Thermodynamics of interspecies electron transfer: from syntrophy to parasitism

Elie Desmond-Le Quéméner, Roman Moscoviz, Jean-Clément Flayac, Eric Trably, Nicolas Bernet

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E. Desmond-Le Quéméner*, R. Moscoviz*, C. Flayac*, E. Trably*, N. Bernet*

* LBE, INRA, 102 avenue des Etangs, 11100, Narbonne
(E-mail: *elie.lequemener@inra.fr*)

Abstract
Interspecies Electron Transfer (IET) is a mechanism that allows energetic coupling between two microorganisms. It plays a key role in a wide range of environmental and biotechnological microbial ecosystems and stimulation of Direct Interspecies Electron Transfer (DIET) appears as an attractive way to boost anaerobic digestion. However, studies dealing with IET have, so far, mostly restrained on the possible syntrophic interactions supported by this mechanism. In the present work, the case of a fermentative bacterium receiving extracellular electrons while fermenting a substrate is considered. By applying microbial thermodynamics to experimental data previously published, we show that IET can promote a wide range of interactions such as mutualism, commensalism or parasitism. In the case of glycerol fermentation IET indeed leads to a diminution of bacterial biomass production concomitantly with an increased production of reduced compounds such as 1,3-propanediol. Thus, our study indicates that IET probably plays an important role nowadays underestimated in microbial interactions networks giving interesting perspectives for the optimization and control of bioprocesses using IET stimulation.
Keywords
Interspecies Electron Transfer (IET); microbial thermodynamics; microbial interactions
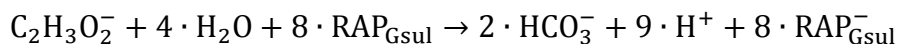
INTRODUCTION

Interspecies Electron Transfer (IET) plays a key role in anaerobic digestion for the syntrophic degradation of volatile fatty acids such as propionate [1]. Recent discoveries indicate that not only hydrogen or formate but also electrons are exchanged between syntrophs [2]. Indeed, when *Geobacter metallireducens* is grown in presence of an electron donor and in absence of soluble electron acceptor, it is able to transfer its electrons to a second microorganism that has no other electron donor and that reduce fumarate (*Geobacter sulfurreducens*) or CO_2 (*Methanosaeta sp.*) [2]. Although these experiments have provided a great insight into the mechanistic of Direct Interspecies Electron Transfer (DIET), this kind of experimental design can only promote syntrophic interactions because each partner is dependent on the other. On the contrary, in a recent co-culture study, *Clostridium pasteurianum*, a fermentative bacterium, was grown with glycerol as fermentation substrate and in presence of *G. sulfurreducens* [3]. Interestingly, *C. pasteurianum* was able to accept electrons resulting from acetate oxidation by *G. sulfurreducens* while fermenting glycerol. As a result, *C. pasteurianum* metabolic patterns were significantly modified and its growth yield was decreased by 39% as compared to mono-culture experiments, thus supporting that *C. pasteurianum* could be parasitized by *G. sulfurreducens*. Using these data we show how microbial thermodynamics allow accounting not only for syntrophism but also for mutualism, commensalism or parasitism associated with IET. This widens our understanding of IET mediated interactions in microbial ecosystems and opens interesting perspectives for the optimization of bioprocesses.

MATERIALS AND METHODS

Standard Gibbs energies ($\Delta_r G°$) for all chemical equations are calculated using data from [4]. More realistic Gibbs energies values ($\Delta_r G°'$) are then estimated taking into account proton activity of 10^{-7} M (pH = 7).

Catabolism of *G. sulfurreducens*

Acetate is considered as the electron donor for *G. sulfurreducens*. It is oxidized into bicarbonate and electrons are released through an electron transport chain to a redox-active protein (RAP$_{Gsul}$, e.g. cytochromes):
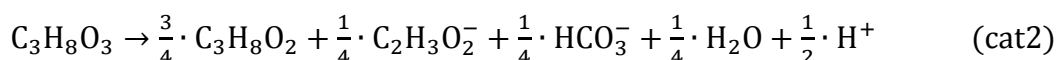
$$C_2H_3O_2^- + 4 \cdot H_2O + 8 \cdot RAP_{Gsul} \rightarrow 2 \cdot HCO_3^- + 9 \cdot H^+ + 8 \cdot RAP_{Gsul}^-$$

