2-Deoxy-d-glucose is a potent inhibitor of biofilm growth in *Escherichia coli*

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*Escherichia coli* strain 15 (ATCC 9723), which forms robust biofilms, was grown under optimal biofilm conditions in NaCl-free Luria-Bertani broth (LB*) or in LB* supplemented with one of the non-metabolizable analogues 2-deoxy-d-glucose (2DG), methyl α-β-mannopyranoside (αMM), or methyl α-β-glucopyranoside (αMG). Biofilm growth was inhibited by mannose analogue 2DG even at very low concentration in unbuffered medium, and the maximal inhibition was enhanced in the presence of either 100 mM KPO₄ or 100 mM MOPS, pH 7.5; in buffered medium, concentrations of 0.02 % (1.2 mM) or more inhibited growth nearly completely. In contrast, mannose analogue αMM, which should not be able to enter the cells but has been reported to inhibit biofilm growth by binding to FimH, did not exhibit strong inhibition even at concentrations up to 1.8 % (108 mM). The glucose analogue αMG inhibited biofilm growth, but much less strongly than did 2DG. None of the analogues inhibited planktonic growth or caused a change in pH of the unbuffered medium. Similar inhibitory effects of the analogues were observed in minimal medium. The effects were not strain-specific, as 2DG and αMM also inhibited the weak biofilm growth of *E. coli* K12.

**INTRODUCTION**

The phosphoenolpyruvate : sugar phosphotransferase system (PTS) catalyses the uptake and concomitant phosphorylation of a number of substrates, termed PTS sugars (for reviews see Deutscher et al., 2006; Postma et al., 1993). Non-metabolizable analogues 2-deoxy-d-glucose (2DG) and methyl α-β-glucopyranoside (αMM) enter *Escherichia coli* cells primarily via the broad-specificity mannose PTS or the glucose-specific PTS, respectively. In an early study, *Kₘ* values of the two PTS permeases (Enzymes II) for sugars and sugar analogues were determined using transport studies in *E. coli* mutant strains. In a strain lacking the mannose Enzyme II (EII*Man*), *Kₘ* values for glucose and αMM were 20 and 400 μM, respectively; mannose, 2DG and methyl α-β-mannopyranoside (αMM) were not substrates. In a strain lacking the glucose Enzyme II (EII*Glc*), *Kₘ* values for glucose, mannose and 2DG were 7, 30 and 200 μM, respectively, and neither αMM nor αMM was a substrate (Adler & Epstein, 1974). Later studies using *Salmonella typhimurium* confirmed, with similar *Kₘ* values, that glucose is a good substrate of both permeases, whereas αMM is a good substrate only of EII*Glc*, and mannose as well as 2DG only of EII*Man* (Rephaeli & Saier, 1978; Stock et al., 1982). These studies did not include αMM, but since the hydroxyl group in the mannose position at carbon 2 should prevent entry via the glucose PTS and the methyl group on the anomeric carbon should prevent entry via the mannose PTS, αMM would not be expected to be a good substrate of either permease, as indicated in the earlier study.

Non-metabolizable PTS sugar analogues, on entering cells, build up as phosphorylated derivatives, causing growth stress; however, their build-up triggers a decrease in the levels of Enzymes II of the glucose or mannose PTSs via a small regulatory RNA, SgrS, limiting further uptake (Rice & Vanderpool, 2011; Vanderpool & Gottesman, 2004). In addition to preventing synthesis of the permeases by inhibiting translation and promoting degradation of both *ptsG* (encoding the membrane PTS component EIICB*Glc*) and *manXYZ* (encoding the components of EII*Man*) mRNAs, SgrS causes activation of a sugar phosphate phosphatase mRNA encoding an enzyme which eliminates the sugar phosphates that have already accumulated (Papenfort et al., 2013). SgrS also encodes a polypeptide, SgrT, which inhibits transporter activity of the glucose PTS (Wadler & Vanderpool, 2007). In rich medium, inhibition of the uptake systems appears adequate to prevent growth stress in the presence of αMM or 2DG, while in minimal medium, the elimination of the sugar phosphates as well as a third unidentified effect are also necessary for full relief (Sun & Vanderpool, 2013). Recent investigations into the actual cause of growth stress resulting from sugar-phosphate...
accumulation provide evidence that it is not sugar-phosphate build-up itself but rather depletion of metabolic intermediates that is responsible (Richards et al., 2013); adding glycolytic intermediates such as glucose 6-phosphate and fructose 6-phosphate to the growth medium decreased the stress caused by αMG.

