PATHOGENESIS AND VIRULENCE FACTORS

Influence of Different Levels of Redox Potential on Fermentative Products Formed by Bacteroides fragilis. M. Goldner, N. Mingot, J. Ph. Emond, and A. Dublanchet. From the Department of Microbiology, Faculty of Medicine, Université Laval, Québec, Canada; and the Laboratory of Microbiology and Serology, Centre Hospitalier de Villeneuve-Saint-Georges, Villeneuve-Saint-Georges, France

The oxidation-reduction (redox) potential (Eh) of a system derives from the measurement of transfer of electrons between a pair of electrodes in relation to the standard hydrogen electrode. The Eh of culture medium can be measured on a scale expressed in millivolts (mV) by use of a platinum electrode connected through a voltmeter to a calomel reference electrode with known Eh.

Bacteroides fragilis can be grown in a chemically defined medium containing cysteine (0.5 g/L) as reducing agent [1, 2]. This potential at the platinum electrode depends on the proportions of oxidized and reduced forms of the added cysteine. The more reduced, the lower the Eh will be, i.e., the more negative on the redox scale (and vice-versa, the more oxidized the added substance is). A special combination platinum-calomel electrode (XM-800; Tacussel, Lyon, France) would be used for Eh (adjusted by calculation for Eh at pH7), and a classic combination glass-Ag/AgCl electrode (TC 100; Tacussel) would be used for pH.

In previous studies, these bacteria have been grown at three initial redox conditions. The amount of cysteine was adjusted to 0.05, 0.25, and 2.5 g/L [3], which provided readings of Eh [4] at, respectively, circa (ca) +100 mV for oxidizing, ca +20 mV for moderating, and ca −60 mV for reducing conditions. By varying the levels of redox potential at start of growth, the studies revealed potential pathogenic characteristics of B. fragilis [3] grown until mid-log phase in the defined medium [1, 2]. A strong penetration into HeLa cells was observed with bacteria grown at initial oxidizing conditions (Eh, ca +100 mV); a weak penetration occurred with bacteria grown at moderating conditions (Eh, ca +20 mV). A compact aggregation at the HeLa cell surface was formed with bacteria grown at reducing conditions (Eh, ca −60 mV).

This sharp distinction in terms of the level of redox potential observed in the interaction between B. fragilis and HeLa cells has revealed a striking adaptation with a pathogenic quality to a change in environment. Mucous material appeared on the bacteria after growth at initial reducing conditions (Eh, ca −60 mV); this may mask receptor sites on the tissue cells and therefore hinder an invasive process [3]. Thus, these previous studies have shown that the pathogenic potential of B. fragilis may be reflected in the redox conditions.

A conceptual argument could be put forward with regard to metabolic change and pathogenic potential. Because the influence shown by variation of the redox potential, the degree of difference could be explored at an initial moderating level for comparison with oxidizing or reducing levels. The accumulation or depletion of certain influential components and the influence of these fermentative products at particular levels of redox potential on formation of other products of this type could manifest a change in character of this potential pathogen. A change in character of the potential pathogen relative to the level of redox potential might reasonably suggest some form of redox switching mechanism. This means that the microorganism, be it in the commensal or the pathogenic state, will have more, or less, accessibility to the pathways in carbohydrate catabolism.

The effect of each of pyruvic, acetic, and lactic acids—constituting the anaerobe/aerobe metabolic interface in the pathway of glucose degradation in B. fragilis [5]—on formation of other components of this type was analyzed after growth under the influence of these different levels of redox potential. The amounts of each acid added were estimates determined from their formation in 48-hour culture under usual conditions of growth (0.5 g/L of cysteine) [1–3]. The amount of cysteine in the chemically defined medium (L-cysteine hydrochloride hydrate, Aldrich Chemical, Milwaukee) was adjusted as previously described to 0.05, 0.25, and 2.5 g/L [3]. The B. fragilis Institut Pasteur (IP) 5-86 clinical isolate was used and conditioned (pH, 7; 37°C) in 10 mL of defined medium [1, 2], containing a concentration of cysteine (0.5 g/L) in sealed, rubber-stoppered serum bottles (95% H₂, 5% CO₂).

