P-106 - HUNTING FOR SOIL NITRIFYING PROKARYOTES: FROM THE NIGHTMARE OF ISOLATION TO THE DREAM OF PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION

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**Background**
Nitrification, the aerobic oxidation of ammonia to nitrate through nitrite, is a chemolithotrophic energy acquiring mechanism performed by ammonia-oxidizing bacteria and archaea (AOM) and by nitrite-oxidizing bacteria (NOB) [1]. This process brings about serious ecological, agricultural and economic impacts. However, a lot about nitrifiers is still unknown, mainly due to their extremely slow growth and the difficulties associated with isolation [2].

**Method**
Enrichment and isolation of nitrifiers and NGS-based microbial profiling analysis was performed for 10 distinct Portuguese soils. Selective enrichment of AOM was achieved using (NH\(_4\))\(_2\)SO\(_4\) (0.2 mM to 20 mM) and urea, to cope with ammonia tolerance and urease production, and spectinomycin addition to enrich archaea. Medium with nitrate was used to select NOB. Enrichments were monitored by measuring ammonium, nitrite and nitrate and genes involved in N-metabolism were detected through PCR. After 175 days, enrichments without antibiotics were plated, morphologically different colonies were microscopically selected and diversity analysed by M13-PCR fingerprinting. FISH combined with flow cytometry (FISH-FCM) is being performed to search for betaproteobacterial AOM, NOB and Thaumarchaeota on enrichments after 240 days.

**Results & Conclusions**
A collection of 477 isolates of putative nitrifiers was obtained, encompassing 72 nitrite-oxidizers, 300 ammonia-oxidizers and 105 urea-oxidizers. Among ammonia-oxidizers, a decrease in number was observed with increasing concentration of ammonia, with only 25% of isolates able to grow at 20 mM.

PCA of monitoring data revealed two major clusters among 10 NOB enrichments and five for 79 AOM ones. For NOB, cluster A enrichments had higher number of isolates and displayed faster nitrite consumption, whereas cluster B enrichments only responded upon medium renewal after 3 months.

For AOM, clusters correlate with different media, with the highest NO\(_2\)\(^+\) production being initially observed in Cluster III, containing only urea-based enrichments, and after media renewal in Cluster II, with the highest number of isolates. Since the highest NO\(_3\)\(^-\) production was observed in clusters V and I, before and after medium renewal respectively, communities of these clusters should be performing both stages of nitrification, consuming produced nitrite. In fact, these soil samples correspond to NOB cluster A, the best nitrite oxidation performers.

From soil microbial profiling analysis and FISH-FCM and targeted-gene PCR of enrichments, role of microbial communities contributing to N-cycle will be evaluated regarding soil type.

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