

**IMAQulate**  
Newton Fund Aquaculture GRP



**Second Annual Progress & Planning Meeting**

**Quantitative and Qualitative Microbiological Analysis**

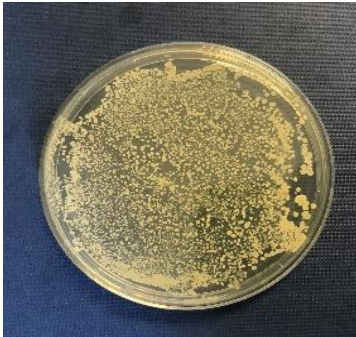
**Neelima Nair and Rachel Lawrence**

9<sup>th</sup> January 2018

# INTRODUCTION

- 52 Probiotic Samples were received from Bangladesh
- Plated in four different culture media:

LB Agar



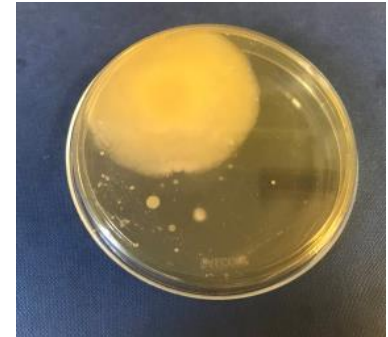
Bacillus Differential Agar



Yeast Malt Agar