Metabolism of *C. pasteurianum*

Electrons from the RAP$_{Gsul}$ are transferred to the RAP$_{Cpast}$ of *C. pasteurianum* through IET and dissipated by producing 1,3-propanediol (PDO) from glycerol according to the following equation:

$$2 \cdot RAP_{Cpast}^- + C_3H_8O_3 + 2 \cdot H^+ \rightarrow 2 \cdot RAP_{Cpast} + C_3H_8O_2 + H_2O \qquad \text{(cat1)}$$

$\Delta_r G_{cat1}^{o\prime} = 6.9$ kJ/mol $+ 2 \cdot F \cdot E^{o\prime}(RAP_{Cpast})$, where F is the Faraday constant ($F = 96485$ C/mol) and $E^{o\prime}(RAP_{Cpast})$ the redox potential of RAP$_{Cpast}$.

This reaction is coupled to glycerol fermentation by *C. pasteurianum*. Here a simplified fermentation of glycerol to PDO and acetate is considered:
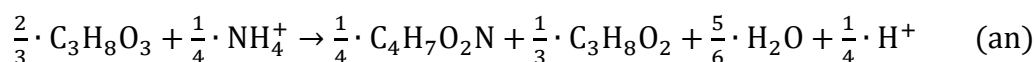
$$C_3H_8O_3 \rightarrow \frac{3}{4} \cdot C_3H_8O_2 + \frac{1}{4} \cdot C_2H_3O_2^- + \frac{1}{4} \cdot HCO_3^- + \frac{1}{4} \cdot H_2O + \frac{1}{2} \cdot H^+ \qquad \text{(cat2)}$$

$\Delta_r G_{cat2}^{o\prime} = -75.7$ kJ/mol

The combination of these two reactions constitutes the global catabolism of *C. pasteurianum*:

$$\alpha \cdot \text{cat1} + (1 - \alpha) \cdot \text{cat2} \qquad \text{(cat)}$$

$\Delta_r G_{cat}^{o\prime} = (-75.7 + \alpha \cdot 82.6)$ kJ/mol $+ 2 \cdot F \cdot E^{o\prime}(RAP_{Cpast})$, with α the fraction of glycerol used for the oxidation of RAP$_{Cpast}^-$ to RAP$_{Cpast}$.

The anabolic reaction is:

$$\frac{2}{3} \cdot C_3H_8O_3 + \frac{1}{4} \cdot NH_4^+ \rightarrow \frac{1}{4} \cdot C_4H_7O_2N + \frac{1}{3} \cdot C_3H_8O_2 + \frac{5}{6} \cdot H_2O + \frac{1}{4} \cdot H^+ \qquad \text{(an)}$$

$\Delta_r G_{an}^{o\prime} = -38.4$ kJ/C–mol$_{biomass}$, with $C_4H_7O_2N$ the formula for a "molecule of biomass" [3].

The global metabolic reaction of *C. pasteurianum* is a combination of anabolism and catabolism:

$$\text{an} + \lambda \cdot \text{cat} \qquad \text{(met)}$$

The factor λ can be evaluated using the "dissipation method" as explained in [4]. Dissipated metabolic energy is calculated as follows:

$$-\Delta_r G_{met}^{o\prime} = 200 + 18 \cdot (6 - NoC)^{1.8} + exp[\{(-0.2 - \gamma)^2\}^{0.16} \cdot (3.6 + 0.4 \cdot NoC)]$$

where γ and NoC stand for the oxidation state and the carbon chain length of the carbon source. For glycerol: $\gamma = -2/3$ and $NoC = 3$, then $\Delta_r G_{met}^{o\prime} = -373.0$ kJ/C-mol$_{biomass}$, thus:

$$\lambda = \frac{\Delta_r G_{met}^{o\prime} - \Delta_r G_{an}^{o\prime}}{\Delta_r G_{cat}^{o\prime}} = \frac{-334.6 \text{ kJ/mol}}{(-75.7 + 82.6 \cdot \alpha) \text{ kJ/mol} + 2 \cdot \alpha \cdot F \cdot E^{o\prime}(RAP_{Cpast})}$$

