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Differential Regulation of the *mcb* and *emr* Operons of *Escherichia coli*: Role of *mcb* in Multidrug Resistance

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The *mcb* operon (which is responsible for microcin B17 production) and the *emr* operon (which encodes a multidrug resistance pump) share a common negative regulator, EmrR. Nevertheless, compounds that induce the *emr* operon repress the *mcb* operon. The pump dedicated to microcin B17 extrusion can also protect the cells against sparfloxacin and other toxic compounds.

The *Escherichia coli mprA* gene (recently renamed *emrR* [9]) represses microcin B17 (MccB17) synthesis (2, 3) and the *emr* operon. The EmrR protein is encoded by the first gene of the chromosomally located *emr* operon (9). Additionally, this operon codes for EmrA and EmrB, which together constitute a multidrug resistance pump (8).

MccB17 production is controlled by a plasmid-encoded *mcb* operon. This peptide antibiotic kills non-microcin-producing bacteria by inhibiting DNA gyrase. It is produced in the stationary phase and presumably relieves starvation at the expense of the killed bacteria (1, 7). The *mcb* operon consists of *mcbABCDEFG* genes (5), the first four of which are involved in MccB17 synthesis (10, 11, 15). The McbEFG proteins perform export and immunity functions (4).

The *emr* operon is induced by a number of toxic compounds which relieve EmrR repression (9). The resulting synthesis of the EmrAB pump confers resistance to several of the same compounds, presumably by enabling the cells to extrude them (9). Since the *mcb* operon is also negatively regulated by EmrR, we wondered whether inducers of the *emr* operon would also induce production of MccB17.

All strains used in this study are described in Table 1. Previous reports have established that *mcb-lacZ* fusions are markedly induced in the stationary phase (5, 6). A single-copy *mcb-lacZ* fusion strain (AMS503 [Table 1; Fig. 1]) was constructed by the method described by Simons et al. (12), as we described previously (9). This strain showed a 25-fold induction in the stationary phase, confirming the results referred to above. We also confirmed that *emrR* is a negative regulator of the *mcb* operon; insertional inactivation of this gene increased the expression of a *mcb-lacZ* fusion (Table 2). This increase affected both the basal levels and the stationary-phase-induced levels; however, the induction ratio was changed by only twofold (data not shown). Thus, this gene has only a minor role in growth-phase regulation of *mcb* operon expression.

We then tested the effects of *emr* operon inducers on expression of the *mcb-lacZ* fusion, using the previously constructed *emr-lacZ* fusions (9) as controls. Salicylate (an *emr* operon inducer) affected the two fusions in opposite ways (Table 2). While it induced the *emr-lacZ* fusion (as reported previously [9]), it repressed the *mcb-lacZ* fusion. The repression of the *mcb-lacZ* fusion by salicylate was EmrR dependent, since it did not occur in strain AMS504, which lacks the *emrR* gene; if anything, salicylate slightly stimulated expression in this genetic background. Similar results were found in response to the addition of 2,4-dinitrophenol (DNP) or carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Table 2).

To directly investigate the effect of salicylate, we determined how its presence affected the killing of sensitive bacteria by MccB17-producing bacteria. Strain AMS6 bearing the plasmid pPY113 (Table 1), which contains all of the genes required for MccB17 synthesis and export, i.e., *mcbABCDEFG* (5), produced a large zone of inhibition on a lawn of sensitive bacteria in the absence but not in the presence of salicylate. However, an *emrR*-deficient strain (OLS101 [Table 1]) containing this plasmid produced a halo even in the presence of salicylate (data not shown). Thus, the salicylate repression of MccB17 synthesis was dependent on EmrR, and the results confirm that the *mcb* operon responds to salicylate in a manner different from that of the *emr* operon (9), even though both are controlled by EmrR.

The *mcbABCDEFG* operon contains an internal promoter (P2) which is regulated in a manner different from that of the primary promoter of this operon, P*mcb*, in that P2 is not induced in stationary phase (5). P2 regulates the extrusion and immunity genes (*mcbEFG*) of the *mcb* operon (5). To determine the role of EmrR in the regulation of P2, we constructed a single-copy P2-*lacZ* fusion (strain AMS505), in which *lacZ* expression is controlled only by P2 (Fig. 1). We found (Table 3) that (i) P2 expression in exponential phase was two- to threefold higher than that in stationary phase; (ii) a null mutation in *emrR* decreased expression, while *emrR* overexpression increased it, indicating that EmrR regulates P2 expression positively; (iii) the growth-phase regulation of P2 was *emrR* dependent; and (iv) salicylate did not inhibit its expression (data on salicylate not shown).