For the redox experiments, the bacterial inoculum (0.05 mL), obtained after usual conditioning (2–4 overnight subcultures), was inoculated into cultures that were then grown in this defined medium, except that the concentrations had been adjusted to the oxidizing, moderating, and reducing conditions. It has been shown that in the defined medium [1, 2] members of the Bacteroides genus have only limited use of amino acids; thus, use of cysteine as a metabolite was probably only of minor importance, and this should be beneficial in achieving Eh [4].

The formation of fermentative products by B. fragilis IP 5-86 after growth at the different levels of redox potential was determined on the 48-hour culture supernatants analyzed by gas chromatography (Hewlett Packard [Detroit] 5890 Series II, Supelco FFAP column, 10 m length by 0.33 mm internal diameter; temperatures: detector [flame ionization detector], 200°C, programmed from 90°C to 130°C, with progression at 5°C/min; extracts prepared according to VPI [Blacksburg, VA] system; sample injection, 2 μL). The percent increase in formation of metabolic components (those distributed through the pathway of glucose degradation) upon addition of the components constituting the anaerobe/aerobe interface (pyruvic, acetic, and lactic acids) was calculated over that of no addition for base-unit growth in each instance under the varying redox conditions (oxidizing, moderating, and reducing).

The addition of lactic acid at oxidizing conditions, in contrast with that at moderating and reducing conditions, resulted in a

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Figure 1. Influence of level of redox potential and pyruvic, acetic, and lactic acids on formation of fermentative compounds (A, B, C, D) in Bacteroides fragilis IP 5-86. The formation of fermentative compounds is presented after growth (as percent increase) with added vs. no added product, under influence of different redox conditions: oxidizing (Eh: ca +100 mV), moderating (Eh: ca +20 mV), and reducing (Eh: ca −60 mV) conditions are depicted as black bars, white bars, and hatched bars, respectively (see also 4th footnote to table 1). Pyruvic, acetic, and lactic acids were included separately in chemically defined medium; solutions of these components, together with glucose, were added in the volume ratios of 1/4 (0.05 mL of acid, 0.15 mL of glucose), 1/2 (0.1 mL of acid, 0.1 mL of glucose), and 3/4 (0.15 mL of acid, 0.05 mL of glucose) per 10 mL of chemically defined medium. The acids were added as their sodium salts (Aldrich Chemical, Milwaukee) to yield the following amounts in the 10 mL of growth medium: pyruvic, 0.625, 1.25, and 1.875 mg, for proportions of 1/4, 1/2, and 3/4, respectively; acetic, 10, 20, and 30 mg, for proportions of 1/4, 1/2, and 3/4, respectively; lactic, 5, 10, and 15 mg, for proportions of 1/4, 1/2, and 3/4, respectively. The D-glucose (BDH Chemicals, Toronto) was correspondingly added in the following amounts in 10 mL of growth medium: 75, 50, and 25 mg for proportions of 1/4, 1/2, and 3/4, respectively. In sum, a volume of 0.2 mL of a separate combination of each component plus glucose was included (at start of growth) per 10 mL of medium. Note particularly the contrasting influence under oxidizing and reducing conditions, as well as the marked effect in general for lactic acid.

striking accumulation of acetic acid at a volume ratio of 1/4 (figure 1A). There was no particular effect due to addition of pyruvic acid. The addition of lactic acid at a volume ratio of 1/4 sharply influenced the formation of propionic acid at oxidizing but not at moderating or reducing conditions (figure 1B); there was no particular effect at this time due to either acetic or pyruvic acids.

However, the addition of acetic acid yielded a modest gradual increase in formation of lactic acid over the scope of reducing conditions, which seemed to reverse at moderating conditions (figure 1C); a similar modest gradual increase in lactic acid at reducing conditions was observed with addition of pyruvic acid (figure 1C). The addition of lactic acid at volume ratios of 1/2 and 3/4 resulted in a substantial influence on the formation of succinic acid at reducing conditions (figure 1D); the equivalent applied over the scope of acetic and pyruvic acid additions (figure 1D). The addition of pyruvic acid exerted at least a noticeable effect on formation of lactic and succinic acids at reducing conditions (figures 1C and 1D).

Varying the initial redox potential produced metabolic changes in B. fragilis IP 5-86 and demonstrated the relative effect of certain fermentative compounds on formation of other products of this type. To elaborate for pyruvic, acetic, and lactic acids, an interesting facet of the analysis has been the marked influence of lactic acid at the volume ratio of 1/4 (figure 1, table 1), followed by acetic acid and then by pyruvic acid at a volume ratio of 1/4. Pyruvic acid, in this sense, served as a useful comparison for the marked responses obtained with lactic and acetic acids.

