Structural and Functional Relationships between *Pasteurella* multocida and Enterobacterial Adenylate Cyclases

MICHÈLE MOCK,¹ MARTINE CRASNIER,² EDITH DUFLOT,¹ VALÉRIE DUMAY,² AND ANTOINE DANCHIN^{2*}

Unité des Antigènes Bactériens (Centre National de la Recherche Scientifique Unité Associée 557)¹ and Unité de Régulation de l'Expression Génétique (Centre National de la Recherche Scientifique Unité Associée 1129),² Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

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The Pasteurella multocida adenylate cyclase gene has been cloned and expressed in Escherichia coli. The primary structure of the protein (838 amino acids) deduced from the corresponding nucleotide sequence was compared with that of *E. coli*. The two enzymes have similar molecular sizes and, based on sequence conservation at the protein level, are likely to be organized in two functional domains: the amino-terminal catalytic domain and the carboxy-terminal regulatory domain. It was shown that *P. multocida* adenylate cyclase synthesizes increased levels of cyclic AMP in *E. coli* strains deficient in the catabolite gene activator protein compared with wild-type strains. This increase does not occur in strains deficient in both the catabolite gene activator protein and enzyme III-glucose, indicating that a protein similar to *E. coli* enzyme III-glucose is involved in the regulation of *P. multocida* adenylate cyclase. It also indicates that the underlying process leading to enterobacterial adenylate cyclase activation has been conserved through evolution.

Cyclic AMP (cAMP) in Escherichia coli is known to play a regulatory role in gene transcription via its receptor protein, catabolite gene activator protein (CAP) (25). Adenylate cyclase (AC) is the enzyme which converts ATP to cAMP. The AC structural gene (cya) of E. coli has been cloned and sequenced (1), and a model for the regulation of AC activity has been proposed. On the basis of genetic experiments, it was suggested that the phosphorylated form of enzyme III-glucose, a component of the phosphotransferase system, is an activator of AC (9, 17). When glucose transport takes place, the intracellular concentration of phosphorylated enzyme III-glucose decreases and correlates with a decrease in intracellular cAMP concentration. It has also been shown that the large increase of cAMP synthesis occurring in crp strains (deficient in CAP) (10) is dependent on the presence of enzyme III-glucose (4). In addition, gene deletion experiments have indicated that the carboxy-terminal domain of the protein is required for the regulation of AC activity by enzyme III-glucose (4, 20).

The cya gene of Erwinia chrysanthemi, another member of the family Enterobacteriaceae, has also been cloned and sequenced (5, 12). Comparison of the amino acid sequences of E. coli and E. chrysanthemi AC indicated that the proteins were very similar. The similarity was too large to permit significant identification of functional residues in the protein. This prompted us to sequence another gram-negative bacterium not closely related to E. coli.

In the present work, we have cloned and expressed in *E.* coli the cya gene of *Pasteurella multocida* from the family *Pasteurellaceae*, a gram-negative bacterium that is pathogenic for humans and animals (2). DNA sequencing data and genetic studies lead us to propose that *P. multocida* AC shares functional organization and regulatory properties with AC of the family *Enterobacteriaceae*. **Bacterial strains, plasmids, and growth media.** The strains used in this work were *E. coli* K-12 derivatives (Table 1). Plasmid pSa206T was a derivative of plasmid pSa206, a low-copy-number plasmid (3). pSa206T was obtained by replacing the kanamycin resistance gene of pSa206 by the tetracycline resistance gene. The growth medium was either Luria broth or minimal medium M63 (14) supplemented with the required amino acids (1 mM each), thiamine (5 μ g · ml⁻¹), and different carbon sources (0.4% each). Transductions using Pl*vir* were performed as described by Miller (14). When required, ampicillin, chloramphenicol, and tetracycline were added at 100, 40, and 2 μ g/ml, respectively.

