Induced Morphological Changes in Bacillus subtilis

Project by: -

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Induced Morphological Changes in *Bacillus subtilis*

Aim: - To study the morphological changes induced in *Bacillus subtilis* under nutrition deficiency condition.

Theory: -

Bacillus subtilis is the model organism used for gram positive bacterial studies. This sporulating bacteria dominates other bacterial colonies in a culture and it also secretes a lot of antibiotics. B. *subtilis* can also form a biofilm and has quorum sensing abilities, which means that it can adjust its gene regulation according to population density in its vicinity. It can also form endospore, which is resistant to lethal effects of heat, radiation and many toxic chemicals. The vegetative or normal cell of B. *subtilis* has one lipid bilayer, and a very thick and complex coat, with nearly 40 layers of murein combined with d-amino acids such as mesodiaminopimelic acid and amino sugars. Flagella is also observed in B. *subtilis*, mostly at polar ends.

The bacteria do not always tend to remain in vegetative condition. When in nutrition deprived state or in anaerobic conditions, it either undergoes sporulation, in which endospores are formed, or a change in shape is observed, which is discussed in this report. The spore formation is a costly process and not all cells are able to perform it. The majority of population just undergoes change in shape, from rod-like to nearly coccus.

Many bacteria are observed to have quorum sensing properties, which allows them to sense the population density near it and adjust the gene expression. To achieve this, each bacterium produces small diffusible molecules needed for cell to cell communication. As the population increases, the relative concentration of the molecules is increased. The cell surface contains receivers, and when threshold of the molecules is reached, sensor proteins transmit a signal to transcriptional regulators to stop cell division.

Hypothesis: -

- Media is very diluted, which creates a hypotonic environment. In these conditions, round shaped cells are more favorable as turgor pressure is distributed evenly. Also, round cells are able to cluster much more efficiently than rod cells, lessening the exposure to hostile environment.
- To face nutrient starved conditions, the cells try to reduce the synthesis of cell wall components and also “consume” the cytosolic components. The amount of cell wall components is reduced but thickness must remain the same, so it changes its shape to the more favorable round shape.
- The surface area of the bacteria is directly proportional to the concentration of the nutrient uptake. So, in higher concentration of the nutrient, the bacteria should be rod shaped as usual, but in lower concentrations the bacteria are expected to show a morphological change due to less nutrition.
- We expect the cells to become smaller in size and spherical, and extended filament formation in which the cells don’t complete their cell cycle, i.e. don’t divide.

Requirements: -

1. LB media (5% and 100% conc.)
2. *Bacillus subtilis*
3. Conical flasks
4. Beaker
5. Distilled water
6. Test tubes
7. Cotton plugs
8. Petri dish
9. Agar
10. Glass slides
11. Spirit lamps
12. Autoclave water
13. Toothpick
14. Weighing machine
15. Incubator (37^{o}C)
16. Refrigerator (4^{o}C)
17. Microscope
18. Spectrophotometer

Protocol: -

PART (A): GROWTH CURVE

Revival from glycerol stock

- Glycerol stock of B. *subtilis* was obtained from TKS lab. LB agar plates were streaked and kept overnight at 37^{o}C.
- A single colony from the plate was streaked onto another LB agar plate (primary culture).

  (NOTE: PLATE WAS RE-STREAKED MULTIPLE TIMES TO KEEP CULTURE FRESH)

Day 0

- Single colony from primary plate was inoculated in 5ml of 5% media and 100% media overnight at 37^{o}C.

Day 1

- 1ml from the overnight mixture was inoculated into 100ml of 5% and 100% media from the respective labeled tubes.
- Multiple OD readings were taken in short time intervals at 600nm.
- Readings were plotted and growth curve was obtained.

PART (B): SPECTRUM READING AND MICROSCOPY

Day 0

- Single colony from primary plate was inoculated in 5ml 100% media overnight at 37^{o}C.
- From obtained data from growth curve, three points in time was chosen to do microscopy, 180, 360, 720 minutes respectively.