Furthermore, lactic acid seemed correspondingly more effective under oxidizing conditions; for example, consider the abrupt change at oxidizing conditions from moderating and reducing conditions for the volume ratio of 1/4 (figures 1A and 1B). According to previous studies [3], this may actually be compatible
with development of potential pathogenic characteristics in lieu of maintenance of the innocuous commensal state. In this respect, it is worth mentioning from the clinical literature that available host lactate will increase, depending on the pathophysiological circumstances (normal serum, ~115 mg/L). However, there was also an apparent change at reducing conditions for volume ratios 1/2 and 3/4 (figure 1D).

The changes particularly refer to the effect of added lactic acid on formation of acetic, propionic, and succinic acids, in depicting metabolic change at varying redox potential (table 1). Likewise, the metabolic effect of added acetic acid on formation of lactic acid resulted in the reverse of an abrupt change from moderating conditions for volume ratio 1/4, but the corresponding effect on formation of succinic acid was evident from reducing conditions over the range of volume ratios. Added pyruvic acid showed a lesser but similar effect on formation of lactic and succinic acids (figures 1C and 1D), in comparison with the effect seen with addition of acetic acid on formation of succinic acid (figure 1D).

In general terms, a relationship between metabolic change and pathogenic potential, where both are reflected in the redox conditions (table 1), may be conceived as dependent on some form of redox switching mechanism (figure 2) [6]. Albright et al. [7] maintain that a series of sensors in microorganisms allows them to produce a fast and coordinated response to changes in the environment. Miller et al. [8] contend that virulence factors are able to determine their expression by common regulators in response to environmental conditions.

It is interesting that Wood [6] has mentioned a redox switch for proline dehydrogenase in *Escherichia coli* that would modulate the respiratory chain function in response to substrate supply. In a similar way, a regulatory process at the anaerobe/aerobe metabolic interface in *B. fragilis* may regulate gene expression of lactic acid dehydrogenase. Thus, reducing/oxidizing levels, as related to the commensal or oxidizing/reducing levels for the pathogen, may coordinate microbial metabolism and gene expression with pathogenic potential.

### Table 1. Metabolic change and pathogenic potential.*

<table>
<thead>
<tr>
<th>Added to chemically defined medium† (volume ratios‡)</th>
<th>Influenced production of</th>
<th>Redox conditions§</th>
<th>Penetration into HeLa cells∥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid (1/4)</td>
<td>Acetic acid</td>
<td>Oxidizing</td>
<td>Strong</td>
</tr>
<tr>
<td>Lactic acid (1/4)</td>
<td>Propionic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid (1/4)</td>
<td>Lactic acid</td>
<td>Moderating</td>
<td>Weak</td>
</tr>
<tr>
<td>Lactic acid (1/2)</td>
<td>Succinic acid</td>
<td>Reducing</td>
<td>None (aggregation of bacteria with apparent mucous material)</td>
</tr>
<tr>
<td>Acetic acid (1/2)</td>
<td>Succinic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvic acid (3/4)</td>
<td>Succinic acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Differential adjustments occurring with respect to influence of redox conditions on potential pathogenic characteristics in *Bacteroides fragilis* IP 5-86.
† Described in [1] and [2]; volume of 0.2 mL of glucose normally added per 10 mL of medium.
‡ On the basis of experimental design (results given in figure 1), these selected fermentative products were added at volume ratios of 1/4, 1/2, and 3/4 for oxidizing, moderating, and reducing conditions (the ratios shown in parentheses); volume ratios refer to 0.05, 0.1, and 0.15 mL of acid metabolite and 0.15, 0.1, and 0.05 mL of glucose added per 10 mL of medium, to give ratios of 1/4, 1/2, and 3/4, respectively. Concentrations for each of the solutions are given in figure 1.
§ Redox potential (Eh) measured at platinum electrode and corrected for pH 7 (Eh7), as in [4]. Initial Eh values: oxidizing, ca +100 mV; moderating, ca +20 mV; reducing, ca −60 mV.
∥ Potential pathogenic characteristics, as ability of *B. fragilis* to penetrate HeLa cells reported in [3].

### References