RESULTS AND DISCUSSION

Bioenergetic mutualism, commensalism and parasitism

To better understand the decrease in growth of *C. pasteurianum* in presence of *G. sulfurreducens*, different energetic partitioning scenarios between the two species have been considered using the fact that both microorganisms' metabolisms are physically separated (see Figure 1).
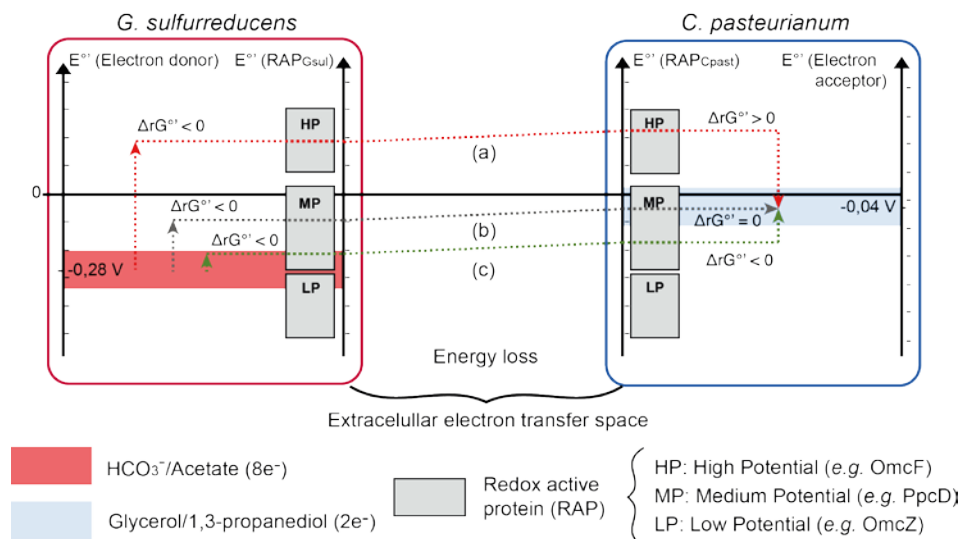
Figure 1. Possible pathways of IET between *G. sulfurreducens* and *C. pasteurianum*: (a) Energetic parasitism; (b) Energetic commensalism; (c) Energetic mutualism. The ranges for redox active proteins were represented according to [5].

For the electron dissipation reaction by *C. pasteurianum* (cat1), three cases are possible depending on the RAP_{Cpast} potential (see Figure 1): (a) $\Delta_r G^{o\prime}_{cat1} > 0$, the reaction is endergonic (energetic parasitism, E°'(RAP_{Cpast}) > -0.036 V); (b) $\Delta_r G^{o\prime}_{cat1} = 0$, the reaction release no free energy (energetic commensalism, E°'(RAP_{Cpast}) = -0.036 V); (c) $\Delta_r G^{o\prime}_{cat1} < 0$, the reaction is exergonic (energetic mutualism, E°'(RAP_{Cpast}) < -0.036 V). In cases (a) and (b), the coupling with fermentation ensures that the global catabolism is still thermodynamically favourable ($\Delta_r G^{o\prime}_{cat} < 0$).

Global mutualism, commensalism or parasitism

Despite appearances, all three cases mentioned above can be detrimental for *C. pasteurianum* biomass yield. Indeed, as glycerol is consumed for the oxidation of RAP_{Cpast} (cat1), this reaction competes with glycerol fermentation (cat2). Therefore, if RAP_{Cpast} oxidation is less exergonic than glycerol fermentation, the amount of energy available for *C. pasteurianum* to sustain its growth will be decreased thus reducing the growth yield.

To assess the dependency of the biomass yield on the RAP_{Cpast} redox potential E°'(RAP_{Cpast}) and to the fraction α of glycerol used for the oxidation of RAP_{Cpast} the growth yield Y (C-mol$_{biomass}$/mol$_{glycerol}$) of *C. pasteurianum* was estimated:

$$Y = \frac{1}{\lambda \cdot Y_S^{Cat} + Y_S^{An}} = \frac{1}{\lambda + \frac{2}{3}} = \frac{1}{\dfrac{-334.6 \text{ kJ/mol}}{(-75.7 + 82.6 \cdot \alpha) \text{ kJ/mol} + 2 \cdot \alpha \cdot F \cdot E^{o\prime}(RAP_{Cpast})} + \frac{2}{3}}$$