Day 1

- Take 1ml from overnight inoculation and add to 100ml 5% media.
- At each chosen time point, spectrum reading was taken from 500-650nm.

- Gram staining and microscopy of those three time points were done following the spectrum.
- Data was exported and plotted.
- Single colony from primary plate was inoculated in 5ml 5% media overnight at 37^{o}C.

Day 2

- Take 1ml from overnight inoculation and add to 100ml 100% media.
- At each chosen time point, spectrum reading was taken from 500-650nm.
- Gram staining and microscopy of those three time points were done following the spectrum.
- Data was exported and plotted.

Data and Plots: -

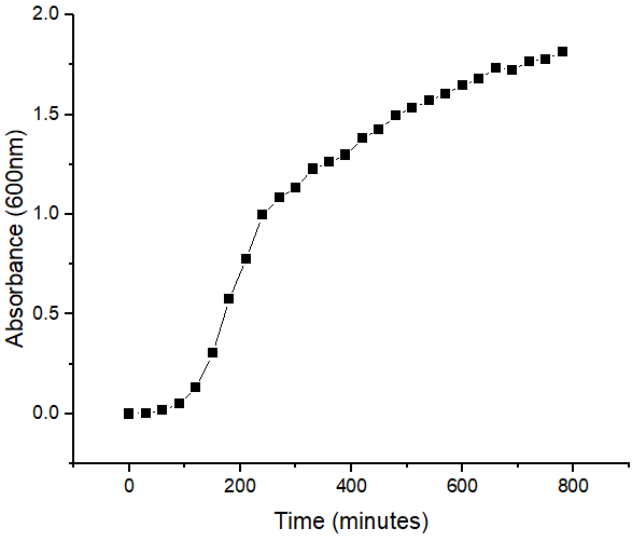
Growth Curves

100% LB media

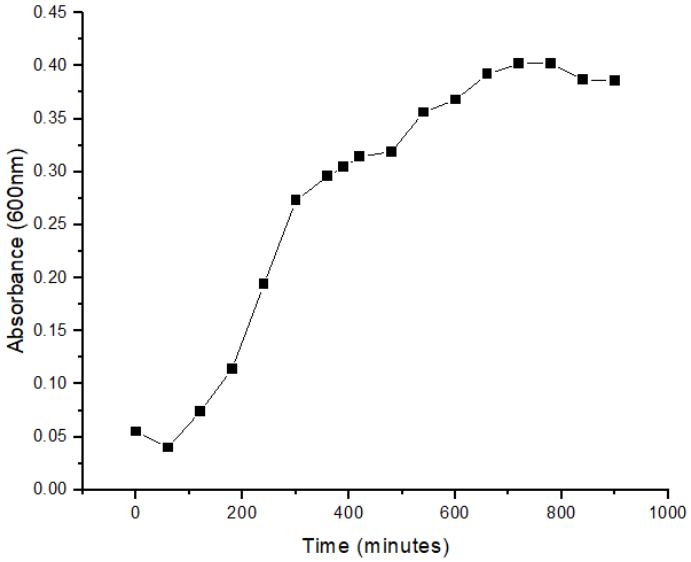
Time (min)	Absorbance (600nm)
0	-0.002
30	0.003
60	0.016
90	0.052
120	0.13
150	0.303
180	0.574
210	0.776
240	0.996
270	1.081
300	1.135
330	1.227
360	1.261
390	1.298
420	1.383
450	1.425
480	1.494
510	1.534
540	1.569
570	1.605
600	1.648
630	1.68
660	1.732
690	1.724
720	1.766
750	1.779
780	1.816

5% LB media

Time (min)	Absorbance (600nm)
0	0.055
60	0.04
120	0.074
180	0.114
240	0.194
300	0.273
360	0.296
390	0.305
420	0.314
480	0.319
540	0.356
600	0.368
660	0.392
720	0.402
780	0.402
840	0.387
900	0.386



100% Media OD Reading



5% Media OD Reading

Microscopy and Image Analysis with Codes: -

Analysis Algorithm:

