9 Isolation of Zinc-Resistant Bacteria

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9.1 Theoretical background

Microorganisms are found everywhere, not only are they found throughout our bodies, but they are also used to produce antibiotics, dairy products, and are of course a staple of all ecosystems. As such, microbiology is one of the most important fields of study, and the identification and study of bacterial species is key to understanding these diverse and vastly important ecosystems. Previous research into the microbes that inhabit the German Wadden Sea found the presence of Marinobacter adhaerens HP15, a bacterium that is now a model organism in the assessment of bacteria and diatom interactions (Gaerdes, et al.). Later characterisation lead to the discovery of two homologous czcCBA gene clusters possessed by it, which are responsible for heavy metal resistance, in particular zinc resistance (Kaeppel, et al.), the role of which is currently under investigation as the concentration that it is able to resist is far higher than the naturally occurring concentration of zinc in the ocean.

The question now is to try and understand this zinc resistance. Is it found in other strains or species in the German byte? Is it related to these previously examined diatom interactions? As such, water samples from different sites were taken from the deepest point and from the chlorophyll max (as it is assumed that this is where diatom-bacteria interactions are higher) and then allowed to grow on zinc-containing medium.

Figure 9.1: Map showing the station number where water samples were taken via CTD.

The figure above shows the different CTD stations where water samples were taken for plating and further microbiological analysis. Over four days, samples from six different areas were taken and their microbial composition examined.

9.2 Instruments used

Equipment:

- Micropipettes (200μL and 100μL)
- Micropipette tips
- Markers
- Tape
- Scissors
- Falcon tubes
- Improvised seawater filter

- Scalpel
- 70% ethanol for sterilising
- MB agar plates containing 1mM of zinc
- Glass beads
- Vortex

Just as with wet labs elsewhere, the above equipment was used filter the water collected at different depths and subsequently plate the bacteria on zinc-containing agar.

A CTD (conductivity, temperature and depth) is a set of sensors that are used to take oceanographic data in the field. Given the background of this experiment, the CTD was used to measure fluorescence throughout the water column and samples were subsequently taken at the maximum depth and at the area of maximum fluorescence, as this is an indicator of the chlorophyll content in the water column. Additionally, the temperature and salinity data at the sample-taking depth was measured. At the given depths for each station, two bottles were closed: one at the maximum depth, the other at the maximum fluorescence area, to take the water samples (OceanExplorer on CTDs)

9.3 Data Collection methods; general methods

1. The improvised seawater filters were prepared by taping a filter of 10μm pore size to a plastic pipe and labelled (one per sample taken). Two falcon tubes were labelled (one per site) and four plates (two per sample site: always one plated with 100 μL of the sample and another with 200μL) were labelled with the station, depth of sample taken, date, volume plated and flask number.
2. The CTD was sent down the water column to record the data by the pilot. Two water samples were then taken: one at the maximum depth and another at the fluorescent maximum.
3. The CTD was brought on board, and 50mL falcon tube was filled with approximately 40μL of the respective water sample.
4. The remaining water from the CTD was allowed to run through the corresponding improvised seawater filter.
5. The filter was cut off the pipe with a sterile scalpel (sterilised by using the 70% ethanol), and placed into its appropriate falcon tube.
6. The falcon tube now containing the filter was vortexed, to resuspend any particulate matter

7. 100μL of the water was put onto the corresponding plate. Glass beads were added and the plate was shaken to spread the water sample over the gel. The beads were thereafter disposed of.

8. This was repeated with 200μL of the water sample, and with the sample taken at another depth.

9. All completed plates were stored in plastic bags, which were sealed once full.

10. The plates were then examined back at Jacobs University, where the colonies were counted for subsequent analysis.

11. Some more unique looking colonies were selected to be restreaked and stored in glycerol stock at -80 $^\circ$C for future use in the microbiology lab group.

9.4 Use of the data and samples

Based on previously conducted experiments and research, and the background described above, the idea of this portion of the excursion was to determine the role and prevalence of zinc-resistance in bacteria. Ideally, the bacteria isolated from this research cruise will be further examined in the future to check for the presence of the czcCBA gene cluster encoding for the cation-proton antiporter and the relation between its incidence and other factors such as fluorescence (which is an indicator of the chlorophyll and such is a marker for the presence of diatoms), temperature, salinity and depth. Maybe, these samples and their associated data will finally help bridge the gap between what we know about zinc resistance in bacteria containing the czcCBA cluster and what we still do not understand about its purpose given the lower zinc concentration present in ocean water.