Results are shown in Figure 2. When α was equal to 0 (i.e. no IET), the *C. pasteurianum* biomass yield was 4.96 g$_{biomass}$/mol$_{glycerol}$. A higher yield could theoretically be obtained if the RAP_{Cpast} potential was lower than -0.43 V vs SHE, but for all other values of RAP_{Cpast} potential and α, *C. pasteurianum* growth yields were lower than 4.96 g$_{biomass}$/mol$_{glycerol}$ indicating that in most cases, IET would be disadvantageous for *C. pasteurianum*. The growth could even be completely stopped in cases of energetic parasitism (E°'(RAP_{Cpast}) > -0.036 V) as electron dissipation would be endergonic.

CONCLUSION

Interactions mediated by IET are not restricted to syntrophy. Indeed, in the case where the electron accepting microorganism catalyses a catabolic reaction independent from the exogenic electron

source, the electrons it receives may be an energetic burden (energetic parasitism). Moreover, even if electron dissipation provides energy (energetic mutualism), it can still hamper its growth if it competes for its catabolic substrate (global parasitism). This study thus shows that IET probably plays an important role nowadays underestimated in microbial interactions networks.

Interestingly, reduced bacterial biomass production due to IET parasitism is concomitant with a redistribution of metabolic patterns towards a better production of highly reduced compounds (e.g. 1,3-propanediol from glycerol). This opens a promising way to optimize carbon recovery during fermentations and widens the interest of stimulating IET for the engineering of bioprocesses.

More generally this work illustrates how microbial thermodynamics allow calculating thermodynamic constraints on IET, thus providing important information on their bioenergetics.
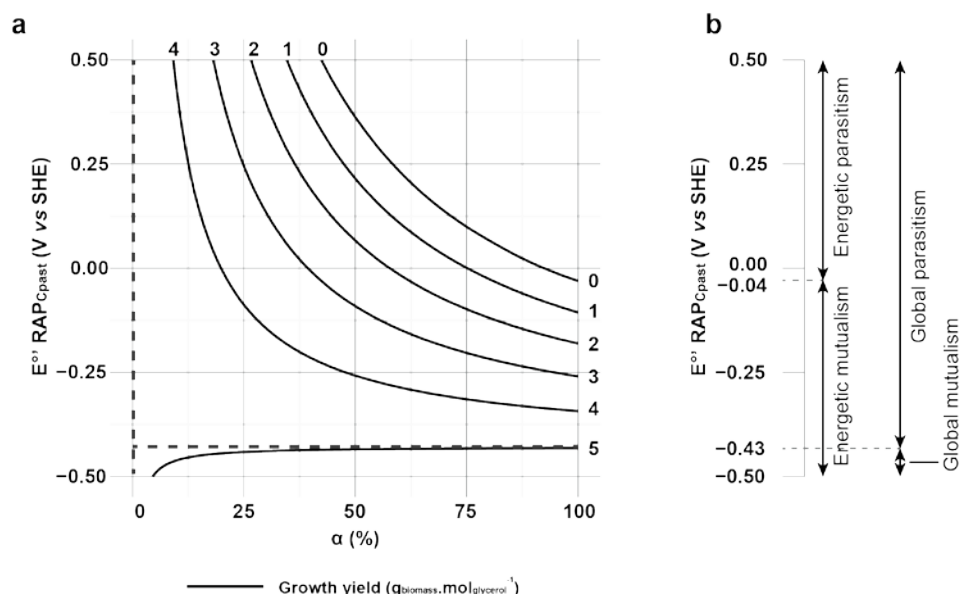

Figure 2. (a) *C. pasteurianum* growth yield map. Solid lines represent contour lines of *C. pasteurianum* growth yield. Dashed lines represent contour line for the specific value of 4.96 $g_{biomass}/mol_{glycerol}$ (fermentative growth yield). α: percentage of glycerol used for dissipating electrons coming from IET (normalized on *C. pasteurianum* consumption for catabolism); E°' RAP_{Cpast}: Biological standard potential of the *C. pasteurianum* redox active protein involved in the electron dissipation reaction; SHE: Standard Hydrogen Electrode. (b) Ecological interaction as function of RAP_{Cpast} potential. Interactions are described as "Energetic" regarding energy partitioning during IET and as "Global" when biomass production of each IET partner is taken into account.

ACKNOWLEDGEMENTS

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