Presence of the ribulose monophosphate pathway in *Bacillus subtilis*

A recent publication of genomic data suggests the existence of the ribulose monophosphate (RuMP) pathway in various non-methylothrophic bacteria, including *Bacillus subtilis* (2). For *B. subtilis*, I recently could validate this finding by using the transketolase (TKT; EC 2.2.1.1)-defective *B. subtilis* strain ATCC 21951.

When the tkt-mutant *B. subtilis* strain ATCC 21951 is grown on D-glucose as the sole source of carbon and energy, D-glucose is partly converted via the oxidative pentose phosphate cycle into D-ribose 5-phosphate and D-xylulose 5-phosphate (Fig. 1). Due to TKT-inactivity, the ketoglutarate group of D-xylulose 5-phosphate is not transferred to D-ribose 5-phosphate, a reaction step that generates D-erythrose 4-phosphate and D-fructose 6-phosphate. *B. subtilis* ATCC 21951 instead converts D-xylulose 5-phosphate (via D-ribulose 5-phosphate) into D-ribose 5-phosphate, which accumulates at the TKT-conversion point. To overcome feed-back inhibition effects exerted by this intermediate, the strain dephosphorylates D-ribose 5-phosphate and secretes it as D-ribose into the medium (1) (Fig. 1).

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**Microbiology Comment**

**The two heresies collide**

These two heresies enter into collision because many of the historical reports on the cancer germ mention the involvement of highly pleomorphic bacteria that undergo complex life cycles (13). The frequency with which apparently highly pleomorphic bacteria have been isolated from cancers is consistently high. In many cases, the life cycles of these supposed cancer germ were said to involve a hidden or filterable phase (12, 13). Recent studies which suggest a role for highly pleomorphic bacteria in cancer aetiology have been published by, amongst others, Wuerthele-Caspe Livingston and Alexander Jackson (12, 15).

The fact that it is received wisdom that highly pleomorphic bacteria do not exist means that it is unlikely that their role in cancer aetiology will be readily accepted. Of course it is possible that bacteria can be highly pleomorphic without being the cause of cancer and vice versa. This begs the question of whether or not *H. pylori* exhibits extreme pleomorphism. Has anyone looked?

We certainly know that this bacterium possesses a coccoid phase (3), but the existence of a complex life cycle, including a hidden or filterable phase, would obviously be missed unless *H. pylori* was exposed to the cultural conditions that favour extreme pleomorphism (i.e. long incubation under stressed nutrient and environmental conditions).

Of course the obvious criticism of the view that cancer is caused by an infectious agent is that it does not appear, at least in the general sense, to be transmissible. However, recent epidemiological evidence suggests that childhood leukaemia may involve an infectious agent (7), and cancer has been linked with infections such as tuberculosis in both the historical and modern literature (6). It is probable that cancer formation is a multi-factorial process that depends upon the presence of one or more cancer-inducing micro-organisms plus environmental, dietary and hereditary factors. We certainly suspect that dietary fibre can alter the bacteriology of the colon and influence the formation of colon cancers (8).

Another frequent criticism of the supposed role of bacteria in cancer aetiology is that, instead of increasing, the frequency of cancers should have declined since the 1940s following the introduction of antibacterial antibiotics. However, the widespread use of antibiotics did not reduce the incidence of stomach ulcers caused by *H. pylori*. As with *H. pylori*, it is possible that cancer-inducing bacteria may fail to respond to single antibiotics like penicillin, but are only eradicated by specific antibiotic cocktails. This is especially likely to be the case if cancers are caused by sympalms phases which lie hidden in the infected cell.

Although there is considerable evidence linking highly pleomorphic bacteria with cancer, it should be noted that the concept of extreme pleomorphism could be dismissed without compromising the view that bacteria in general cause cancer (11, 12). It is also worth noting that extremely pleomorphic bacteria have recently been associated with a wide range of infections, including rheumatic fever and Crohn's disease (4).

Hopefully, bacteriologists will now be encouraged to re-examine the question of whether or not bacteria exhibit extreme bacterial pleomorphism and, together with cancer experts, re-assess the role of both pleomorphic and non-pleomorphic bacteria in cancer aetiology. For the sake of balance, the reader is also referred to papers by Winogradsky (14) and an anonymous article (1), which, respectively, are critiques of pleomorphism and the existence of specific cancer-causing bacteria.