As mentioned in the Method, some of colonies were selected by Professor Dr. Matthias Ullrich to be restreaked by a lab technician and thereafter stored in glycerol stock. This is so that members of the lab group can later look at the bacteria isolated over the course of this excursion, and such that they can be further characterised in the future.

9.5 Results and Discussion

Once the plated samples arrived at Jacobs University, they were examined visually for an overview of the types of colonies that were collected and managed to grown on the zinc-containing medium. Many different types of colonies were observed, and a collection of these are shown below.

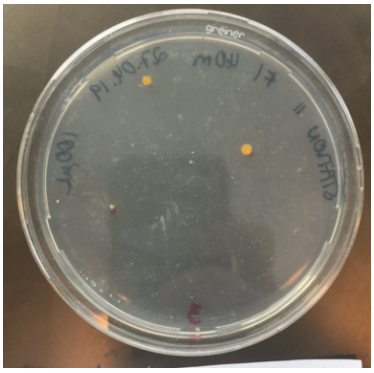

Figure 9.2: Resulting plate from a water sample at station 11-1, with water taken from flask 1 of the CTD at a depth of 40m.

The above image shows a group of orange/yellow colonies which were found present in most of the older plates, but not the more recent ones. This could be due to two reasons: it is either a contamination which came in during the earlier sample preparation steps and were avoided later on, or it is a very slow growing organism which needs time to grow as it is not at its optimal conditions. If it is the latter case, it would appear to be ubiquitously spread out in the German Byte, at least the area around Helgoland, as it was found in plates regardless of depth, fluorescence, temperature, salinity and location. A contamination also becomes less likely, when we consider that the plates contained zinc, so it is less likely that something with a higher zinc-tolerance came in from the outside.

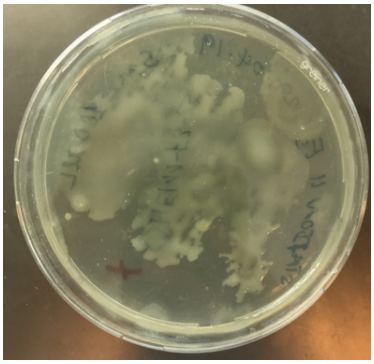

Figure 9.3: Resulting plate from a water sample at station 11-1, with water taken from flask 3 of the CTD at a depth of 25m.

This plate clearly shows how some of the colonies were not clearly distinguishable from one another - sometimes they were more like smears which merged into one another, that could not be counted as they were not individual colonies. That is not to say that this data was disregarded or that it is not valid, it is just a different colony morphology to what was observed elsewhere.

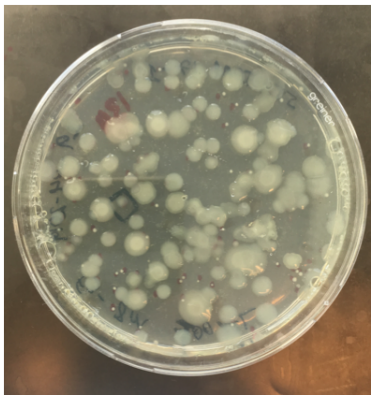

Figure 9.4: Resulting plate from a water sample at station 17-1, with water taken from flask 3 of the CTD at a depth of 8m.

Other colonies were more in line with what was expected of the plates, and displayed many different colonies which were mostly rounded in shape, with clear edges, white/milky in colour and were quite flat.

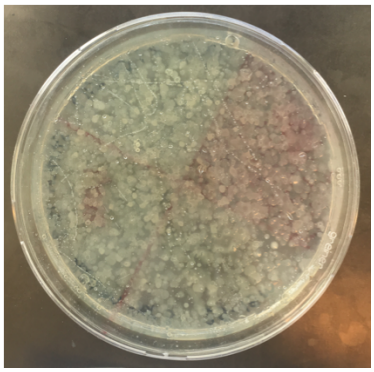

Figure 9.5: Resulting plate from a water sample at station 19-2, with water taken from plankton net 3 which opened at 25m and closed at 15m.

Some water samples were also taken from the plankton net in addition to the CTD, just to collect further data, just like the one shown above. These samples were very rich in colonies and could therefore not be counted as individual colonies could no longer be distinguished. These colonies, however, were quite homogeneous in that they were all small, flat, rounded and white in colour.

