Investigation of salt tolerance in *Bacillus*

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Abstract

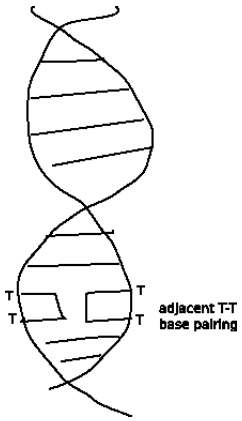
In this project, we intend to sudy how *Bacillus* can survive under various salt concentrations; and how mutagenesis can affect their ability to survive.

1 Introduction

Many bacteria show some degree of salt tolerance. We intend to observe if such salt-tolerant effects can be enhanced by UV mutagenesis. There are many bacteria whcih are useful in agricultural situations for example - nitrogen-fixing bacteria. If their salt tolerance is increased, it would indeed help a great deal.

1.1 UV Mutagenesis

UV works by breaking bonds in the DNA and forming new ones between adjacent bases (thymine-thymine, cytosine-thymine and cytosine-cytosine). Out of these thymine-thymine base pairing is most common. This base pairing causes a change in the proteins that are expressed from genes containing that portion of DNA. These proteins may become malfunctional; or even express new characteristics. We hope that some of these will turn out to be salt-tolerant. A schematic showing the damage to the DNA is shown below:

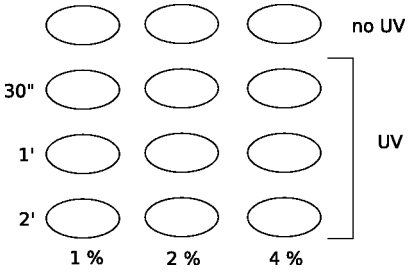


2 Procedure

First we should prepare the LB (Luria Bertani) media, which is the growth media for *Bacillus*. Its composition is as follows:

- Tryptone 10 g/L
- Yeast extract 5 g/L
- NaCl 10 g/L
- Agarose (for the gel) 2-5 %.

Then we prepare 12 plates:



Finally we plate the *Bacillus* culture on the media (of salt concentrations 1%, 2% and 4%), do the mutagenesis (for the time periods shown), and incubate at 37°C, taking care to cover the plates on which UV mutagenesis was done, so that no light repair takes place.

3 Results

The results were quite unexpected:

[NaCl]	1 %	2 %	4 %
0	150/100	180/115	168
30”	1	4	0
1’	0	0	6
2’	0	0	0

I have highlighted the inconsistencies with a different colour. For the first row, I have put some numbers after a /; those are the number of smaller colonies which we counted. The reason I put them separately is that their size was distinctly different from the big ones.

4 Endnote

Interpretation, Conclusion, Precaution

Normally, we would have expected only one kind of colony – not two kinds as we saw here. This could be caused by some contamination in the initial sample. Also another curious fact I noticed was that for the *no UV* and *30”* case, as the salt concentration went up, the number of colonies actually went **up**, instead of decreasing. This problem could also be caused by contamination with some other species or strain of the bacteria, which is not affected much by salt. I also saw 6 colonies in the 1 minute case which is also surprising, as we expected no colonies at all because of the exposure time.

Thus, we cannot conclude anything substantial from this experiment, as it is quite evident that contamination took place, producing data which does not correlate with theory at all. In the future we need to follow proper decontamination procedures, and ensure that only pure strains are used.

5 Acknowledgements

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