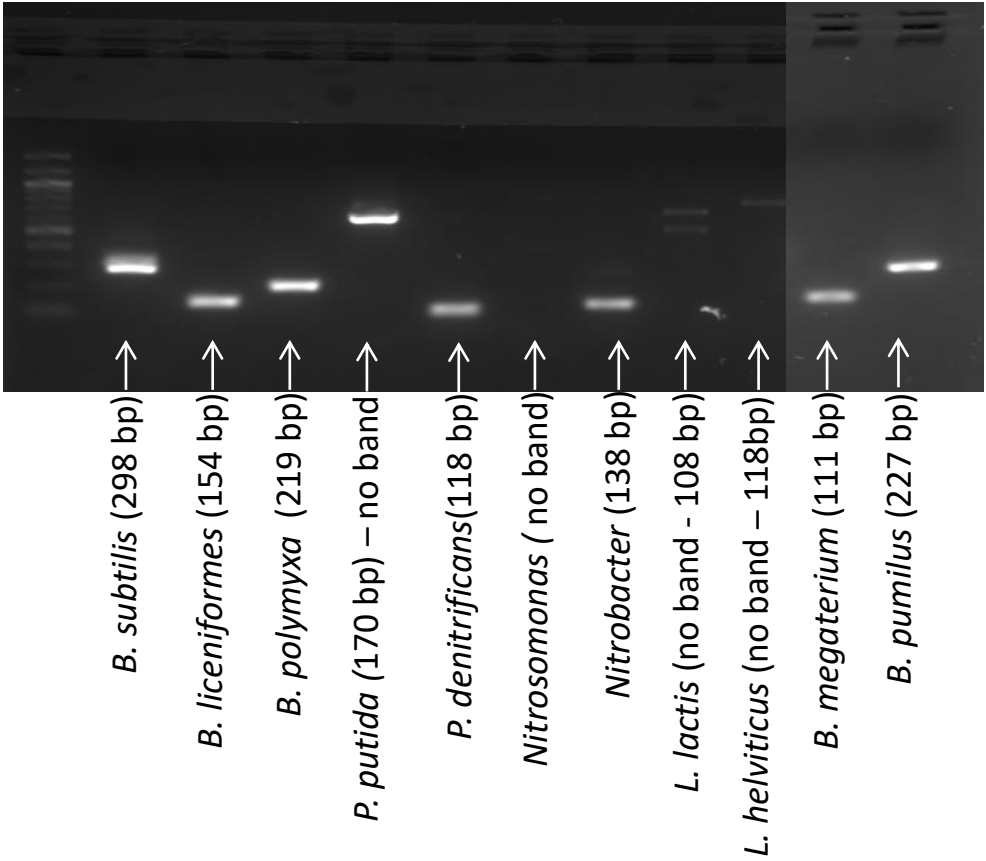


MRS Agar

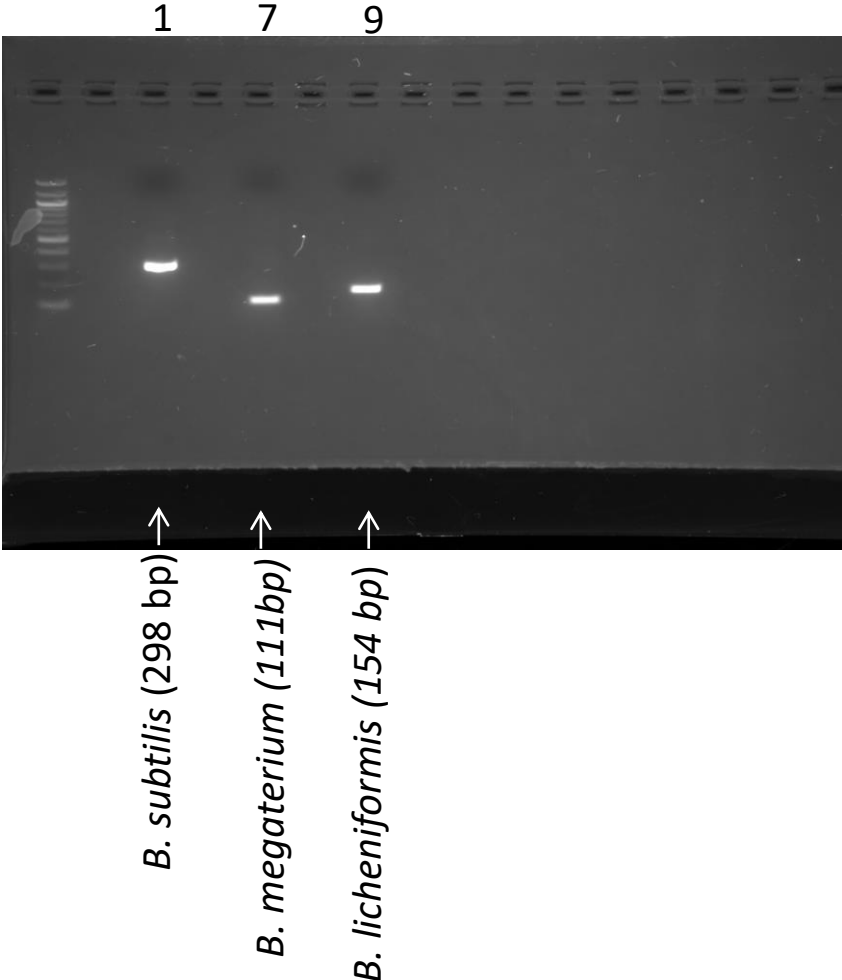


- Colonies were scraped and DNA was extracted
- Primers were designed for the respective 16s region of the bacteria

**Eg: Probiotic Sample 28** – *B.subtilis*, *B.licheniformis*, *B. polymyxa*, *P.putida*, *P.denitrificans* ,  
*Nitrosomonas*, *Nitrobacter*, *L.lactis*, *L.helveticus*, *B.megaterium*, *B.pumilus*, *Aspergillus niger*,  
*Saccharomyces cerevisiae*

