Microbiology Comment provides a platform for readers of **Microbiology** to communicate their personal observations and opinions in a more informal way than through the submission of papers.

Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful piece of methodology that we would like to share.

The Editors hope that readers will take full advantage of this new section and use it to raise matters that hitherto have been confined to a limited audience.

Jon Saunders
Editor-in-Chief

Bacillus megaterium **CcpA does not cross-** **complement *Escherichia*** ***coli* FruR**

Current research is revealing a mechanism of catabolite repression in species of *Bacillus* that is mediated by the CcpA DNA-binding protein (2, 7). The proposed mechanism involves a metabolite-sensing protein kinase that phosphorylates the small phosphocarrier protein, HPr, of the phosphoenolpyruvate:sugar phosphotransferase system at seryl residue 46 (4, 8). This phosphorylated protein binds to the CcpA transcription factor which binds to *cis*-acting, catabolite-responsive elements (CREs) in the promoter regions of catabolite-sensitive operons to effect catabolite repression (3).

The applicability of this catabolite repression mechanism to other low GC Gram-positive bacteria is not known, but HPr(Ser-P) has been identified in all such organisms in which it was sought, and CREs have been detected in catabolite-sensitive operons of many of them. Recently, a homologue of CcpA was identified in *Clostridium acetobutylicum*, and it was shown to mediate catabolite repression of

Table 1. Growth response of *E. coli* wild-type (LJ2724) and *fruR* mutant (LJ2725) strains bearing plasmids encoding FruR of *E. coli* or CcpA of *Bacillus megaterium*

Growth was monitored after 48 and 96 h at 37 °C on medium 63 minimal agar plates supplemented with 0.5 % of the indicated carbon source and 1 mM IPTG. Sensitivity to xylitol was assayed on LB agar plates containing 0.2% xylitol. The *E. coli* strains LJ2724 and LJ2725 have been described previously (6). Plasmid pFRUR1 expresses the *fruR* gene of *E. coli*, and plasmid pAT153 served as the corresponding control plasmid (6). Plasmid pWH1564 expresses the *B. megaterium* *cspA* gene upon induction with IPTG, and plasmid pQE16 served as the corresponding control plasmid (2). All strains grew at apparently the same rate on minimal glucose plates. Growth or lack of growth on the gluconeogenic carbon sources is indicated by + or -, respectively.

Strains	Xylitol sensitivity	Growth on pyruvate, lactate or alanine
LJ2724	Resistant	+
LJ2725	Sensitive	-
LJ2724(pAT153)	Resistant	+
LJ2725(pAT153)	Sensitive	-
LJ2724(pFRUR1)	Resistant	+
LJ2725(pFRUR1)	Resistant	+
LJ2724(pQE16)	Resistant	+
LJ2725(pQE16)	Sensitive	-
LJ2724(pWH1564)	Resistant	+
LJ2725(pWH1564)	Sensitive	-

amylase and catabolite activation of acetolactate synthase in a *cspA* mutant of *Bacillus subtilis* (1). This protein, designated RegA, exhibited 38–40% identity with the *cspA* gene products of bacilli and was proposed to be the functional analogue of CcpA in *C. acetobutylicum*. These proteins are members of the LacI–GalR family of helix–turn–helix-containing transcription factors, a family that includes the pleiotropic

regulator of carbon metabolism in enteric bacteria, FruR, previously termed Shl (5). Davison *et al.* (1) reported that the cloned *regA* gene of *C. acetobutylicum* complemented the defect in an *Escherichia coli fruR* mutant. This report prompted us to examine the possibility that CcpA could substitute for FruR with respect to its identified roles in the regulation of the *fru* and *pps* operons of *E. coli* (5, 6).

As shown in Table 1, the *fruR* mutation renders *E. coli* sensitive to xylitol since it renders the fructose operon constitutive. Xylitol sensitivity is readily reversed by inclusion of a plasmid expressing FruR (pFRUR1) but not by inclusion of one expressing CcpA (pWHI564). Additionally, pFRUR1 but not pWHI564 readily reversed the negative growth phenotype of the *fruR* mutant LJ2725 in a minimal medium containing either pyruvate, lactate or alanine (Table 1; 6). Finally, we have previously shown that FruR does not bind to CREs of *B. subtilis* (6, 7). We therefore conclude that contrary to expectation based on the reported complementation of both the *B. subtilis* *ccpA* mutant and the *E. coli* *fruR* mutants by the *C. acetobutylicum* *regA* gene product, the two homologous proteins CcpA of *Bacillus*

► GUIDELINES

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of ***Microbiology*** do not necessarily agree with the views expressed in ***Microbiology* Comment**.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.

megaterium and FruR of *E. coli* are not functionally interchangeable.

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Authors' reply

Clostridium acetobutylicum RegA mimics *Escherichia coli* FruR in a *supH leu* mutant

Davison *et al.* (1) reported the cloning and characterization of a *Clostridium acetobutylicum* P262 *regA* gene and showed that the gene product exhibited 38–40% identity with the *acpA* gene products of *Bacillus* strains. Furthermore, the *C. acetobutylicum regA* gene complemented a *Bacillus subtilis acpA* mutant and mediated catabolite repression of amylase and catabolite activation of acetolactate synthase in the *B. subtilis acpA* mutant.

Since the *C. acetobutylicum* RegA protein showed 21% identity to the *Escherichia coli* FruR protein, previously termed Shl (2, 3), its ability to complement FruR was investigated (1). The system utilized to determine complementation of FruR did not involve an *E. coli fruR* mutant. However, Leclerc *et al.* (2) showed that the *E. coli fruR* gene on a multicopy plasmid inhibited the growth of *E. coli* GD40 (*supH leu*) cells on minimal medium. It was assumed that the FruR protein inhibited the expression of the *supH*

gene and therefore the *leu* auxotrophy was not relieved. The *C. acetobutylicum regA* gene was similar to the *E. coli fruR* gene in that both *regA* and *fruR* on multicopy plasmids inhibited the growth of *E. coli* GD40 (*supH leu*) on minimal medium (1).

The ability of the *C. acetobutylicum regA* gene to substitute for FruR in an *E. coli fruR* mutant has not been determined. In view of the ability of RegA to complement FruR in an *E. coli supH* mutant it will be interesting to investigate whether RegA could also substitute for FruR with respect to its identified roles in the regulation of the *fru* and *pps* operons of *E. coli* (3). It would also be interesting to determine whether the CcpA protein could inhibit growth of the *E. coli* GD40 (*supH leu*) on minimal medium.

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