Short Term Scientific Mission (STSM) Report

COST action: FA1302 – METHAGENE

COST STSM Reference Number: ECOST-STSM-FA1302-091016-080901

Applicant: Stav Eyal

Home institution: Dept. of Life Science, Ben-Gurion University of the Negev, Israel

Host: Dr. Samantha Noel

Host institution: Dept. of Animal Science, Aarhus University, Denmark

Period: 09/10/2016 to 29/10/2016

STSM Topic: Interactions between Methanogens and Bacteria and their Effect on the Rumen Ecosystem Functionality

Purpose of the STSM

The overall goal of this STSM was to acquire cultivation techniques used by Dr. Noel for the enrichment of methanogenic archaea belonging to the Rumen Cluster C (RCC) group of methanogens (Borrel, 2013); to further grow rumen fluid consortia previously cultivated in the Mizrahi Lab; and the enrichment cultures obtained by Dr. Noel. These cultivations will be used for further experiments to identify and characterize possible methanogens-bacteria interactions in the rumen microbial community and their contribution to the global environment.

Description of the work carried out during the STSM

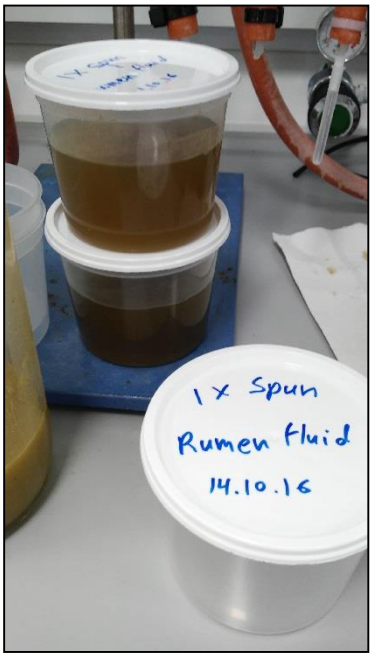
Learning media preparation techniques. During my visit at Aarhus University in Foulum I learned anaerobic enrichments techniques of rumen RCC methanogens from Dr. Noel, as well as anaerobic cultivations on agar plates to obtain methanogens-bacteria paired colonies for the study of possible cross domain interactions. Dr. Noel and I went over the full process of preparing

Rumen Media (RM), modified by her for the specific enrichment of RCC methanogens, along with its supplements of vitamins concentrate and clarified rumen fluid based mixes. I have Learned the key steps for the successful and efficient execution of these techniques and discussed possible ways to further improve the cultivation techniques. For the clarified rumen fluid based mixes, we sampled fresh rumen fluid and together went through the clarifying process and the preparation of the mixes. Other than obtaining the necessary components for the culturing media, the work enabled us to detected difficulties in the protocols and refining them accordingly.

collecting fresh rumen fluid

Calibrating methanogens culturing. 16 of Dr. Noel's RCC enrichment cultures, found to share bacterial and archaeal Operational Taxonomic Units (OTUs) with cultures from the Mizrahi Lab, were chosen to be revived in order to optimize the cultivation of the methanogens. To this end, the 16 cultures were revived in RM supplemented with two different rumen fluid mixes – with or without yeast extract, as the omission of yeast extract from the media was considered as a possible benefit for the methanogens growth. The cultures were then grown on anaerobic agar plats of the same two modifications of RM to calibrate the cultivation of cross-domain pairs of

colonies for the further study of transferred metabolites between RCC methanogens and their co-inhabitant bacteria.



rumen fluid in the clarifying process for media supplements

Methane production measurements. All cultures were checked for methane production using gas-chromatography for the estimation of the methanogens growth with the different supplements in liquid and on solid media.

Description of the main results obtained

Methane production measurements showed successful growth of methanogens in more than 90% of the revived cultures in liquid media. Higher methane was detected in the cultures grown in media not supplemented with yeast extract. Methane emission was not detected from cultures on solid media. These results might be due to the relatively short time of cultivation and the large volume of the anaerobic culturing canisters. The revived cultures were shipped to the Mizrahi Lab in Israel for the further study of the interactions between their members.

Further Plans

The revived cultures that arrived to Israel will undergo experiments of antibiotic supplementation, targeting the bacterial community, to estimate the effect of the bacterial growth on the methanogens growth and predict possible interactions between the two domains. Additionally, cultivation of the methanogens-bacteria paired colonies on solid media will be used to identify transferred metabolites between the co-housing domains in the rumen environment and their functional contribution to the ecosystem.

Description of benefits from the STSM to the METHAGENE network

Further work for the identification of yet unknown discrete methanogens-bacteria interactions in the rumen environment will lead to a better understanding of the factors contributing to methane emission by ruminants. This knowledge has a high potential of providing the METHAGENE society, as well as the overall study of the ruminant methane production, with new perspectives regarding the rumen microbiome functionality. Novel strategies and targets for methane mitigation in ruminants' agriculture can then be inferred for reduced contribution of methane emission to global warming and for the preservation of the environment.

Borrel, G. e. (2013). Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis. Genome biology and evolution 5.10, 1769-1780.

Samantha Noel

Dept. of Animal Science
Aarhus University
Blichers Allé 20, Postboks 50
DK-8830 Tjele

27-12-2016

To whom it may concern,

I hereby confirm that Stav Eyal visited Aarhus University at Foulum for the period of Oct 9th to 29th for a Short-Term Scientific Mission (STSM) under COST Action FA1302. The visit was very productive and Stav has acquired new techniques for the cultivation of methanogens including the collection of rumen samples and the production of anaerobic media components. In her visit Stav produced subcultures of enriched methanogens cultures which were shipped to Israel for her to further study bacteria – methanogen interactions from the rumen.

Yours faithfully,

Dr. Samantha Noel

Tel.: +45 8715 8068
Email: Samantha.Noel@anis.au.dk

Tel.: +45 8715 6000
Web: www.au.dk