Proposition de stage M2 à l'UMR GenPhySE (INRA Toulouse)

Estimation of the cultivability of gut microbes

Keywords : antibiotic resistance, gut bacterial communities

Context

The concept of cultivability of microbes has been evolving. While initially reserved to defined media and macroscopic colonies, now it is extended to partly undefined media such as sludge slurry and microcolonies (Ferrari et al. 2005). Some environments are known to contain many cells that are not cultivable. For example, 1% of marine bacteria and 15% of bacteria in wastewater treatment plants are cultivable. Some bacteria of the gut are cultivable as well, but the exact proportion remains elusive. Indeed Browne and coll evidenced that 60% of the metagenomic reads can be obtained in cultivable isolates but that is not exactly the number of cultivable cells (Browne et al. 2016). One could imagine the situation where all the reads are found, but that the repartition between the cells is unique. For example, if bacteria A carries gene 1 and gene 2, and bacteria B carries gene 3 and 4, then genes 1 to 4 are found if bacteria A and B are cultivable. Yet a non-cultivable bacteria C harboring gene 2 and 3 could be uncaptured even if it is dominant in the initial sample.
Recent publication also suggest that the anaerobic/ aerobic barrier is not as clear-cut as previously thought (Dione et al. 2016).
In this context, culture of bacteria could be a major asset to study gut microbes, yet clear data is missing from the litterature. We aim to provide an estimate of the percentage of cultivable gut microbes.

Aims of the master student

The aim of the master student is to test and/or optimize the culture in complex medium. To this end, he will is expected to :
- estimate of the cell counts in the fecal sample
- count the number of cells cultivable as micro- or macro- colonies using wide angle microscopy available through the cytonote device (iprasense).
- sequence the 16S rRNA gene of the isolates and develop metrics to report the fraction and nature of the cultivable microbes.

The planning for the master student is the following :

1. *Collect 10 fecal samples from pigs*
2. *Spread the cultures on 6 different media with different complex nutrients and redox potentials*
3. *Use wide-angle microscopy to count the number of cultivable cells*
4. *Use qPCR , optical microscop and cytometry to quantify the total number of cells in the fecal samples.*
5. *collect and sequence the strains that are were cultivable*

Depending on the results, the rest of the internship can be directed in different directions accoding on the student's preferences.

Abilities

The student should understand english and be highly motivated. Previous knowledge about handling microbial communities would be appreciated but is not necessary.

Contact

If interested please send CV to Olivier ZEMB (GenPhySE) olivier.zemb@inra.fr ; Tel : 05 61 28 50 99
https://sites.google.com/site/olivierzembwebsite/home

Practical details

- 6 months from Jan to Jun 2018
- INRA Toulouse – UMR GenPhySE à Auzeville (31)
- paid about 550 €/mois – (lunch available on site for ~ 2.50 eur)

Browne, H.P., Forster, S.C., Anonye, B.O., et al. (2016) Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature* **533**, 543-+.

Dione, N., Khelaifia, S., La Scola, B., Lagier, J.C. and Raoult, D. (2016) A quasi-universal medium to break the aerobic/anaerobic bacterial culture dichotomy in clinical microbiology. *Clin Microbiol Infect* **22**, 53-58.

Ferrari, B.C., Binnerup, S.J. and Gillings, M. (2005) Microcolony cultivation on a soil substrate membrane system selects for previously uncultured soil bacteria. *Appl Environ Microbiol* **71**, 8714-8720.