In a previous study (Sutrina et al., 2015), we determined that E. coli strain 15 (ATCC 9723), a WT strain with respect to the PTS, forms robust biofilms on borosilicate glass in buffered and unbuffered NaCl-free Luria Bertani broth, (LB*). We found that low concentrations of sugars, including glucose and mannose, activate biofilm growth of this strain, but that high concentrations inhibit. Glucose was inhibitory at relatively low concentrations (0.3–0.4 %, or 17–22 mM, and higher) compared with mannose (0.6–0.7 %, or 33–39 mM, and higher). At the lowest inhibitory concentrations of both glucose and mannose, exogenous cAMP reversed the inhibition, but it became less effective as the sugar concentration increased.

In vivo, the adhesin FimH located on type I pili binds to mannose-containing surface molecules, contributing to the attachment of E. coli to the surfaces of host cells. Mannose binds to FimH, inhibiting the attachment (Hung et al., 2002). Other possible inhibitors of FimH have been investigated. Glucose and αMG bind very weakly to FimH relative to mannose; αMM binds relatively tightly, but removal of the hydroxyl group on C-2 results in a decrease of 100-fold in its binding affinity (Bouckaert et al., 2005). This is not surprising as this hydroxyl group has been shown to be involved in interactions, directly and indirectly via a water molecule, with residues in the mannose binding pocket of FimH (Hung et al., 2002). The analogue 2DG was not tested, but the missing hydroxyl group at C-2 suggests that it would not interact strongly with FimH.

![Graphs showing the effect of non-metabolizable analogues on biofilm growth of E. coli strains in buffered and unbuffered LB*.](image-url)

**Fig. 1.** Effect of non-metabolizable analogues on biofilm growth of E. coli strains in buffered and unbuffered LB*. Strain 15 (a, b, c) was incubated at 29°C for 24 h in the presence of the indicated levels of non-metabolizable sugar analogues in unbuffered (a), phosphate-buffered (b) and MOPS-buffered (c) medium. Biofilm growth was then quantified as described in Methods. For each run, biofilm quantities were calculated relative to the uninhibited control; means of at least three separate runs with standard error are shown. Mean control values (A590 using the crystal violet assay described in Methods) were: unbuffered 1.669±0.228; phosphate-buffered 0.730±0.172; MOPS-buffered 0.701±0.116. The experiment was repeated using strain K12 in unbuffered LB*, incubated for 48 h (d). The data represent the means of five runs ±SEM. For K12, the control A590 was 0.315±0.066. ▲ 2DG; ♦ αMM; ◇ αMG.
In a study published in 1998, Pratt & Kolter (1998) showed that mannose and its non-metabolizable analogue, αMM, inhibit biofilm growth in LB of E. coli strain 2K 1056, a motile laboratory strain, on several abiotic surfaces, including borosilicate glass, to an extent dependent on concentration. On PVC, 25 mM (0.45 %) αMM inhibited about 50 %, 50 mM (0.9 %) about 70 %, and 100 mM (1.8 %) about 90 %. Glucose did not inhibit biofilm formation. The authors speculated that the binding of mannose or its analogue to FimH may alter its conformation, masking a region that interacts with abiotic surfaces and is critical for attachment.

Since our results with strain 15 were much different than the results of the study just described in that glucose was a stronger inhibitor of biofilm growth than mannose, and since non-metabolizable PTS sugar analogues should give further insight into the mechanism of effects of PTS sugars on biofilm growth, we investigated the effects of mannose analogues αMM and 2DG and also glucose analogue αMG on biofilm growth of this strain.