Cloning and nucleotide sequence analysis. Genomic DNA from P. multocida CNP1 (NCTC 10322) (6) was kindly provided by F. Escande. After partial digestion with Sau3A, the DNA fragments, in the 2- to 10-kb range, were cloned into the unique BamHI site of plasmid pBR322. Nucleotide sequence analysis was performed by using subclones in the single-stranded phage vector M13mp19 (15). Unidirectional deletions were generated by using the Cyclone system (IBI) as recommended by the manufacturer. Nucleotide sequence was determined by the dideoxynucleotide chain termination method (22) when using PolIk or by a modified dideoxynucleotide chain termination method when using Sequénase (23). Restriction enzymes, T4 DNA ligase, and PolIk were from Boehringer-Mannheim. Modified T7 DNA polymerase (Sequenase) was from USBC. Oligodeoxyribonucleotides used as primers in DNA sequencing were purchased from Pharmacia. Sequence analysis was performed using the facilities of the Unité d'Informatique Scientifique of the Pasteur Institute.

Analysis of plasmid-encoded proteins. Minicells of strain AR1062 were purified as described by Rambach and Hogness (18). Plasmid-encoded proteins labeled with $[^{35}S]$ methionine were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under denaturing conditions (13).

MATERIALS AND METHODS

^{*} Corresponding author.

TAF	SLE.	1.	E.	coli	strains
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Strain	Genotype	Source or reference
TP610	F ⁻ thi-1 thr-1 leuB6 pro lacY1 tonA21 supE44 hsdR hsdM recBC lop-11 lig ⁺ cya-610	12
TP9500	\mathbf{F}^{-} xyl argH1 Δcya	4
TP2339	F^- xyl argH1 $\Delta cya \Delta crp-39 \Delta lac X74$	21
TP9510	F^- xyl argH1 ilvA Δcrp -39 Δcrr Km ^r	4
TP9512 ^a	F^- xyl argH1 $\Delta cya \Delta crp-39 \Delta crr Km^r$	$\begin{array}{l} \text{TP9510} \times \text{P1} \\ \text{(TP9500 } ilv^+) \end{array}$

^a Bacterial strain derived during this work.

cAMP assays. The excretion of cAMP by bacteria was analyzed on maltose MacConkey plates by using strain TP610A as an indicator bacterium (4). Strain TP610A is a spontaneous mutant of strain TP610 producing red colonies on maltose MacConkey agar when supplemented with a small amount of cAMP. A drop of an overnight culture of the strain to be analyzed was plated on a lawn of TP610A and then incubated at 37°C overnight. Strains excreting significant levels of cAMP produced a red halo on the plate around the culture drop because of the fermentation of maltose by the indicator bacterium. cAMP production was quantified by a radioimmunological assay (11) with cultures grown in minimal medium M63. Anti-cAMP antibodies were kindly provided by Agnès Ullmann. The amount of cAMP, including intracellular cAMP and cAMP excreted into the culture medium, was expressed in picomoles per milligram (dry weight) of bacteria. The background level was <5 pmol/mg. Total cAMP per milligram (dry weight) of bacteria was constant during the exponential phase of growth. The values are the means of three determinations.

Nucleotide sequence accession number. The *P. multocida* cya sequence has been assigned GenBank accession number M68901.

RESULTS AND DISCUSSION

Cloning of *P. multocida* AC (*cya*) gene. We transformed an *E. coli* Δcya mutant (TP610) with a plasmid library of *P. multocida* chromosomal DNA and screened for Cya⁺ clones on MacConkey maltose plates. Of several thousand transformants a few Mal⁺ clones were found in which the *cya* deficiency of the recipient was complemented. To confirm that the phenotype was due to cAMP production, the variations in the amount of cAMP per milligram (dry weight) of bacteria in cells grown with pyruvate were measured. The cAMP values ranged between 35 and 2,000 pmol/mg (dry weight) of bacteria. The plasmid DNAs of the recombinants were then analyzed. Restriction site analysis of four plasmids indicated that they all had in common a 0.7-kb *Hind*III-*Eco*RI fragment (Fig. 1).

