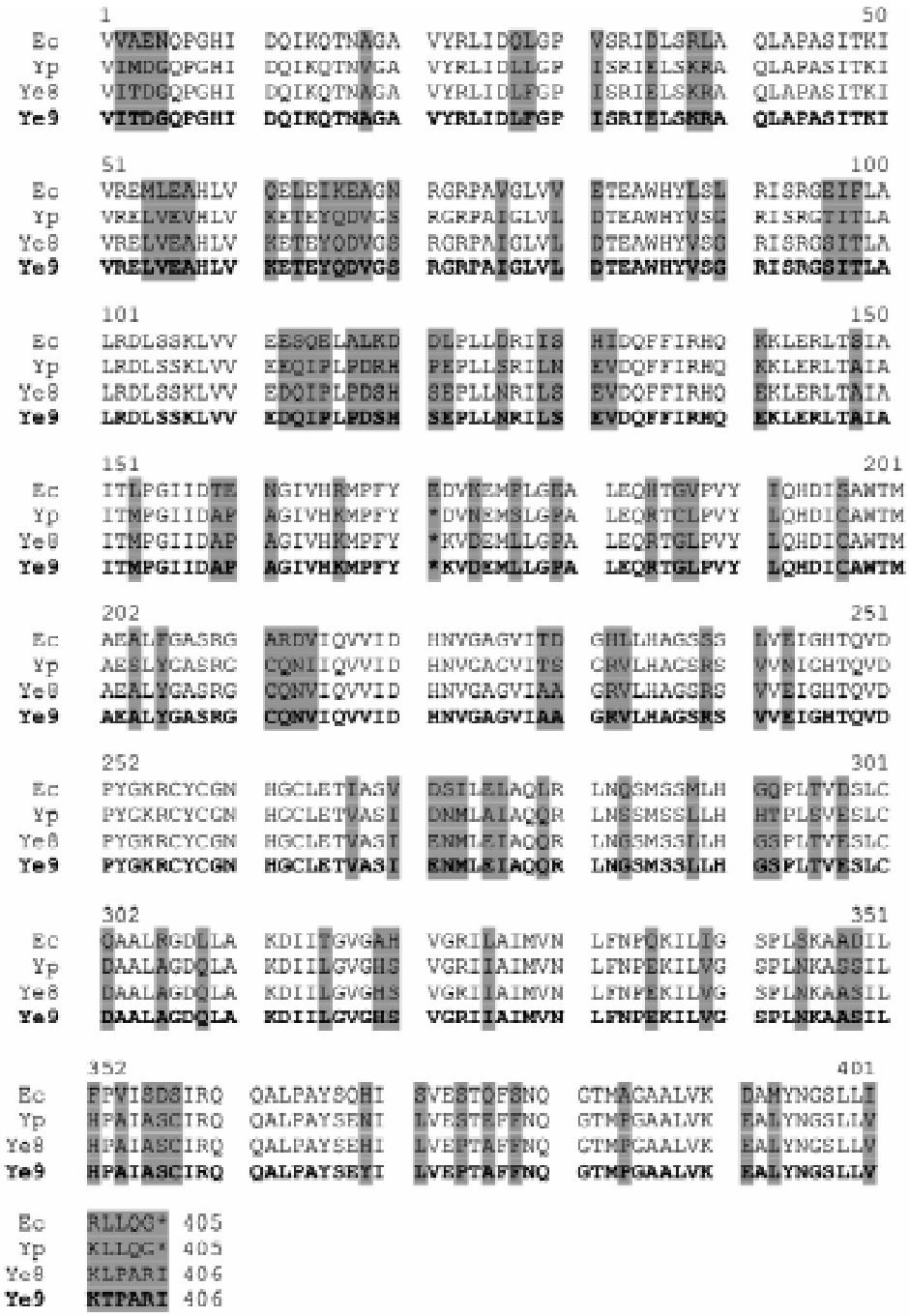

Fig. 4. Amino acid sequence alignment of the *Y. enterocolitica* Ye9 Mlc with *Y. enterocolitica* serotype O8 (Ye8), *Y. pestis* (Yp), and *E. coli* (Ec) homologues.

Changes in amino acids are indicated by grey background. The amino acid sequence of *Y. enterocolitica* O8 was obtained from the Sanger Institute (http://www.sanger.ac.uk), *Y. pestis* and *E. coli* from NCBI (http://www.ncbi.nlm.nih.gov/).

Confirmation of the effect of a lack of functional Mlc protein should have emerged from the complementation experiments. To carry out genetic complementation, the pBBRM7 plasmid expressing Mlc was constructed. Unfortunately, the complementation of the *mlc* mutation in the strain AR2 was not achieved with plasmid pBBRM7. We were able to introduce pBBRM7 only to Ye18 (Mlc, *malT*'::*lacZYA*) yielding Ye20 strain. When Mlc was expressed from pBBRM7 an inhibitory effect of this protein was observed. The level of *malT* transcription was six-fold decreased in Ye20 compared with Ye18 strain (Table II). These results suggest that Mlc may control *mal* gene expression by regulating the amount of MalT protein.

Taken together, our data suggest that the level of *malT* transcription may be a result of the functioning of two opposing factors, the cAMP-CRP complex (activator) and the Mlc protein (repressor). These results are in agreement with the data obtained for *E. coli* and may reflect a common feature of genes belonging

to the Mlc regulon which possess at least one cAMP/CRP binding site as well as the Mlc binding site and thus are under dual control (Decker *et al*., 1998, Plumbridge, 1999). Basing on current knowledge it is probable that Mlc protein, protecting a region extending from +1 to the +23 with respect to the transcriptional start of *malT* gene, directly interfers with RNA polymerase binding. Moreover, it has been proved that binding of the cAMP/CRP complex at position –70.5 upstream of the transcriptional start has no effect on Mlc binding (Decker *et al*., 1998).

To summarize, the presented work sheds light on the mechanisms responsible for the control of maltose regulon in *Y. enterocolitica* via regulation of *malT* expression. Our results indicate the influence of catabolic repression and the inhibitory effect of Mlc protein on the level of *malT* transcription. Moreover, our findings point out that MalT does not regulate the production of Yop proteins.

Acknowledgements

This study was supported by the Polish Ministry of Science through the Faculty of Biology, Warsaw University intramural grant, BW-501-68-1680-46.

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