**METHODS**

**Curli and cellulose.** To test for curli production, bacteria were streaked onto NaCl-free LB* agar plates supplemented with Congo red (40 µg ml⁻¹) and Coomassie Brilliant Blue (20 µg ml⁻¹) and incubated at 29 °C (Römling et al., 1998). To test for cellulose production, cells were streaked onto LB* agar plates supplemented with Calcofluor white (fluorescent brightener 28, 50 µg ml⁻¹), incubated at 29 °C and viewed under UV light (366 nm) (Römling et al., 2000).

**Biofilm growth.** Biofilms were grown in borosilicate glass tubes (13 × 100 mm). Rich medium experiments were done using unbuffered LB* medium or the same medium buffered with either 100 mM KPO₄, pH 7.5 or 100 mM MOPS, pH 7.5. Minimal medium was M63 [50 mM KPO₄, pH 7.2, 0.02 % MgSO₄, 0.2 % (NH₄)₂SO₄] supplemented with 0.2 % or 0.5 % lactose, fructose, mannitol, mannose or glucose. Tubes were prepared by adding 250 µl of fourfold-concentrated LB* and 500 µl of 200 mM buffer or 500 µl water to each, or by adding 500 µl of two-fold-concentrated M63. Sugars and analogues plus other components (cAMP, glucose 6-phosphate) were added to give the desired concentrations, 20 µl of an overnight culture of bacteria was added and the total volume was brought to 1 ml with water. This gave an initial OD₅₆₀ of 0.05–0.1. After mixing, three 250 µl aliquots were transferred to three tubes and the remaining 250 µl served as a fourth replicate. The replicates were then incubated at 29 °C for the time indicated (usually 24 h).

**Biofilm and planktonic cell quantification.** The planktonic cells from each set of four replicate tubes were removed carefully with a pipette and pooled, and the OD₅₆₀ was determined. The tubes were then rinsed three times with tap water and drained. Crystal violet (0.4 ml of 0.1 %) was added to each tube. After incubating for 15 min at room temperature, the crystal violet solution was removed, and the tubes were washed five times with tap water and drained. The crystal violet was eluted with 95 % ethanol (2 ml). The eluates were read at 590 nm against a blank prepared by running 0.25 ml of uninoculated medium through the biofilm growth and staining procedure. Thus, we quantified as biofilm only cells that adhered tightly enough to the glass to withstand fairly extensive washing: aggregative behaviour such as clumping was noted, but would not have been detected as biofilm.

**RESULTS AND DISCUSSION**

**Inhibition in rich medium**

In unbuffered LB*, 2DG inhibited biofilm growth of E. coli strain 15, strongly (about 70 %) and from a low concentration; αMG also inhibited, but less strongly (about 35 %), and αMM, which should not enter the cells, did not inhibit (Fig. 1a). The non-metabolizable analogues had no effect on the pH of the medium and no inhibitory effect on the 24 h accumulation of planktonic cells. For E. coli, biofilm growth involves production of cellulose and thin, proteinaceous, aggregative fibers called curli fimbriae on the cells’ surfaces (Römling et al., 1998). As shown in Fig. 2, colonies grown on LB* Congo red plates in the presence of 0.005 %, but not 0.02 % or higher, 2DG glowed under ultraviolet light, indicating cellulose production, and colonies grown on LB* Congo red plates showed inhibition of curli development, as reflected by red colour (Römling et al., 2000), at 0.02 % 2DG and higher but not at 0.005 %. Inhibition of cellulose and curli production thus correlated with inhibition of biofilm growth. Buffering LB*, with either KPO₄ or MOPS (100 mM, pH 7.5), led to a decrease in biofilm levels to about 45 % of the unbuffered level, in agreement with our previous study (Sutrina et al. 2015). Moreover, inhibition of biofilm growth by 2DG and αMG was much stronger in buffered than in unbuffered LB*, 2DG at 0.02 % or higher inhibited biofilm growth nearly completely (Fig. 1b, c). Planktonic cell accumulation was not inhibited, and αMM had little effect over the low range tested.