Nucleotide sequence of the cya gene and deduced amino acid sequence of its gene product. The 0.7-kb *HindIII-EcoRI* fragments of two plasmids, pPMA140 and pPMA150 (Fig. 1), were sequenced and were shown to be identical. The nucleotide sequence contained an open reading frame which was incomplete in that it lacked a termination codon. A complete open reading frame sequence was obtained after cloning a 4-kb *HindIII* fragment (Fig. 1) in plasmid pBR322 yielding plasmid pPMA155. The pyruvate-grown cells carrying pPMA155 produced more cAMP (20,000 pmol/mg) than cells carrying pPMA150 (2,000 pmol/mg) or pPMA140 (35 pmol/ mg). The nucleotide sequence of 2.8 kb of the 4-kb *Hind*III insert, together with its deduced amino acid sequence, are presented in Fig. 2. The coding region was 838 codons in length. The deduced molecular size of 92 kDa was in agreement with the size of the protein synthesized in minicells containing plasmid pPMA155 (data not shown). The presumed start codon is TTG, a feature in common with the AC structural genes of *E. coli* (21) and *E. chrysanthemi* (5).

Comparison of the primary structures of P. multocida and enterobacterial ACs. As shown in Fig. 3, the P. multocida protein shares several regions of identity with E. coli and E. chrysanthemi ACs. There are 325 identical amino acid residues in the three proteins, and an additional 139 residues are conservative replacements. The lengths of the proteins are very similar (838 residues for P. multocida, 848 for E. coli, and 851 for E. chrysanthemi), and very few insertions or deletions are necessary to produce the best alignment. In general, deletions and insertions are in regions that are likely to be folded into loops of variable length (as seen from the presence of proline and glycine residues in their immediate vicinity). The 23 C-terminal residues of the E. coli protein, which are completely different from those of the E. chrysanthemi counterpart, had been thought to be dispensable (5); they are replaced by a set of only 5 residues in *P. multocida*, thus substantiating this hypothesis. Regions of identity are clustered into four major and several minor groups, suggesting a modular organization of the protein.

The main regions of divergence are located near the NH₂ terminus of the protein and in the region of residues 510 to 550. The former region cannot at present be related to a specific function of the protein, whereas the latter is a region that can be considered a hinge joining the catalytic and the regulatory domains of the protein (20). Another feature of these proteins is the high amount of cysteine and histidine residues. This may be related to a metal requirement for activity or some yet uncovered regulatory process. P. multocida AC also shares with the enterobacterial enzymes a common regulatory pathway affecting gene expression. The start codon of the three genes is the unusual UUG codon. The same observation was also made in the case of the enterobacterial species Salmonella typhimurium, from which the nucleotide sequence of the cya gene transcription regulatory region has been determined (8, 24). In the case of the E. coli cya gene, replacement of the UUG initiation codon by GUG or AUG has shown that the UUG codon has the lowest efficiency of translation (19). It was therefore proposed that the UUG codon provides a mechanism for limiting cya expression.

Regulation of *P. multocida* **AC activity in** *E. coli.* The CAP-dependent activation of *E. coli* **AC leading to the synthesis of a large amount of cAMP is easily visualized on MacConkey plates (see Materials and Methods). This cAMP**



FIG. 1. Partial restriction map of the *Hind*III fragment encoding the *cya* gene of *P. multocida*. The filled box represents the coding region of the *cya* gene. The vertical arrows indicate the 3' end of the truncated forms of the *cya* gene carried by plasmid pPMA140 (I) and plasmid pPMA150 (II).

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assay evaluation was used to analyze the production of cAMP by *P. multocida* AC in the *E. coli crp* strain.

The 4-kb HindIII fragment (Fig. 1) was inserted into HindIII-digested pSA206, a low-copy-number plasmid (3), to give pDIA1955. This plasmid was used to transform a Δcya strain (TP9500) and a $\Delta cya \ \Delta crp$ strain (TP2339). The secretion of cAMP by the $\Delta cya \ \Delta crp$ strain containing the cya_{PM} plasmid (pDIA1955) was analyzed on maltose MacConkey plates and compared with that of the same strain containing the $cya_{\rm EC}$ plasmid (pDIA1900), which carries the cya gene of E. coli (4). cAMP was excreted by both strains, as visualized by the red halos, but a smaller halo was observed with the strain containing the cya_{PM} plasmid (pDIA1955). No halo was observed when the same experiment was carried out with a $\Delta cya \ \Delta crp \ \Delta crr$ strain (TP9512) deficient in AC, CAP, and enzyme III-glucose and containing the cya_{EC} or cya_{PM} plasmid. These results indicate that P. multocida AC activity in E. coli crp strains is dependent on the presence of E. coli enzyme III-glucose. In order to quantify cAMP production, the $\Delta cya \ \Delta crp$ strain containing either the $cya_{\rm PM}$ plasmid or the $cya_{\rm EC}$ plasmid was grown on pyruvate as the sole carbon source and total cAMP production during exponential growth was measured. The levels of cAMP thus obtained were compared with those obtained with the Δcya strain containing the same plasmids (Table 2). When the $\Delta cya \Delta crp$ strain was used as the host cell, higher levels of cAMP were observed with both plasmids. The level of cAMP obtained with the $\Delta cya \ \Delta crp$ strain containing the cya_{PM} plasmid was lower than that obtained with the same strain containing the $cya_{\rm FC}$ plasmid, a result in agreement with the cAMP secretion level observed on MacConkey plates. With the Δcya S K T F K A G W Y V V N Q T P S V A G F V Q K R Y T E Y S E S L N K L V A W A Y

