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► **To cite this version:**

Magali Roger, Xavier Marbehan, Pascale Infossi, Louis Delecourt, Cassandra Backes, et al.. Deciphering nutrient cross-feeding interaction network in an artificial bacterial consortium. *Biochimica biophysica acta (BBA) - Bioenergetics*, 2022, 1863, pp.148824. 10.1016/j.bbabi.2022.148824. hal-03846172

**HAL Id: hal-03846172**

**<https://hal.science/hal-03846172>**

Submitted on 31 Jan 2023

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## **Deciphering nutrient cross-feeding interaction network in an artificial bacterial consortium.**

In Nature, bacteria usually exist within diverse communities. The function, structure and stability of microbial communities lie on their capacity to communicate, interact, and trade biological materials (including metabolites) within community members. Obtaining a comprehensive picture of how bacterial communities function is challenging, especially in Nature, on account of community complexity. This requires designing simplified artificial model.

Based on the process of bio-H<sub>2</sub> production from the anaerobic digestion of organic matter, which involve many microbes working in syntrophy, previous work in the laboratory led to the construction of an artificial consortium constituted by *Clostridium acetobutylicum*, and *Desulfovibrio vulgaris*. Upon nutritional stress, they form a cell-cell interaction network [1] allowing carbon exchange between both bacteria [2]. Since *D. vulgaris* cannot utilize glucose, its survival lies on metabolite(s) provided by *C. acetobutylicum* (obligate cross-feeding). Furthermore, based on its metabolic activity *C. acetobutylicum* also benefit from this interaction, although the nature of metabolites exchanged and how bacteria benefit from this cooperative interaction remained obscure.

Here, we combined for both pure cultures and the consortium, (i) metabolic analysis to identify main pathways involved, (ii) label-free quantitative proteomics to validate the presence of enzymes and identify new pathways (iii) metabolic modelling to fill the gaps and uncover the whole extend of the metabolic switch occurring upon bacterial pairing. Finally, the first model of the syntrophic association between *C. acetobutylicum* and *D. vulgaris* under anaerobic fermentative conditions was proposed. The current model points towards a large network of bidirectional cross-feeding interactions. This work has the potential to greatly improve our ability to predict metabolic profile and the function of microbial communities.

[1] S. Benomar, D. Ranava, M. L. Cárdenas, E. Trably, Y. Rafrafi, A. Ducret, J. Hamelin, E. Lojou, J-P. Steyer, M-T. Giudici-Ortoni, Nutritional stress induces exchange of cell material and energetic coupling between bacterial species. Nat Commun, 6 (2015) 6283.

[2] D. Ranava, C. Backes, G. Karthikeyan, O. Ouari, A. Soric, M. Guiral, M.L Cárdenas, M-T. Giudici-Ortoni, Metabolic exchange and energetic coupling between nutritionally stressed bacterial species: Role of quorum-sensing molecules, mBio, 12 (2021) e02758-20.