To determine whether the inhibitory effects of 2DG and αMG were specific to strain 15, we investigated E. coli strain K12. Our K12 strain formed only weak biofilms in LB*, giving a low signal to noise ratio and an unreliable baseline. Accumulation of biofilm by K12 for 48 h averaged less than 1/5 the accumulation by strain 15 for 24 h. Nevertheless, 2DG clearly inhibited its weak biofilm growth, from a low concentration, αMG inhibited less strongly and αMM had little effect (Fig. 1d). In buffered LB*, biofilm growth of

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**Fig. 2.** Effect of 2DG on cellulose (a) and curli (b) production by strain 15. Calcofluor (a) and Congo red (b) plates containing 2DG were streaked with strain 15 and incubated for about 24 h at 29 °C. The two plates are in the same orientation: (1) control, (2) 0.005 %, (3) 0.02 %, (4) 0.05 % 2DG.
K12 was undetectable after 48 h, so inhibitors could not be tested.

Our results suggest that a non-metabolizable analogue must enter the cells in order to have an effect on biofilm growth. To further investigate this point, we tested the analogues with *S. typhimurium* strain SB2950 (Sutrina et al., 2007). This strain has a deleted *pts* operon, which encodes the general PTS energy-coupling proteins Enzyme I (EI) and HPr as well as the regulatory protein Enzyme IIA^Glc_. Due to the lack of EI and HPr, no PTS sugar or analogue can enter the cells (Postma et al., 1993). This strain forms robust biofilms (our unpublished observations). Neither 2DG nor the other analogues affected biofilm growth of this strain, or its positive response on Calcofluor or Congo red plates, consistent with our conclusion.

The inhibitory effect of the non-metabolizable analogues on entering cells may possibly be due to a build-up of the phosphorylated form. Phosphorylated N-acetylglucosamine has been shown to inhibit biofilm growth in *E. coli* (Barnhart et al., 2006). Growth stress caused by sugar-phosphate build-up is minimized by induction of the small regulatory RNA SgrS; aMG at 0.1% was shown to induce sgrS 126-fold while the same concentration of 2DG induced just 36-fold (Rice & Vanderpool, 2011). SgrS-dependent reduction of the level of *manXYZ* mRNA also takes longer than reduction of *ptsG* mRNA (Balasubramanian & Vanderpool, 2013). Inability of the cells to prevent 2DG-phosphate build-up as effectively as build-up of aMG-phosphate could account for 2DG being a stronger inhibitor of biofilm growth than aMG. The stress-response system normally allows recovery from the negative effects on growth rate of sugar-phosphate build-up following a temporary slowing of growth (Vanderpool & Gottesman, 2004), which is consistent with our finding that the analogues did not affect the density of planktonic cells measured at 24 h.

Although its analogue aMM did not inhibit biofilm growth over the range tested (up to 0.1%), mannose inhibits biofilm growth of strain 15 at concentrations of 0.6–0.7% and higher (Sutrina et al., 2015), and it is possible that its binding to the adhesin FimH could be a contributing factor (Pratt & Kolter, 1998). We tested the mannose analogue aMM over a high concentration range similar to that used in the study cited. At concentrations up to 1.8%, at which biofilm growth of the *E. coli* strain tested in the 1998 study was inhibited 90%, we saw no strong inhibition of biofilm growth of strain 15 (Table 1). Thus it seems unlikely that binding to FimH contributes to inhibition of biofilm growth by mannose in strain 15 since aMM, which would be expected to bind with similar affinity (Bouckaert et al., 2005), did not inhibit even at similar high concentrations.