Editor - E:\IISER\LVL 5\LS3104 (BioLab V)\LS3104 Final project\Part-A\microscopy data\selected\5%-100X\analysis.m

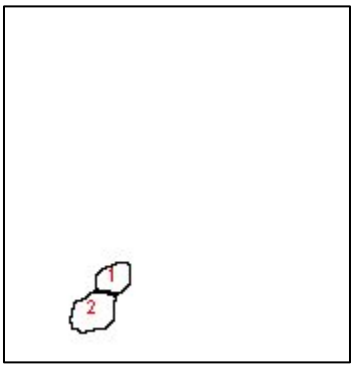
analysis.m | LB_5_720m_100X.m | test.m | crop.m | first1.m | LB_5_180m_100X.m

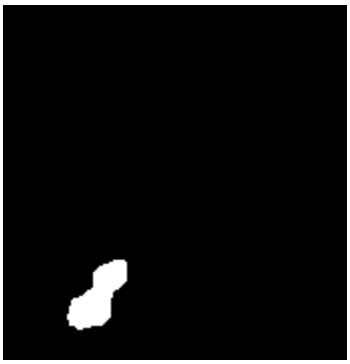
```
Image = imread('180m.TIF');
I = imcrop(Image,[551.5100  348.5100  506.9800  462.9800]);
Ic = imcomplement(I);
I2 = imbinarize(Ic,0.77);
I3 = edge(I2);
se90 = strel('line',2,90);
se0 = strel('line',2,0);
I4 = imdilate(I3,[se90 se0]);
I5 = imclearborder(I4,4);
I6 = bwareaopen(I5,250);
I7 = imfill(I6,'holes');
I8 = I7 - bwareaopen(I7, 400);
seD = strel('diamond',1);
I9 = imerode(I8,seD);
imshow(I)
```

Analysed in 5% media: -

(a) 180minutes

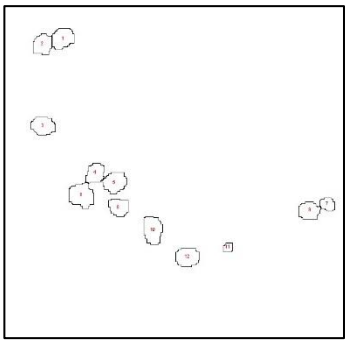
Sl.no.	area	perimeter	circularity
1	0.023	0.571	0.868
2	0.038	0.758	0.826

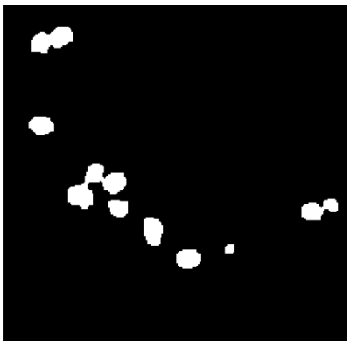




(b) 360minutes

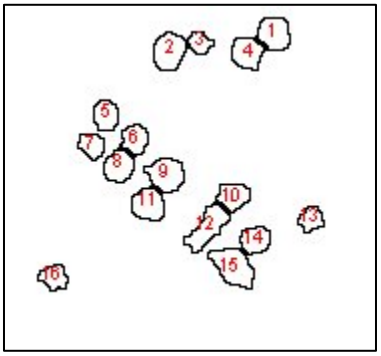
Sl.no.	area	perimeter	circularity
1	0.157	1.578	0.791
2	0.137	1.493	0.77
3	0.152	1.551	0.796
4	0.122	1.45	0.727
5	0.164	1.645	0.761
6	0.204	1.884	0.723
7	0.067	1.018	0.818
8	0.121	1.386	0.789
9	0.131	1.477	0.757
10	0.184	1.758	0.748
11	0.032	0.689	0.859
12	0.161	1.579	0.809