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**Fig. 1.** Glucose catabolism in the presence of formic acid by the D-ribose-producing and TKT-defective *B. subtilis* strain ATCC 21951. The numbers depict the major enzymes that are involved, namely: 1, formaldehyde dehydrogenase; 2, formate dehydrogenase; 3, hexulose-6-phosphate synthase; 4, hexulose-6-phosphate isomerase; 5, TKT (EC 2.2.1.1); 6, lactate dehydrogenase (EC 1.1.1.27); 7, acetoin reductase (EC 1.1.1.4). The double bar at the TKT-conversion point symbolizes TKT-negativity. PPC, pentose phosphate cycle.
D-glucose was consumed (after 22 h at 37 °C) by growing the tkt-mutant strain on D-glucose (30 g l⁻¹), yeast extract (10 g l⁻¹), K₂HPO₄ (5 g l⁻¹), KH₂PO₄ (5 g l⁻¹) and MgSO₄·7H₂O (1 g l⁻¹). The initial pH was 7.0. The experiment was performed with glass tubes (2.5 cm diameter, 15 cm length), provided with 5 ml growth medium. The data were recorded when all D-glucose was consumed (after 22 h at 37 °C, 200 r.p.m. on a rotary shaker). The supplied formic acid concentration was determined as cell dry weight by drying a culture sample until constant weight at 150 °C. The amount of formic acid that was oxidized to CO₂ was not determined. The results are mean values obtained from three independent experiments. The statistical deviation is 5-7%.

### Table 1. Influence of formic acid (1-3 g l⁻¹) on D-glucose conversion by *B. subtilis* ATCC 21951

<table>
<thead>
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<th>Formic acid (g l⁻¹)</th>
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<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
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<td>D-Ribose</td>
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<td>1.6</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>AB</td>
<td>12.2</td>
<td>15.0</td>
<td>15.0</td>
<td>13.5</td>
</tr>
<tr>
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<td>3.0</td>
<td>4.0</td>
<td>4.7</td>
</tr>
<tr>
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<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Biomass</td>
<td>9.6</td>
<td>9.1</td>
<td>8.6</td>
<td>9.5</td>
</tr>
</tbody>
</table>

By growing tkt-mutant strain *B. subtilis* ATCC 21951 on D-glucose, and by supplementing the medium with a low concentration of formic acid (1-3 g l⁻¹), the presence of the RuMP pathway in *B. subtilis* could be revealed. The rationale behind the experiment was that if the conversion of formic acid in the presence of D-glucose leads both to a decreased titre of D-ribose and an increased amount of glycolytic end-products (lactic acid, acetic acid, acetoin and 2,3-butanediol) in the medium, the RuMP pathway must be present (Fig. 1). Formaldehyde formed by the reduction of formic acid (formaldehyde dehydrogenase; EC 1.2.1.46) withdraws D-ribulose 5-phosphate from its conversion into D-ribose-5-phosphate, and channels it into the RuMP pathway (Fig. 1). The condensation of formaldehyde with D-ribulose 5-phosphate (hexulose-6-phosphate synthase) generates 3-oxo-6-phosphate hexulose, which is next isomerized into D-fructose 6-phosphate (hexulose-6-phosphate isomerase). Due to the high glycolytic activity in *Bacillus* spp., the latter is predominantly converted into glycolytic metabolites (1). A decreased concentration of D-ribose and an enhanced amount of glycolytic end-products (lactic acid, acetic acid, acetoin and 2,3-butanediol) in the presence of formic acid thus should illustrate the existence of a RuMP pathway in *B. subtilis*.

The D-ribose yield obtained in control cultures (no formic acid added, 3 g D-ribose l⁻¹ generated from 30 g D-glucose l⁻¹) dropped to 1.6, 1.8 and 2.0 g l⁻¹ in the cultures provided with 1, 2 and 3 g formic acid l⁻¹, respectively (Table 1). Interestingly, the amount of acetic acid produced simultaneously increased from 2 g l⁻¹ in the control sample to 3, 4 and 7 g l⁻¹, respectively (Table 1). The concentration of acetoin plus 2,3-butanediol also increased, from 12.2 to 15 g l⁻¹ (in the presence of 1 and 2 g formic acid l⁻¹), and to 13.5 g l⁻¹ in medium supplied with 3 g formic acid l⁻¹. Lactic acid (0-6 g l⁻¹) was only produced in medium containing 3 g formic acid l⁻¹.

In conclusion, the physiological data from this experiment can be explained only by the presence of a RuMP pathway in *B. subtilis*. This is because no other known metabolic route can withdraw D-ribulose 5-phosphate in the presence of formic acid, leading to an increase in glycolytic end-products and a decrease in D-ribose synthesis when a tkt-mutant *B. subtilis* strain is used.

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