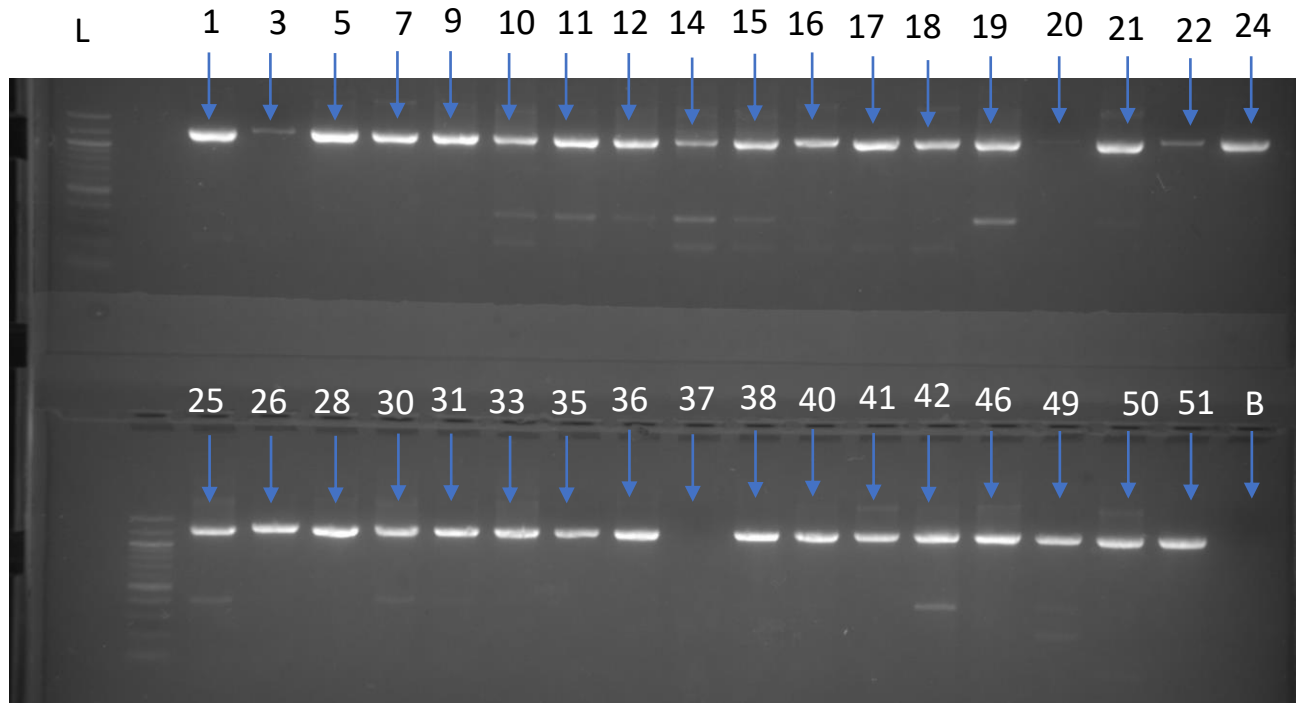


# Cross-Reaction of 16s Primers



# Endoglucanase Gene Can Be Used For Identification Of *Bacillus subtilis*

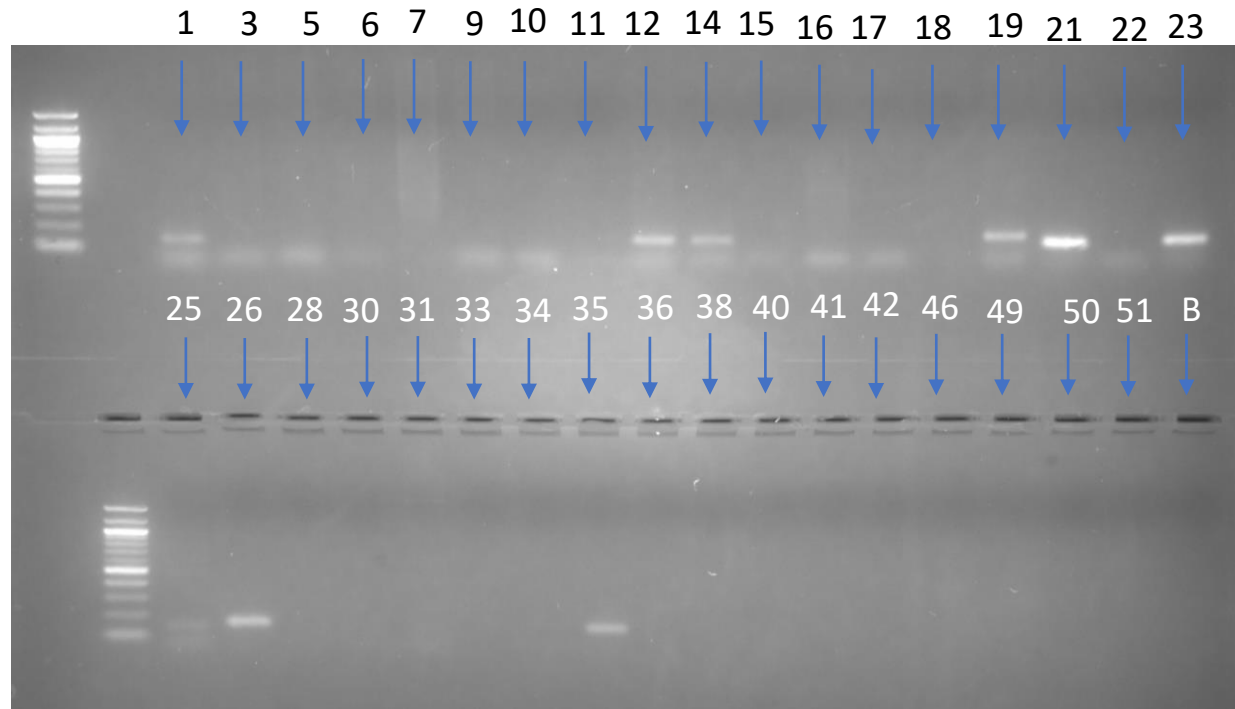
|            |                        |        |
|------------|------------------------|--------|
| B.sub EN1F | CCAGTAGCCAAGAATGGCCAGC | 1341bp |
| B.Sub EN1R | GGAATAATCGCCGCTTTG     |        |




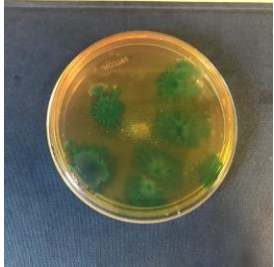