These different modes of regulation of genes responsible for MccB17 synthesis and its extrusion prompted us to consider the possibility that the *mcbEFG* genes have a role in addition to MccB17 extrusion. This is consistent with the finding that, in spite of the fact that MccB17 is not produced in exponential phase (see above), the microcin pump genes are nevertheless

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TABLE 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Genotype or description	Reference or source
E. coli		
AMS6	K-12Δ*lacU169*	Our laboratory collection
MLM63	AMS6λRS45[ϕ(*emrRA-lacZ*)]	9
MLM635	AMS6λRS45[ϕ(Δ*emrRA-lacZ*)] *emrR*::Km	9
OLS103	AMS6*emrB*::Km	9
OLS101	AMS6*emrR*::Km	9
AMS503	AMS6λRS45[ϕ(*mcbA-lacZ*)]	This study
AMS504	AMS503*emrR*::Km	This study
AMS505	AMS6λRS45[ϕ(*mcbE-lacZ*)]	This study
AMS506	AMS505*emrR*::Km	This study
Plasmids		
pRS415	Vector for constructing transcriptional fusion to the *lacZ* gene; Ap^r	12
pUC18	Cloning vector	New England Biolabs
pEmrR	pGEM3Z cloning vector carrying *emrR* gene	9
pSE-AB	pSE380 cloning vector with *emrAB* genes	8
pPY113	pBR322 carrying *mcbABCDEFG* genes	5
pMM3	pBr322 carrying *mcbEFG* genes	4

expressed. We wondered whether this role was protection against other deleterious agents.

An *E. coli* strain carrying plasmid pPY113, which overproduces proteins mcbABCDEFG, exhibited increased resistance to the fluoroquinolone antibiotics sparfloxacin (eightfold), tosulfloxacin (fourfold), and levofloxacin (twofold). These experiments were done by using twofold serial dilutions in microtiter plates that were incubated overnight. When the plasmid pMME3 (containing *mcbEFG* genes) was used, the same degree of protection was observed, indicating that the increased resistance was due to the overexpression of the pump genes. None of these strains showed increased resistance to other

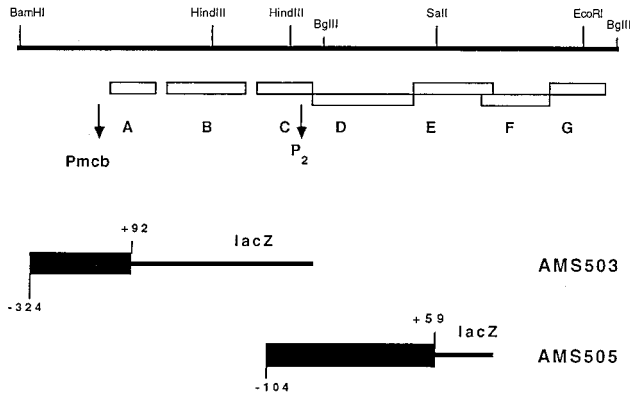

FIG. 1. Schematic representation of the fusions constructed with the transcriptional vector pRS415 (●). *lacZ* expression in strains AMS503 and AMS505 is controlled by P*mcb* and by an internal promoter within the *mcb* operon (P2), respectively. The top horizontal line gives the restriction pattern of the genomic fragment bearing the *mcb* operon. Solid boxes (the bottom two lines) represent *mcb* sequences. The negative numbers in both fusions refer to the corresponding transcriptional start sites, while the positive numbers refer to translational start sites of *mcbA* (AMS503) and *mcbE* (AMS505) genes.

TABLE 2. Effects of different compounds on expression of the *mcb-lacZ* fusion in *emrR*-proficient and -deficient backgrounds[a]

E. coli strain	Fusion	*emrR*	β-Galactosidase activity			
			No addition	Salicylate	DNP	CCCP
AM503	*mcb-lacZ*	+	12,000	1,690	1,630	6,210
AMS504	*mcb-lacZ*	−	46,000	63,333	55,200	48,130
MLM63	*emr-lacZ*	+	296	2,338	2,500	444
MLM635	*emr-lacZ*	−	14,414	18,110	18,900	13,000

[a] Samples were removed from cultures grown overnight in Luria-Bertani medium (37°C) in the presence of sodium salicylate (5 mM), DNP (0.1 mM), or CCCP (10 mM) and were assayed with chlorophenol red–β-D-galactopyranoside as described previously (9). Values are the averages of at least three experiments; standard deviations did not exceed 5 to 10%.

quinolone antibiotics, viz., ciprofloxacin, norfloxacin, temafloxacin, lomefloxacin, and oxolinic acid, nor was resistance to kanamycin, tetracycline, or chloramphenicol increased. Thus, it appears that the *mcb* pump is most effective against sparfloxacin. The fact that sparfloxacin is unusual in containing 5-amino and 8-fluoro groups may be related to this phenomenon. Other quinolone pumps can also exhibit substrate specificity toward particular quinolones, e.g., the *Staphylococcus aureus* NorA (14) and the *Mycobacterium smegmatis* LfrA (16) proteins, which have a predilection for hydrophobic quinolones. However, different degrees of susceptibility, as reflected by MICs, are not necessarily a reflection of the specificity of the pump, since other factors, viz., membrane permeability and gyrase sensitivity to different antibiotics, can also have a role. Accumulation experiments are in progress to determine the importance of the pump specificity in this phenomenon.

