# Materials and methods

## Bacterial Strains

The following bacterial strains were used in this study: *E. coli* DH5α, *B. subtilis* NCIB3610, *Clavibacter amylovora ???,*  All strains were taken from the lab stocks at Volcani Center.

Bacteria were transferred from pure cultures stored at -20 0C (with 20% glycerol solution), were grown on LB agar medium (add make and origin) and incubated at 37 0C overnight. Several well isolated colonies were harvested, suspended in a 10 or 15 ml volume of LB broth and incubated at 37 0C with shaking for overnight to prepare a stock.

The stocks were later used in the sample preparation procedure in different ways for different experiments.

## OD to CFU calibration

In order to link optical density with microbial load, an experiment was set up where liquid bacterial cultures were diluted to OD 1. The cultures were then serially diluted between 1:10 and 1:1010 and 100µL were plated on agar plates and incubated overnight in 37°C. Each dilution was plated 3 times for replicates, and 3 plates were incubated without inoculation as controls. After incubation, colonies were counted on the plates and the bacterial concentration of a 1 OD liquid culture was calculated to be ~108 CFUs/ml in both *E. coli* and *B. subtilis*.

## Survival test under sample preparation, transfer and radiation

## Sample preparation for Raman scanning

Prior to scanning with Raman instrument, 15ml of bacteria culture were grown overnight in optimal conditions. The bacterial culture was centrifuged for 10 minutes at 4991 g. The supernatant was thrown away and the bacterial pellet re-suspended in 15ml of distilled water or saline (depending on the experiment). This washing stage was repeated 3 times, and the final pellet was re-suspended in distilled water or saline to the volume necessary to reach optical density of 1. The sample was later diluted according to each experiment in serial dilutions. The samples were kept at room temperature at all times after this and were measured 1-3 hours after the preparation stage. Different sample preparations were used in part of the experiments and will be described here:

### “schlichta” experiment

In this experiment the goal was to scan bacteria as a pellet, with a low concentration of water and with as few other substances as possible. To achieve this bacteria were washed as in the standard sample preparation but the final pellet was not re-suspended but scraped and loaded onto the aluminium cup. In order to get a large enough pellet, 2 stater cultures (15 ml each) were combined before the final centrifugation step.

* + 1. Saline

In this experiment the goal was to improve the bacterial Raman signal by suspending the bacteria in saline (0.9% NaCl). The rationale was that bacterial cells might be under osmotic stress in distilled water and are changing their optical properties. In this experiment all distilled water was replaced by autoclaved saline but the sample preparation was otherwise unchanged.

* + 1. Glass slides

In experiments done on glass slides the goal was to reduce scatter and reflectance from aluminium cup and (in some experiments) to reduce water content effect on the signal by drying the samples on the slides. After standard sample preparations, microscope glass slides (???) were loaded with 50 uL droplets of the samples. The slides were put into petri dishes and air-dried in an incubator at 37 dC overnight.

* + 1. Aluminium slides

In this experiment the goal was to reduce the effect of water content on the signal by drying the samples. Roughly 10x3 cm cutting of thick aluminium foil (???) were made and 50 uL droplets of samples were placed on top of the cuttings. The slides were put into petri dishes and air-dried in an incubator at 37 dC overnight.

* + 1. “Dirty” experiment

In this experiment the goal was to keep the bacteria’s natural chemical environment in order to get a signal from both the bacteria and their environment. For this purpose, 15ml of overnight cultures were diluted in LB broth to OD of 1 and serial dilutions were made in LB broth to get a range of concentration between 106 – 10 CFU/ml. Samples were kept in ice to inhibit growth during transfer and scanning procedure, but brought to room temperature for actual Raman scanning.

* + 1. Membrane prep
    2. Milk

# Water sampling

Water samples were taken from X drinking water drillings in Israel between July 2017 and X 2018 before and after standard chlorination treatment with X. The list of the drillings, the number of sampling events and the frequency of sampling are listed in Table X. Water was collected in cleaned glass bottles and transported in coolers to the laboratory where it was stored in 4°C until analysis.

Table X. Water sampling schedule

|  |  |  |
| --- | --- | --- |
| Well (code) | Sampling Frequency | Number of samples |
| Alonei Ha’Bashan 5 (AAI05) | Monthly |  |
| Alonei Ha’Bashan 8 (AAI08) | Monthly |  |
| Kidmat Tzvi 1 (KDZ) | Quarterly |  |
| Shimron 7 (SH7) | Monthly |  |
| Einan 3 (QP003) | Summer (April – November): 2 per month  Winter (April – March): 2 per week |  |
| Einan 6 (QP006) | Summer (April – November): 2 per month  Winter (April – March): 2 per week |  |
| Total samples |  | ? |

# Water sample standard testing

All water samples were sent simultaneously to be analysed in a service lab according to standard methods (ref) for: coliforms, fecal coliforms, fecal streptococci, heterotrophic bacterial counts and turbidity. In chlorinated samples chlorine concentrations were also measured.

# Water sample preparation

250 or 500 ml of water from drillings were sampled by trained personnel according to standard water sampling procedure (ref – min. of health) and transported in coolers to lab in Volcani center (Rishon L’etzion). The water was put in 4°C until scanning. Prior to scanning the water was brought to room temperature by leaving the bottles in room temp for approx. 3 hours. The cuvvett was washed 3 times (6 ml) with distilled water before the beginning of sampling, and washed by 4 ml of sample water between each scan and 2 ml were loaded for scanning. The cuvette was wiped with kimwipes before each scan and was aligned to the same side for scanning.

# Fluorescence Scanning

Using a fluorometer (make and model), 2 ml of water samples were scanned for fluorescence in the ranges of excitation 210-400 nm (resolution of 5nm) and emission 220-410 nm (resolution of 2nm). Each sample was scanned twice.