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V GTC/	T 400	N 3441 181	E TGA 10	D GGA1	L 171	T AAC1 820	H CAT	A GC1	C TGT 830	GAN	1 A11	R CGG 184	S AGC 0	L TTA	I 185	V ITGI IO	A GCAG	V 577 1	N AAC 860	L TTA	T	V GTT0 1870	D GAC	P	T ACCA 188	к Алл О	K AAA	1 1 TCAG 189	r q Caca Po	AGTO	K 190	S rcti 0	R CGC/	I 171C. 191	Q A AAGO O	CAG	D TGAT 1920
L	F	S IAG1 19:	F TTT 30	G TGGJ	P 100	к GAAA 940	E IGAA	E GAV	S MGT 1950	L TTA	V GTO	G GGT 196	S AGT O	I ATC	D GATA 197	1 (TT) '0	T ACTI	Y AC	R CGT. 980	N AATO	L CTT	W TGG/ 1990	N NAT D	E GAN	1 111A 200	R GAA O	T CCT	L H TACJ 201	4 F ATTT 10	E TGAJ	G GGA 202	P CCA	N AATO	A GCGA 203	1 L TCC1 0	. L ICTT	A AGCC 2040
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R CGC	C rg1	1 1411 21	S TAG 70	I TATO	Q (CA 2	1 AATI 180	G GGGC	D GA	A 16CA 2190	L ICTT	р СС/	P CCA 220	Q (CAA 10	N AAT.	N AATO 221	L TA	L TTG	R CGT 2	V GTG 220	A GCAI	G GGT	K 223	N NAC D	W TGGI	Q CAGT 224	F T T T O	F TCT	F 1 TTG/ 22!	E E VAGA 50	R	G GGC 226	I ATC. 0	S AGCI	L 1TAC 227	Q E AAGA O	i I Mit	H TCAC 2280
S TCA	H 441	E 16A 22	E AGA 90	L ATT/	E AGA 2	A AGCI 300	T	G	F 111 2310	D GAT	T	A GCG 232	L ICTA	о с м	T ACCO 233	E 544 30	V GTGI	E GAA 2	Е GAA 340	к ААА	E GAA	S IAGCI 235	A GCC 0	L TTA	P CCCG 236	D ATA O	T ICCT	S 1 CACI 23	ς τ 5ΑΑC 70	r y Ctai	P CCCO 238	р ССА 0	E GAG/	1 4TTG 239	D I ATC/ 0	I F ACTT	A TGCC 2400
S AGT	E	G GGG 24	F CTT 10	L TTV	Q ACA 2	F ATT 420	۶ TTTC	F TT	E TGAV 2430	D IGA1	H M	S 11CT 244	D GAT	G GGT	S TCC1 24!	F 111. 50	N AAT	V Бтс 2	Y TAT 460	I ATT	L 11/	D GAT 247	E GAA 0	A GCC	N AATC 248	R GC <i>I</i> 10	I TCG	E AAA 24	1 1 1117 90	(R NCCG	N FAAC 250	C TGC 0	D GATO	G GGAC 251	Q 1 AAA 0	(E NAGA	K AAAG 2520
K AAG	I ATI	L 111 25	E GGA 30	I GAT	H TAA 2	H CCAI 540	I CATI	Y TA	Q TCAV 2550	S NTCI	S ITC	G AGGC 256	L :TTC :0	D	E GAN 25	N VAT 70	N	N AA1 2	P CCG 580	Y TAT	к	1 MTT 259	V GTG 0	Q	R CGTC 260	D (AT1 (0	F	N ACT 26	Y F Atco 10	GCA	F ATTC 262	Y TAT O	Q	L 1TAT 263	L I TAT 10	L Q TACA	E AGAG 2640
N AAT	6 (),)	V Tan	K	I AATI	V	P	F	H	S	R	L	A	H	S	TAA	TAT	ATT	000	GGT	TAT																	