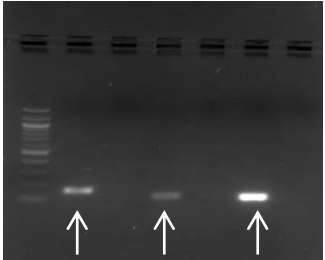

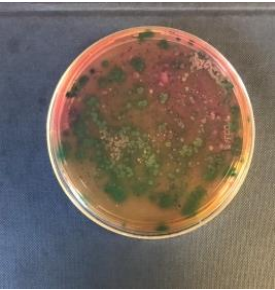
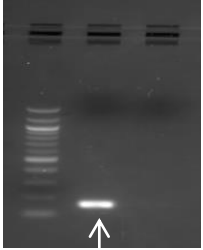
Ashe, S., Maji, U. J., Sen, R., Mohanty, S., & Maiti, N. K. (2014). Specific oligonucleotide primers for detection of endoglucanase positive *Bacillus subtilis* by PCR. *3 Biotech*, 4(5), 461–465. <http://doi.org/10.1007/s13205-013-0177-6>

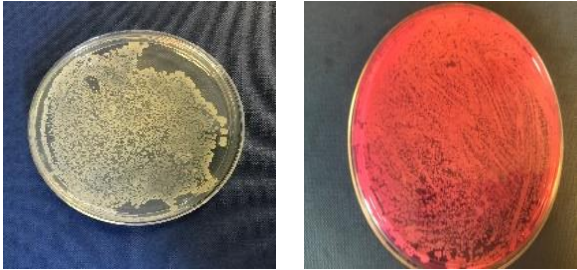
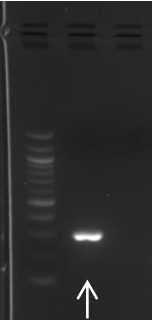