The colonies were then counted for each plate, and the salinity, fluorescence, temperature, depth, and colony count data for each sample was compiled from the CTD data into the table below:

Table 9.1: CTD data collected for each station where water samples were taken to be plated and the final colony count.

Date (2019)	Station	Latitude	Longitude	Flask (#)	Volume Plated (µL)	Salinity (PSU)	Fluorescence (mg/m^3)	Temperature (˚C)	Depth (m)	Colony Count
27.04	11-1 CTD	54˚08.392'N	007˚52.852'E	1	100	34.0175	1.0718	7.9488	40	1
27.04	11-1 CTD	54˚08.392'N	007˚52.852'E	1	200	34.0175	1.0718	7.9488	40	1
27.04	11-1 CTD	54˚08.392'N	007˚52.852'E	3	100	33.9722	1.2095	7.9488	25	-
27.04	11-1 CTD	54˚08.392'N	007˚52.852'E	3	200	33.9722	1.2095	7.9488	25	-
28.04	14-1 CTD	54˚10.910'N	008˚01.741'E	1	100	33.7547	0.9771	8.1252	28	9
28.04	14-1 CTD	54˚10.910'N	008˚01.741'E	1	200	33.7547	0.9771	8.1252	28	9
28.04	14-1 CTD	54˚10.910'N	008˚01.741'E	3	100	32.0111	1.5926	9.6844	5	88
28.04	14-1 CTD	54˚10.910'N	008˚01.741'E	3	200	32.0111	1.5926	9.6844	5	444
29.04	17-1 CTD	54˚17.940'N	007˚38.917'E	1	100	33.7024	1.2886	8.0563	25	22
29.04	17-1 CTD	54˚17.940'N	007˚38.917'E	1	200	33.7024	1.2886	8.0563	25	50
29.04	17-1 CTD	54˚17.940'N	007˚38.917'E	3	100	32.6126	2.5345	8.9166	8	134
29.04	17-1 CTD	54˚17.940'N	007˚38.917'E	3	200	32.6126	2.5345	8.9166	8	123
29.04	19-1 CTD	54˚08.240'N	007˚38.674'E	1	100	33.9294	0.8587	7.9764	45	70
29.04	19-1 CTD	54˚08.240'N	007˚38.674'E	1	200	33.9294	0.8587	7.9764	45	28
29.04	19-1 CTD	54˚08.240'N	007˚38.674'E	5	100	33.2059	2.1451	8.6464	5	7
29.04	19-1 CTD	54˚08.240'N	007˚38.674'E	5	200	33.2059	2.1451	8.6464	5	14
30.04	21-1 CTD	54˚06.261'N	007˚58.541'E	1	100	33.7756	1.3509	7.9609	25	55
30.04	21-1 CTD	54˚06.261'N	007˚58.541'E	1	200	33.7756	1.3509	7.9609	25	80
30.04	21-1 CTD	54˚06.261'N	007˚58.541'E	3	100	32.2615	1.7153	9.1055	5	76
30.04	21-1 CTD	54˚06.261'N	007˚58.541'E	3	200	32.2615	1.7153	9.1055	5	56
30.04	23-1 CTD	54˚05.060'N	008˚07.210'E	1	100	33.3071	1.1727	8.2202	19	12
30.04	23-1 CTD	54˚05.060'N	008˚07.210'E	1	200	33.3071	1.1727	8.2202	19	9
30.04	23-1 CTD	54˚05.060'N	008˚07.210'E	3	100	31.9926	1.3104	9.3203	8	30
30.04	23-1 CTD	54˚05.060'N	008˚07.210'E	3	200	31.9926	1.3104	9.3203	8	54
30.04	24-2 CTD	54˚04.410'N	008˚14.310'E	1	100	29.5873	1.6761	10.4844	10	20
30.04	24-2 CTD	54˚04.410'N	008˚14.310'E	1	200	29.5873	1.6761	10.4844	10	12
30.04	24-2 CTD	54˚04.410'N	008˚14.310'E	2	100	30.8031	2.5636	9.8772	5	7
30.04	24-2 CTD	54˚04.410'N	008˚14.310'E	2	200	30.8031	2.5636	9.8772	5	17
30.04	25-1 CTD	54˚07.970'N	008˚06.780'E	1	100	30.0893	3.2903	10.3000	15	5
30.04	25-1 CTD	54˚07.970'N	008˚06.780'E	1	200	30.0893	3.2903	10.3000	15	5
30.04	25-1 CTD	54˚07.970'N	008˚06.780'E	2	100	33.0303	1.3397	8.4940	5	19
30.04	25-1 CTD	54˚07.970'N	008˚06.780'E	2	200	33.0303	1.3397	8.4940	5	55