FIG. 2. Nucleotide sequence of the *P. multocida cya* gene and deduced amino acid sequence of the AC. The numbering of the

nucleotides starts at the HindIII restriction site.

 $\Delta crp \ \Delta crr$ strain containing pDIA1956, a derivative of pSa206T containing the *P. multocida cya* gene, the level of cAMP was very low. It may be argued that the increase of cAMP synthesis in the *crp* strain (about fourfold) could be accounted for by an elevated level of expression of AC (the transcription of the *cya* gene of *E. coli* has been shown to be negatively regulated by CAP-cAMP). However, the fact that the $\Delta cya \ \Delta crp \ and \ \Delta cya \ \Delta crp \ \Delta crr \ strains expressing the$ *P. multocida cya*gene produced different levels of cAMP demonstrated that enzyme III-glucose is involved in the activation of*P. multocida*AC.

When glucose was used as the carbon source, the level of cAMP of the Δcya strain containing the cya_{PM} plasmid was lower (550 pmol/mg) than that obtained with pyruvate as the sole carbon source (about an eightfold increase) and very similar to that obtained with the Δcya strain containing the cya_{EC} plasmid (400 pmol/mg). Thus, *P. multocida* AC activity (like *E. coli* AC) was inhibited during the glucose transport, as proposed by Postma (16), i.e., because of a decrease of the intracellular concentration of phosphorylated enzyme III-glucose.

Different C-terminal truncated forms of *E. coli* AC have been shown to retain enzymatic activity (20) but have lost the regulation leading to the high production of cAMP in *crp* strains (4). On the basis of these experiments, it has been proposed that *E. coli* AC is composed of two functional domains: the amino-terminal catalytic domain and the carboxy-terminal regulatory domain (20), the latter being required for both the glucose effect and the CAP-dependent activation process (4). In the case of *P. multocida* AC, both truncated forms were active (as shown by the complementation experiments) but no cAMP excretion was obtained on MacConkey plates with the $\Delta cya \ \Delta crp$ strain containing either pPMA140 or pPMA150 carrying truncated genes (Fig. 1). When the minicell-producing cell AR1062 was transformed with plasmid pPMA140, a polypeptide of the ex-