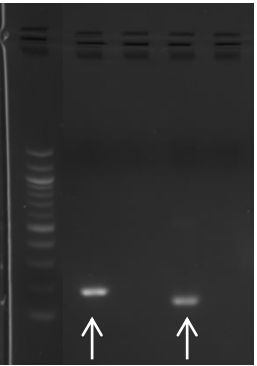
## Identification Of *Saccharomyces cerevisiae*

|        |                         |       |
|--------|-------------------------|-------|
| Sach F | GCGCTTTACATTCAGATCCCGAG | 150bp |
| Sach R | TAAGTTGGTTGTCAGCAAGATTG |       |


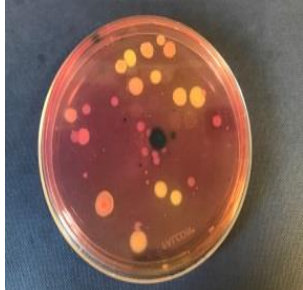
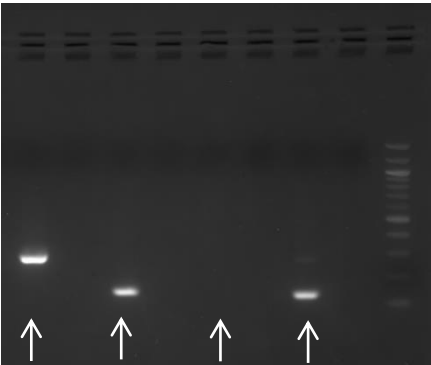


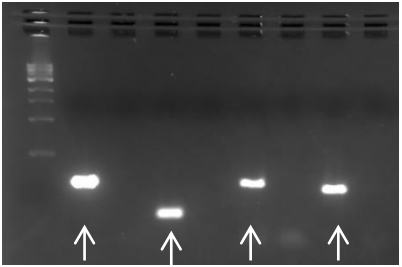




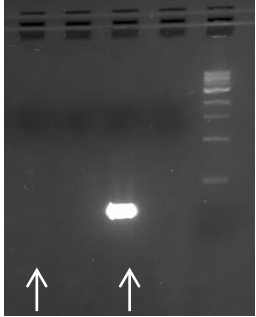


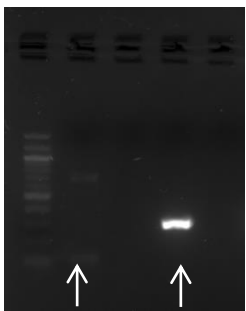
*Hana Suranska, Dana Vranova, Jinna Omelkova; 2016; Isolation, identification and characterization of regional indigenous Saccharomyces cerevisiae strains; Brazillian Journal of Microbiology, 47; 181-190*



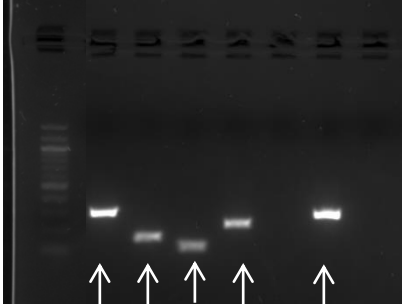

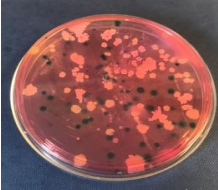
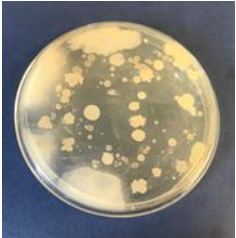
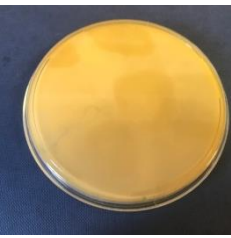
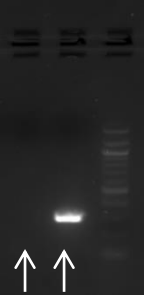
| #  | Bacteria   |             | Culture Plates  |   | Gel  |
|----|--|-------------|---|---|--|
| 1. | B. mesentericus (Bacillus sp.)<br>Enterococcus Faecalis<br>Clostridium butyricum | ✓<br>?<br>? | LB<br>  | BDA<br>  |  <p data-bbox="1553 451 1586 622">Bacillus sp. (154 bp)</p> <p data-bbox="1644 451 1676 712">Enterococcus faecalis (110 bp)</p> <p data-bbox="1734 451 1767 722">Clostridium butyricum (104 bp)</p> |
| 2. | None   |             |   |   |  |
| 3. | Bacillus sp.   | ✓           | LB<br> | BDA<br> |  <p data-bbox="1630 1143 1663 1315">Bacillus sp. (154 bp)</p>  |