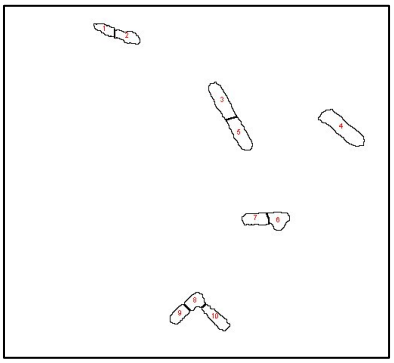


(c) 720minutes

Sl.no.	Area	Perimeter	Circularity
1	0.023	0.571	0.897
2	0.026	0.603	0.898
3	0.011	0.39	0.878
4	0.021	0.559	0.863
5	0.017	0.471	0.941
6	0.017	0.505	0.816
7	0.013	0.438	0.882
8	0.02	0.526	0.925
9	0.026	0.624	0.843
10	0.018	0.518	0.855
11	0.023	0.559	0.928
12	0.027	0.756	0.604
13	0.012	0.432	0.819
14	0.018	0.494	0.904
15	0.032	0.737	0.74
16	0.014	0.482	0.763

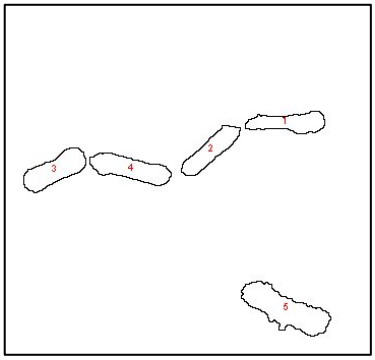


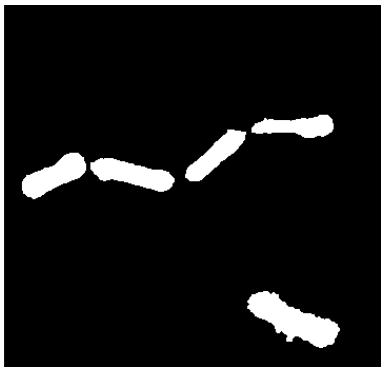
Analysed in 100% media: -



(a) 180minutes

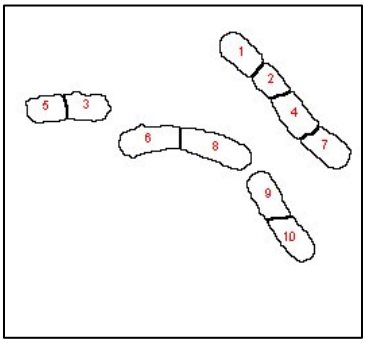
Sl.no.	Area	Perimeter	Circularity
1	0.032	0.817	0.599
2	0.045	0.965	0.606
3	0.092	1.44	0.559
4	0.133	1.761	0.538
5	0.082	1.316	0.593
6	0.054	0.93	0.789
7	0.053	1.005	0.655
8	0.044	0.836	0.795
9	0.042	0.815	0.801
10	0.057	1.122	0.573





(b) 360minutes

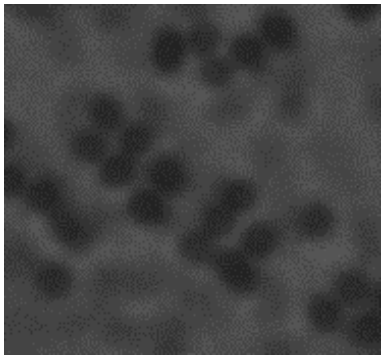
Sl.no.	Area	Perimeter	Circularity
1	0.122	1.957	0.4
2	0.123	1.71	0.53
3	0.15	1.772	0.601
4	0.158	2.029	0.483
5	0.245	2.585	0.462

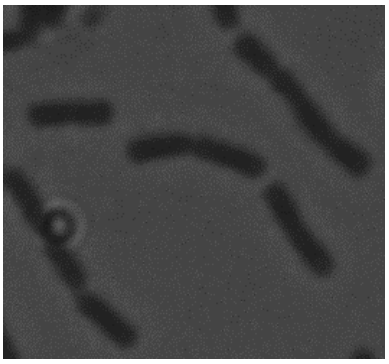


(c) 720minutes

Sl.no.	area	perimeter	circularity
1	0.07	1.037	0.813
2	0.046	0.856	0.781
3	0.062	1.043	0.72
4	0.073	1.129	0.719
5	0.056	0.928	0.817
6	0.087	1.29	0.66
7	0.071	1.081	0.764
8	0.107	1.473	0.618
9	0.074	1.142	0.711
10	0.072	1.126	0.713