In addition, albeit only partially, the *mcb* pump protected against CCCP and tetrachlorosalicylanilide (TCS), which are the substrates for the EmrAB pump. A mutant strain, OLS103, devoid of the EmrAB pump exhibited better growth in the presence of CCCP when it contained different *mcb* plasmids (Fig. 2); the presence in this mutant of plasmid pSE-AB (encoding the EmrAB pump) provided better protection. Similar results were obtained with TCS (data not shown).

These studies were undertaken to address an apparent physiological paradox. The *emr* and the *mcb* operon genes that code for MccB17 synthesis represent survival strategies of different kinds. The former is essentially a defensive mechanism designed to protect the cell against toxic compounds; the latter is an aggressive strategy which kills competing bacteria. Yet they both share a common negative regulator (EmrR), implying that both may be induced by the same environmental sig-

TABLE 3. Regulation of P2 expression[a]

Growth phase	β-Galactosidase activity with		
	emrR^{+}[b]	*emrR*[c]	pEmrR[d]
Log	1,180	260	2,800
Stationary	440	227	3,590
Fold induction[e]	2.7	1.1	0.8

[a] Samples of various strains were removed from cultures growing either exponentially (optical density at 660 nm, ~0.3) or after overnight growth in Luria-Bertani medium at 37°C and were assayed with chlorophenol red–β-D-galactopyranoside. At least three independent experiments were performed; standard deviations were within 5 to 10%.

[b] Strain AMS505.

[c] Strain AMS506 (*emrR* deficient).

[d] AMS505 carrying the multicopy plasmid pEmrR.

[e] Fold induction values are stationary phage-to-log phase ratios.

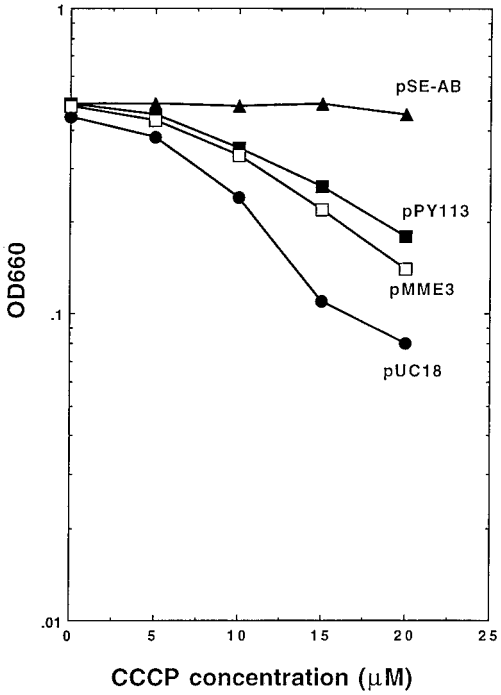

FIG. 2. Effects of different plasmids on the CCCP sensitivity of the *emrB* mutant strain OLS103 containing different plasmids. Cells were grown in Luria-Bertani medium at 37°C to late log phase and were diluted in Luria-Bertani medium with different concentrations of toxic compounds to an A_{660} of approximately 0.1. After 2.5 h of growth, the A_{660} values of the cultures were measured and plotted as a function of drug concentrations. pUC18, control; pPY113, contains the entire *mcb* operon; pMME3, contains *mcbEFG* genes; pSE-AB, contains the *emr* operon. Individual points are the averages of at least four measurements; standard deviations were within 5%. A_{660}, optical density at 660 nm.

nals. The *emr* operon is induced by a number of toxicants which serve as substrates for the EmrAB pump, making this induction protective in the presence of such compounds (9). However, concurrent induction of the MccB17 synthesis-encoding genes by these compounds has no obvious advantage. These studies show that *E. coli* has successfully resolved this paradox by repressing one operon and inducing the other in response to the presence of the same toxicants, even though the two operons share the same regulator. The underlying molecular mechanism of this differential effect remains undetermined. Our preliminary results with cell extracts show that EmrR is a DNA-binding protein whose affinity to *emr* and *mcb* operons is differently affected by a given compound; studies with purified EmrR are in progress. On the basis of our preliminary findings, it appears that EmrR enables the cell to selectively switch on the survival response appropriate to individual environmental conditions.

Our investigation of whether the internal promoter of the *mcb* operon is affected by EmrR and inducers of the *emr* operon led to the unexpected finding that McbEFG proteins, which are thought to be a dedicated pump and immunity mechanism against MccB17, can confer resistance against the unrelated compounds sparfloxacin, CCCP, and TCS. Thus, bacteria can recruit what appears to be a dedicated function to gain protection against other antimicrobial agents. This realization is important for our present efforts to combat emerging bacterial multidrug resistance.

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