Data from the water samples was taken over four days at different locations and stations throughout the excursion. The final colony count that was assessed at Jacobs University is also included, along with salinity, fluorescence and temperature at the given depth and the flask of the CTD that each sample was taken from is also indicated. Some water samples were taken from the plankton nets to be plated, however these values could not really be used for further analysis as the plankton nets take samples over a range of depths (e.g. one opens at 25m, goes up, and closes at 15m). As such all the related data would be over a range and does not have a specific value. The data was then compiled into four graphs with the number of colonies counted on the respective plate on the 07.05.2019. Each sample was plated twice: once in 100μL of the plate and once in 200μL. For consistency, the graphs below all have the same key: data collected from the 100μL of the sample is shown in blue, while that from 200μL of the sample is shown in orange.

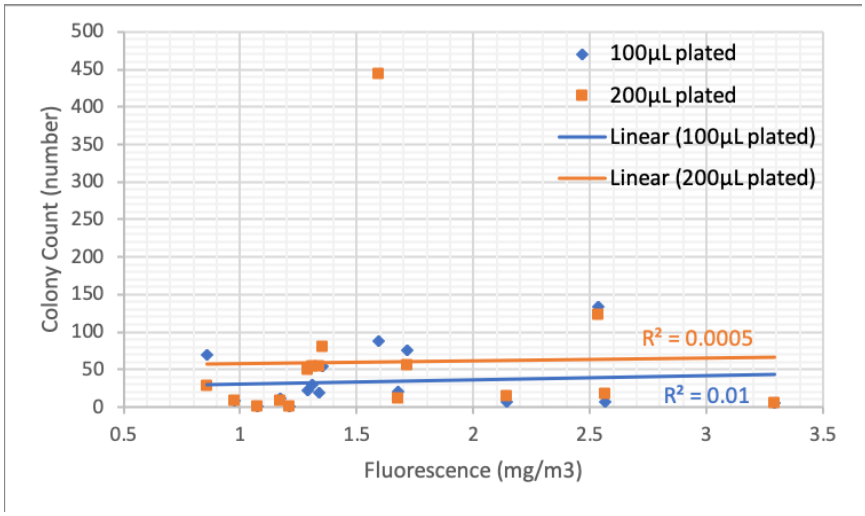

Figure 9.6: Graph showing relation between the final colony count and fluorescent signal at the sample depth.

The above graph shows the colony number plotted against fluorescence at the givent depth. As the graph illustrates there is no correlation between them, the R^2 value for the plates plated with 100μL of the sample was only 0.0005, while that for 200μL was 0.01 indicating no statistical relationship. While most of the colony counts were in the range of 0 to about 150, there is a clear outlier in the 200μL data, which shows a count just below 450 colonies on the plate but a relatively low fluorescence of about 1.5 mg/m^3.

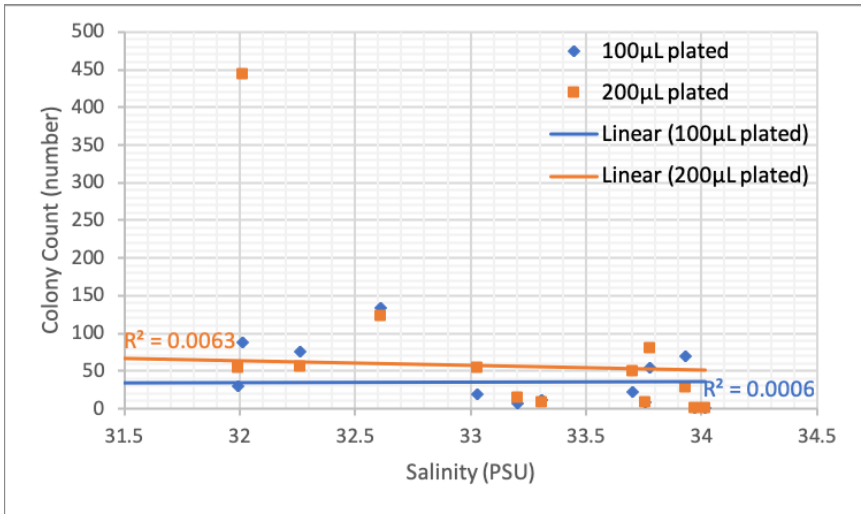

Figure 9.7: Graph showing relation between the final colony count and salinity at the sample depth.

