Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA17108

Grantee name: Morozov Alexandr

Details of the STSM

Title: DETECTION OF BACTERIA IN MOSQUITOES USING METAGENOMICS WITH NANOPORE SEQUENCING

Start and end date: 21/08/2022 to 05/09/2022

Description of the work carried out during the STSM

During the Short Term Scientific Mission, almost all the goals were achieved. During the first week after the training course and safety training, DNA extraction was to be performed. To perform the extraction of the DNA from the mosquitos, first, they had to be homogenized, but homogenization should be performed without reaping apart the bacteria cells. It took two days to find the right combination of metal beads size and the time in the homogenizer. For each try, the DNA was extracted with the use of 2 different methods, usual DNA extraction (precipitation method) and Extraction with depletion of the host DNA. After each extraction, the quality and the amount of the extracted DNA were measured using Qubit and Nanodrop. After finding the correct combination for homogenization and achieving the satisfying quality of the extracted DNA, the 19 mosquitos were homogenized, and DNA was extracted using two different methods. At the start of the second week, the extracted DNA from both methods was purified, then amplified using a 16s barcoding kit. Then the amplification products were pooled and prepared for Nanopore sequencing. In the last two days of the STSM, the data were analysed using different software and methods. The group of Rafi Ahmad (host institution – INN University, Norway) was supportive and taught the principles of bioinformatic analysis of the results of the Nanopore sequence, working with databases and several specific software.

Description of the STSM main achievements and planned follow-up activities

During work in INN we were able to make a strong team with the group of Rafi Ahmad and performed most of the planned activities. It was very interesting not only from my part, but the host institution was also very involved as the they had never worked with vectors objects. In the results of the conducted work we have collected the results of the 16s sequencing of the 19 mosquitos (*Aedes, Culiseta,Coquillettidia*). Thirteen of the samples gave good results after the sequence, we were able the obtain the pilot data about the whole mosquito bacteria screening. The results are very promising.

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

We have agreed with the host institution to prolong the collaboration and write the short communication about the experience in using Nanopore sequence and specialized host DNA depletion kits when working with mosquitos.

There is a lot of data, and the analysis is still ongoing with the collaboration from both sides. The acquired knowledge can be passed to the local scientists working with vector-borne diagnostics.

The PCR-free metagenomics part did not get us the expected results, because the amount of the gDNA collected from the single mosquito was too low to perform the sequencing. One of the possible solution to this problem is to pool the mosquitos or to do the metagenomics without host DNA depletion, and to deplete host DNA from the bacteria DNA using bioinformatics after the sequence.

In the result we have tested and approved the not expensive method of the arthropod whole bacteria vector screening by Nanopore sequencing, with the small adjustments of the homogenization part, which varies depending on the species size and exoskeleton rigidity, this method can be applied to any arthropod vector.