### Inhibition in minimal medium

We found that strain 15 formed biofilms in minimal medium M63 supplemented with fermentable carbon sources (both PTS and non-PTS sugars). Whereas biofilm growth in LB* was a ring at the air/liquid interface, in minimal medium it covered most of the liquid/solid interface. Römling & Rohde (1999) reported a similar phenomenon using a semi-constitutive *csgD* mutant of *S. typhimurium*. In minimal medium with the non-PTS sugar lactose as carbon/energy source, 2DG inhibited biofilm growth strongly, aMG inhibited less strongly and aMM had little effect (Fig. 3a). Results were similar using either of the two PTS sugars fructose and mannitol (Fig. 3b, c). The concentrations of sugar used in these experiments, 0.2–0.5%, were those that we found to support maximal biofilm growth. At these concentrations of the carbon source in the absence of analogue, most of the cells were in the biofilm; as the concentration of inhibiting analogue increased, an increase in planktonic cell accumulation correlated with the decrease in biofilm growth (Fig. 4). This was in contrast to growth in rich medium, in which planktonic cell density was high (OD_{600} averaged about 2 in unbuffered and 3 in buffered LB*) and remained high in the presence of inhibitors; a slight increase observed may reflect redistribution of some biofilm cells to planktonic. Thus in both rich and

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**Table 1. Effect of aMM (high range) on biofilm growth of *E. coli* strain 15 in buffered and unbuffered rich medium**

Cells were grown in the medium indicated for 24 h. Biofilms were quantified as described in Methods, and for each run the values were calculated relative to the control lacking aMM. Data shown represent the mean for at least two (2–5) separate runs ±SEM. The mean values for the controls (A_{590}±SEM using the crystal violet assay described in Methods) are shown in bold type.

<table>
<thead>
<tr>
<th>aMM concn (%)</th>
<th>Unbuffered LB*</th>
<th>KPO_{4}-buffered LB*</th>
<th>MOPS-buffered LB*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>0.05</td>
<td>1.019±0.089</td>
<td>1.017±0.046</td>
<td>0.885±0.046</td>
</tr>
<tr>
<td>0.10</td>
<td>1.023±0.065</td>
<td>1.003±0.062</td>
<td>0.862±0.052</td>
</tr>
<tr>
<td>0.20</td>
<td>1.085±0.129</td>
<td>0.942±0.089</td>
<td>0.989±0.110</td>
</tr>
<tr>
<td>0.50</td>
<td>1.080±0.150</td>
<td>0.992±0.132</td>
<td>0.833±0.052</td>
</tr>
<tr>
<td>0.70</td>
<td>0.962±0.096</td>
<td>0.927±0.075</td>
<td>0.767±0.078</td>
</tr>
<tr>
<td>1.0</td>
<td>1.045±0.102</td>
<td>0.878±0.058</td>
<td>0.788±0.072</td>
</tr>
<tr>
<td>1.8</td>
<td>0.907±0.015</td>
<td>1.069±0.182</td>
<td>0.914±0.104</td>
</tr>
<tr>
<td>Control (A_{590})</td>
<td>1.010±0.131</td>
<td>0.653±0.078</td>
<td>0.455±0.085</td>
</tr>
</tbody>
</table>
minimal medium, 2DG and αMG inhibited not overall cell growth but biofilm growth. In minimal medium with fructose as carbon/energy source, inhibition was consistently stronger than with other sugars. This may be due to the fact that the fructose transcriptional repressor protein, which is also a global transcriptional regulator, will have low activity in the presence of fructose. This regulator, FruR or Cra, has been shown to activate biofilm growth (Reshamwala & Noronha, 2011). Low levels of an activator may enhance the inhibitory effect of the analogues.

In contrast to the other sugars tested (Fig. 4a, b), when mannose was the carbon source, much higher levels of 2DG were required for strong inhibition (Fig. 4c). We tested 2DG over a high concentration range (Fig. 5), and found that with mannose, in contrast to lactose, levels that caused substantial inhibition of biofilm growth also inhibited planktonic growth. With glucose, similarly to mannose, as carbon/energy source, high concentrations of 2DG were required to inhibit biofilm growth (Fig. 4d); however, planktonic growth was not inhibited even at high concentration.