P.m E.c Erw.c S.t	10 MULTESAOK MULTESAOK MULTESAOK MULTESAKO MULTESAKO	RVENLIKLRI RLDNINGLRV RLDRINGLRV RLDRINGLRV	ERAISGESGE DRAIAAMGPA DRAIEMIRPA DRAIEMIRPA	roeve glitti roove slitti roove slitte roove slitte roove slitte	TLEINEN NLE TLEINEN IMP TLEINEN IMP TLEINEN IMP	59 GYL-ADARG GYLDGNVIRG GYLECKVIRG GYLDGNVIRG
P.m E.c Erw.c S.t	IADIVISPYD ICLATPDETO ICLFSPDERO ICFATPDETO	ROTILETVPS REVINELELY OHYLOSVELR REVINELELY	LEANQBLLPS RGMSVQDPPK WGELSAPDRK RGMTPQDPPK	FSYRSINAIL Gelpirgvyt Gelpirgvys Gelpirgvyt	GVYVNGSIAS MGSTSSVGQS MGSTSSIGQS MGSTSSVGQS	119 1507778502.0 CSS02.0 CSS02.0 CSS02.0
P.m E.c Erw.c S.t	TWVCERDERS INVCEQSWED INVCEQSWED INVCEQSWED	TREACHION SPEROLICIU NEERO-LCOM GEEROLLON	THE ICHARO CSCIENDAS CSCIENDAG CSCIENDAG CSCIESMAS	FNI FIN VIA LGVEVS FII QGVDVS FII LGVEVS FII	CORRECTRY DENREMENS DENREMENES DENREMENES	179 AEFITALICG GS-LGGEDCG GS-LGGEDCG GS-LGGEDCG
P.m E.c Erw.c S.t	SAQIMULIDE STORI LLIDE STORI LLIDE STORI LLIDE STORI LLIDE	FYRSATRLAG FYRTAVRLAG FYRTAVRLAG FYRTAVRLAG	NPLLWLRLL NRLWNMAPC NRLWNMAPM NRLWNMAPM	DE EENYESEV DE EENYESEV DE EENYDDYV DE EENYDDYV	ERIVRTQQIC MTUYAQGQLT LSUYARGALA MTUYAQGVLT	239 LODIMOFICI PNEHLOLGGL PNEHLOLGGL PNEHLOLGGL
P.m E.c Erw.c S.t	GOLSANEYFG SSLSADEYFG SALSADEYFG SSLSAEEYFG	ASLWQLYNGI ASLWQLYNSI ASLWQLYNSI AFLWQLYNSI	DAP YKSVIRI DSPYRAVIRI DSPYRAVIRI DSPYRAVIRI	LILLET KESEY LILLEA KSWEY LILLEA KSWEY LILLEA KSWEY	PHTYLIAROF PHPRLIARDI PHTRLISSEI PHTRLISSEI	299 Nebîla Tâkî N Norlihogel v Norlihogel v Norlihogel v
P.m E.c Erw.c S.t	PSHHPDPYLA -SFGLDPYCM -SFGLDPYCM -SFGLDPYCM	NLOR ATTYLT MERCHANY AND NLOR ATTYLT MERCHANY AND	KHNELKRIGF ATTOFTRIOL ATTOTRIOL ATTOPTCIOL	VRRSVYLKAT VRRSPYLKVC VRRSPYLKVC VRRSPYLKVC	EGHCHOOPNN 1991 SRERA ERUSRERA ERUSRERA	359 TINNITE CHILD CVCHIRRAVES CTANIRE JES CVCHIRE JES
P.m E.c Erw.c S.t	Kutornonso Quvsengroe Quvsengroe Quvsengroo	at the fingra artamionra eruvmionra artimionra	NNIK DOQVIOO NNIK DOQVINEA NNIK DOQVINEA NNIK DOQVINEA	HINSLITKFIAL HINSLITKFIAL HINSLITAANNO HINSLITAANNO HINSLITAANNO	SYRNLFAFAR SYRNLERFAR SYRNLERFAR SYRNLERFAR	419 RHKVNSSIMP RNNLSVSASP RNNLSVSASP RNNLSVSASP

pected size (35 kDa) was detected (data not shown). It therefore appears that the activity of the truncated 35-kDa polypeptide corresponding to the N-terminal part of P. *multocida* AC is not activated in the presence of enzyme III-glucose. It can then be proposed that E. *coli* and P. *multocida* AC belong to a class of proteins organized in functional domains. It is worth noting that different point mutations affecting the regulation of E. *coli* AC activity by enzyme III-glucose (4) were located in highly conserved regions of the COOH-terminal part of P. *multocida* AC.

Another class of bacterial AC includes the calmodulindependent AC toxins secreted by two pathogens, *Bordetella pertussis* and *Bacillus anthracis*. Although these ACs are quite different and are produced by taxonomically distinct organisms, they are antigenically related to each other and share regions of striking similarity (7). Since *P. multocida* is a toxigenic pathogen, we also investigated the possible effect of calmodulin on its AC. The enzymatic activity appeared to

TABLE 2. cAMP levels in strains containing different plasmids

Strain	Total cAMP [pmol/mg (dry wt) of bacteria] with plasmid:										
(genotype)	pDIA1900 (E. coli)	pDIA1955 (or pDIA1956) (P. multocida)									
TP9500 (Δcya)	1,000	4,500									
TP2339 ($\Delta cya \ \Delta crp$)	32,000	17,000									
TP9512 ($\Delta cya \ \Delta crp \ \Delta crr$)	400	300									