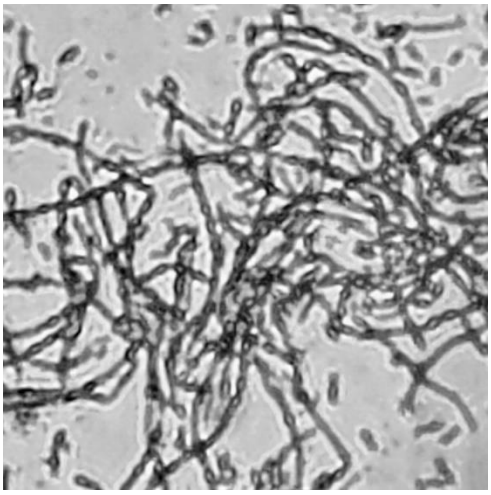
Result and Conclusion: -

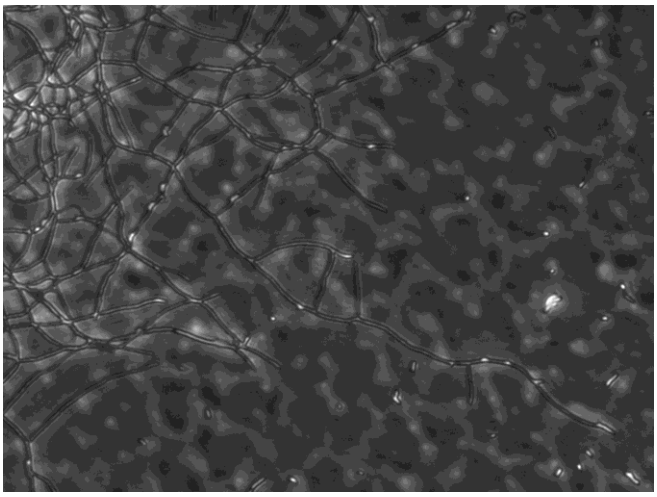




As expected, the cells grown in 5% media have an average circularity index closer to 1 i.e., they are more spherical. Also, their size is small in comparison to the cells grown in 100% media. At time 720minutes, these are the images captured: - (5% and 100% respectively)

Hence, we were successfully able to see morphological changes induced in the bacterium. If allowed the cells to grow in the nutrient deficient media for prolonged days, we could have been able to seen endospores.





Note: We used LB media and not a media specific for B. subtilis study. This may have affected our experimental results.

We have also seen the formation of filaments and cell aggregations, suggesting that the cells adopt a method of colony co-existence, to increase nutrient uptake with each cell having less surface area, but as an aggregate having more surface area. (This was done in no nutrient media, MQ. Only nutrition was the initial inoculum.)

Acknowledgement: -

We are grateful to Bidisha Sinha ma'am for her guidance throughout the project. We would also like to express our gratitude towards Punyasloke Bhadhury sir, Lekha ma'am, Debabrata sir, Sudhangsu sir, Madhura Di, Jeebak Da and Ananya Di for their helpful inputs and support throughout the project.

Reference: -

- *Analysis of Peptidoglycan Structure from Vegetative Cells of Bacillus subtilis 168 and Role of PBP 5 in Peptidoglycan Maturation*, ABDELMADJID ATRIH, GEROLD BACHER, GU¨NTER ALLMAIER, MICHAEL P. WILLIAMSON, AND SIMON J. FOSTER
- *Bacillus Subtillis*, A. DANCHIN. doi: 10.1006/rwgn.2001.0099
- *Bacillus subtilis isolated from the human gastrointestinal tract*, Huynh A. Hong, Reena Khaneja, Nguyen M.K. Tam, et al.