Thus when the analogue was not a substrate of the uptake system for the carbon/energy source, a strong inhibitory effect of 2DG was observed, similar to that seen in rich medium. However, when mannose was the carbon source, 2DG competed with mannose for entry via the mannose PTS. The affinity of EII\text{Man} for mannose is greater than that for 2DG (K_m 30 vs 200 µM; Adler & Epstein, 1974), meaning that under these growth conditions a higher concentration of 2DG will probably be required to build up enough internal 2DG-phosphate to affect biofilm growth, and also to induce sgrS and reduce the level of the permease. Once the level of the permease is reduced, the uptake of the metabolizable sugar (carbon/energy source) will decrease and growth, planktonic and biofilm, will be inhibited. Similarly, with glucose as the carbon source, 2DG will compete with glucose for EII\text{Man}, which also has a much higher affinity for glucose than for 2DG (K_m 7 vs 200 µM; Adler &
Epstein, 1974), and higher levels will be necessary to see an inhibitory effect. As glucose enters via either the mannose or the glucose PTS and can also enter via the galactose permease, a non-PTS transporter (Saier et al., 1973), it is not surprising that inhibition of planktonic growth due to lack of a carbon/energy source was not observed even at relatively high 2DG concentration.

Effect of cAMP on inhibition

Inhibition of biofilm growth by the lowest inhibitory concentrations of most PTS sugars, including glucose and mannose, is reversed by cAMP (Sutrina et al., 2015). To investigate the effect of cyclic AMP on the inhibition of biofilm growth by 2DG, we added exogenous cAMP (3 mM) to the medium, for selected concentrations of the inhibitor. Results are shown in Fig. 6. In unbuffered LB* (Fig. 6a), phosphate-buffered LB* (Fig. 6b) and minimal medium with 0.2% lactose as carbon/energy source (Fig. 6d), cAMP had little effect on biofilm growth in the absence of 2DG. In MOPS-buffered LB* (Fig. 6c), it showed some (about 40%) stimulation. This is in agreement with previous results (Sutrina et al. 2015).

Addition of cAMP did not prevent inhibition of biofilm growth by either the lowest concentration of 2DG that showed strong inhibition (0.02% in unbuffered, 0.005% in MOPS-buffered) or by a higher concentration (0.05% and 0.02%, respectively) in unbuffered or MOPS-buffered LB*, although there was some lessening of the inhibition in the former. In KPO4-buffered LB*, cAMP limited the inhibition by the lowest strongly inhibitory concentration (0.005%), but was much less effective at a high concentration (0.02%). However, in minimal medium (which was buffered with KPO4), cAMP did limit the inhibition by even a high concentration (0.02%) of 2DG.

CRP-cAMP activates expression of sgrS during sugar-phosphate stress by increasing the activity of SgrR (Sun & Vanderpool, 2011). Thus cAMP, where it reversed the inhibition of biofilm growth by 2DG, may have done so via sgrS, which would minimize accumulation of 2DG-phosphate. It is also possible that the SgrS system was

Fig. 4. Effect of 2DG on biofilm and planktonic growth of E. coli strain 15 in minimal medium. The medium was supplemented with 0.2% lactose (a), 0.5% fructose (b), 0.2% mannose (c) or 0.2% glucose (d). After incubation for 24 h at 29 °C, planktonic cell density (OD_{600}) was determined and biofilms were quantified as described in Fig. 1. Values represent the means of at least three separate experiments ±SEM. Uninhibited control values were: lactose 1.73±0.42; fructose 2.18±0.04; mannose 1.22±0.08; glucose 1.08±0.15. • Biofilm; ○ planktonic.
expressed more strongly in minimal medium, where we observed reversal of 2DG inhibition by cAMP, than in LB* or MOPS-buffered LB*, where we did not see reversal, as the level of growth stress experienced by E. coli in the presence of αMG or 2DG is more severe in minimal than in rich medium and requires more of the multiple effects of the system for full relief (Sun & Vanderpool, 2013). It is also interesting that there was reversal in phosphate-buffered LB*, at low levels of 2DG. Reversal of inhibition of biofilm growth by the lowest inhibitory levels of non-PTS sugars by cAMP also occurs in phosphate-buffered but not unbuffered LB* (Sutrina et al., 2015). The phosphate buffer in the minimal medium may prove to be relevant.