						479
P.m E.c Erw.c	ODISVLTRKI ODIGVLTRKI ODIGVLTRKI	NTAFES APGR NAAFBAARGR NAAFBAARGR	utionpoise Vitionpoise Vitionpoise	NLSERVILLEE DLSERVILLEE DLSETVLEET	EVINGSKTERA Yvepgranrs Yveagranrs	GWYUVNOITS GWYLYNRAFN GWYLYNOAFS
P.m E.c Erw.c	VAGPVORRYT TESTISHOPL MDATUSHOPL	EYSESATIAA CANRY LARAA EYNRY LARAA	NKAY INRI AT NON PIGLAT NYAY INGLAT	ANTO GET SP SPERICUTINGN SSERVITINGH	N-VSEППЕН СТУПЕРКЕДЕ БІССПЕНКЕДЕ	538 FVTDLRLSFP MVAOVSHHFP LVSDVSSHFP
P.m E.c Erw.c	VIVSSVINED LRLPAPTEKA LRVAAFTEKA) ПТНАСТІРІЗІ. 1 ЦУЗРСКІВНІ. 1 ЦУЗРСКІВНІ.	IVAVNITVDP ATTVNLEYDP ATTVNLEHDP	TKKI TQVK TAAFRNQVVH TAAFRNQVVH	SRIQASDLFS FDFRKLDVFS FDFRQLDVFS	596 FGPKEESLVG FGENQNCLVG FGQQQQCLVG
P.m E.c Erw.c	599 Siddi i YRNL A Svort arrise Siddi arrise	NET RTLEIFEG NEVRTLEIFNG NEVRTLEIFSG	FNATELATRV EQSMIEATRT EQSMIEATRT	LISNKITHRGAP TILGKONODAN TILGKONODAN	SPKLIQVISI PROSVEVICS LPESLEVICS	656 Shrmrtisn Sonirgiart Sonirgiart
P.m E.c Erw.c	IVPHLINRCI RVQQLVSECI RVQQLVSECI	STOTGOALP- ELRLSSTRQE ELRLSSTRQE	-PQNNLLRVA TGRFKALRVS PGRFKAVKVA	GRINGLEFFE Gotngleffer Getngleffer	RGISLOPIHS LNVSVORLEN LSVSAORLEN	714 NERLEATGED AIRFYGAISH AVEFYGAISN
P.m E.c Erw.c	TATCHEVERK NKIRGLSVOV NKIGGLPVOV	ESALPDTSRT ETNHVKL ETNHIHL	YPPETDHFAS -PAVVDSFAS -PPVVDSVAS	EGELQFFFED EGELQFFFED EGELQFFFED	NSDG-SIFNVY TQDENGIPNEY QHDNQGIPNEY	773 LDEARRIEL LDESRUEU LDESRUEU
P.m E.c Erw.c	YRNCDGQKBK YHHCBGSKBE YHHCBGSKBE	KTILE INHI YQ LVRDVSRFYS LVRDVSRFYS	SSGLDENNNP SSHDRFTYGS SSHDRFTYGS	YKIVQHIFNY SFINFNI SFINFNI	5ÖLIÖINÖID 5ÖLIÖINKAD 5ÖLIÖIIKAD	833 NGVKIVPIHS GREQVIPIRT GRTQVIPIRS
P.m E.C	838 RLAMS KSIGNMPPAN	QDHDTPLLQQ	YFS			

FIG. 3. Comparison of the amino acid sequences of P. multocida (P.m), E. coli (E.c), E. chrysanthemi (Erw.c) and S. typhimirium (S.t, first 412 residues only) adenylate cyclases. Identities are indicated in darkly shaded boxes and conservative amino acid changes are shown in lightly shaded boxes. Numbers refer to P. multocida AC residues.

be insensitive to calmodulin. Therefore, the *B. pertussis* and *B. anthracis* ACs remain the only known examples of prokaryotic enzymes activated by eukaryotic protein. In conclusion, *P. multocida* AC clearly belongs to a class which was, until the present work, believed to be limited to enterobacterial ACs. Further experiments will be required to establish the presence of a phosphotransferase system in *P. multocida* as suggested by our data.

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