The above graph describes the relationship between the colony count and salinity at the

given depth, which has no correlation given the R^2 values of 0.0006 for 100μL data and 0.0063 for 200μL data. Just as with the data shown above in Figure 9.6, there is an outlier from the 200μL data showing a count of more than 400 colonies, when the remaining data does not even exceed 150 colonies.

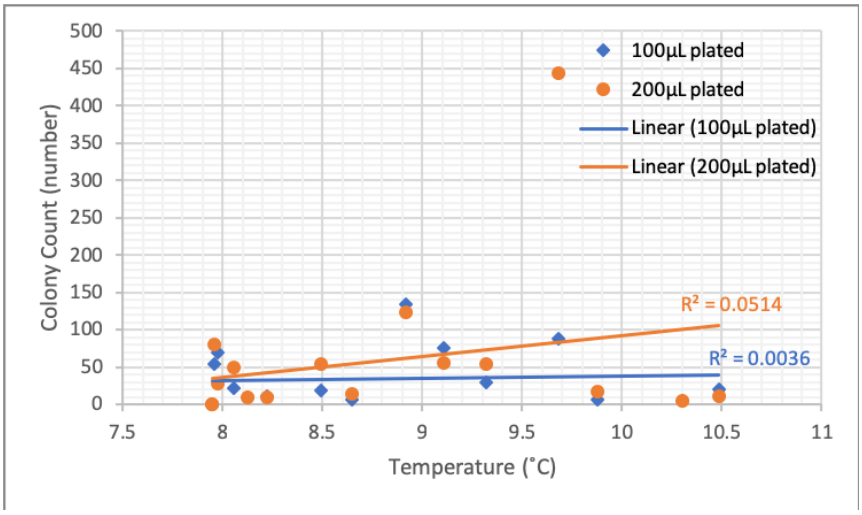

Figure 9.8: Graph showing relation between the final colony count and temperature at the sample depth.

The above graph shows the relation between colony count and temperature. Again, there is no correlation, the R^2 values for the 100μL data is 0.0036 and for the 200μL data is 0.0514. At around 9.7 ° C there is a high number of around 450 colonies once again, while the rest of the samples had lower amount of colonies.

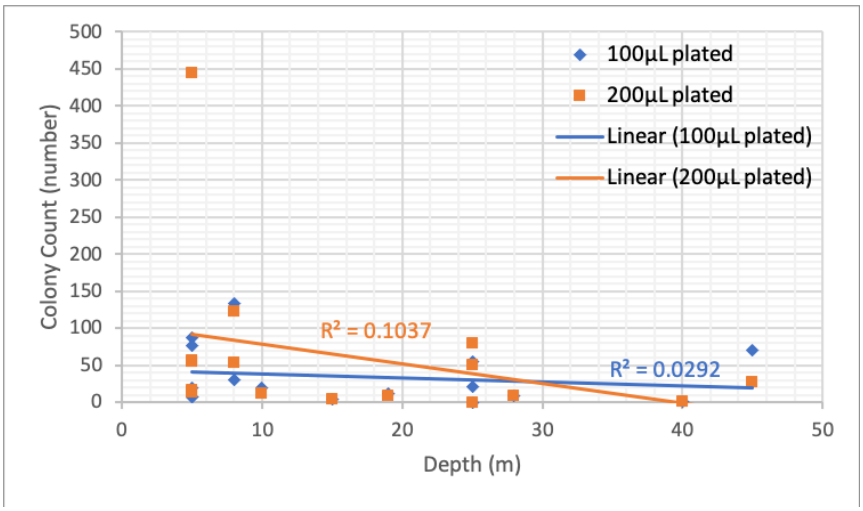

Figure 9.9: Graph showing relation between the final colony count and the sample depth.

The final graph demonstrates the connection between the number of colonies counted in respect to the depth at which the samples were taken.Here also it shows no correlation between those two variables with R^2 values of 0.1037 for the 200μL data and 0.0292 for the 100μL data. At the depth of 0.5 there is a high number of colonies which was counted in a 200μL plate.

From all the data expressed in the graphs above, it can be inferred that, as there is no statistical correlation between these four variables with number of colonies, the data collected doesn't have a clear implication about the conditions at which more bacteria grow and thrive.So, we cannot estimate anything give this inconclusive data.