**Effect of glucose 6-phosphate on inhibition**

Since glycolytic intermediates such as glucose 6-phosphate relieve growth inhibition or stress caused by αMG in SgrS mutants or WT E. coli, respectively (Richards et al., 2013), we investigated the effect of adding 0.1 % glucose 6-phosphate on inhibition of biofilm growth by 2DG. There was no effect in LB*. In minimal medium (Fig. 7), 0.1 % glucose 6-phosphate was not a good carbon/energy source; it supported very little biofilm or planktonic growth.

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**Fig. 5.** Effect of 2DG (high range) on biofilm and planktonic growth of E. coli strain 15 in minimal medium. The medium was supplemented with 0.2 % lactose (a) or 0.2 % mannose (b). After incubation for 24 h at 29 °C, biofilms and planktonic cell densities were determined as in Fig. 4. Values represent the means of at least three separate experiments ± SEM. Uninhibited control values were: lactose 1.731±0.426; mannose 1.227±0.083. • Biofilm; o planktonic.
growth. In minimal medium supplemented with 0.2% lactose, inclusion of 0.1% glucose 6-phosphate led to a decrease in biofilm growth and also to marked clumping of the planktonic cells. However, it also prevented the usual decrease in biofilm growth in the presence of 2DG. In the presence of glucose 6-phosphate, the biofilm accumulated to the same level as in the absence of 2DG, and the planktonic cells again showed a high degree of clumping.

Thus glucose 6-phosphate appeared to cause the cells to stick to each other more and to the glass surface less, but to resist the anti-aggregative effect of the inhibitor 2DG, possibly by replenishing glycolytic intermediates that are diminished when the 2DG enters and accumulates as 2DG-phosphate. This would suggest that, as with growth stress, the actual inhibitory effect of non-metabolizable analogues on biofilm growth may not result entirely from build-up of their phosphorylated forms per se but from a concomitant decrease in glycolytic intermediates. This may include a decrease in the phosphoenolpyruvate pool, or, in other words, to a shift of the phosphoenolpyruvate:pyruvate ratio toward pyruvate, which plays a role in ‘classic’ cAMP/Enzyme IIA^{Ac} mediated catabolite repression by both PTS and non-PTS sugars (Hogema et al., 1998), and which may play a role in inhibition of biofilm growth by these sugars (Sutrina et al., 2015).

Possible applications of this study

Biofilms play a role in many inflammatory and infectious diseases caused by bacteria, including infections associated with implanted medical devices. Furthermore, cells growing as biofilms are much more resistant to both the body’s immune response and to treatment with antibiotics than are planktonic cells (for a review, see Lebeaux et al., 2014). A potent inhibitor of biofilm growth such as 2DG, possibly in combination with antibiotics, could prove invaluable in the treatment of such medical problems. Because 2DG has
Fig. 7. Effect of glucose 6-phosphate on inhibition of biofilm growth of E. coli strain 15 by 2DG in minimal medium supplemented with lactose. Cells were incubated in minimal medium supplemented with 0.2% lactose, with 0.1% glucose 6-phosphate, or with both, in the presence or absence of 2DG. After incubation for 24 h at 29°C, biofilms were quantified as described in Fig. 1. The mean biofilm values (A590 using the crystal violet assay described in Methods) from nine separate runs ±SEM are shown. Black, no 2DG; light grey, 0.005% 2DG; dark grey, 0.02% 2DG. G6P, Glucose 6-phosphate.

several effects on mammalian cells that have led to its being investigated in the treatment of cancer (e.g. Leung et al., 2012; Zhang et al., 2006), its suitability as a therapeutic agent with respect to toxicity, etc., has already been assessed. Thus it is a promising candidate.

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