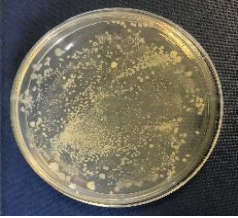

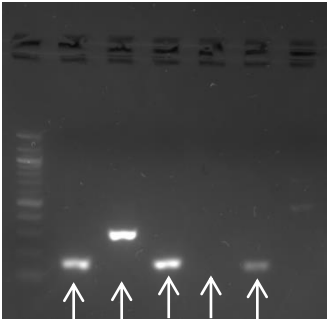
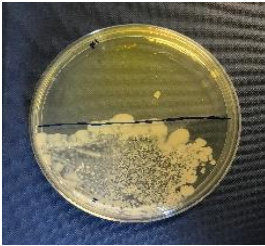

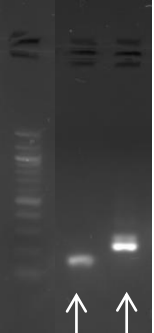
| #  | Bacteria                        | LB plate   | Gel   |
|----|---------------------------------|--|---|
| 4. | None                            |  |   |
| 5. | Bacillus subtilis               | <p style="text-align: center;">LB                      BDA</p>   |  <p style="text-align: center;">Bacillus subtilis (298 bp)</p>   |
| 6. | Bacillus sp.<br>Pediococcus sp. | <p style="text-align: center;">LB                      BDA</p>  |  <p style="text-align: center;">Bacillus sp. (154 bp)</p> <p style="text-align: center;">Pediococcus sp. (118 bp)</p> |



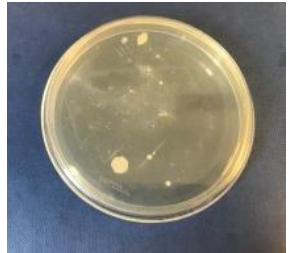
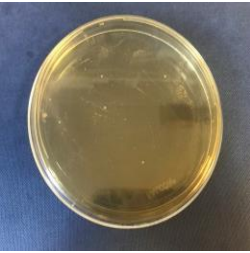
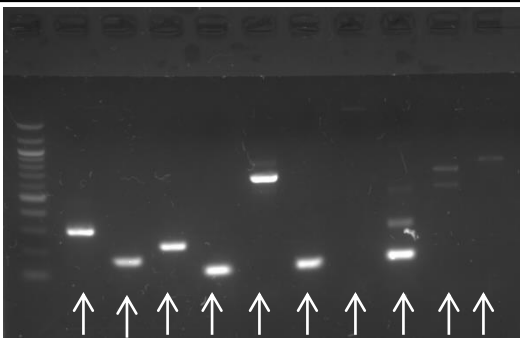


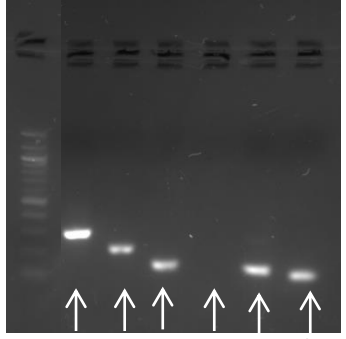


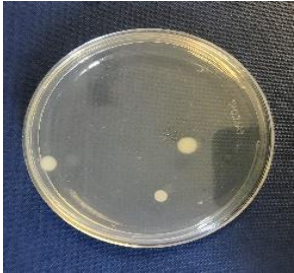
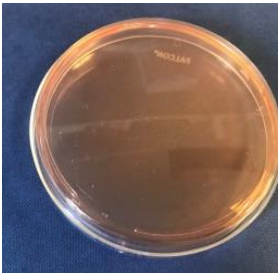
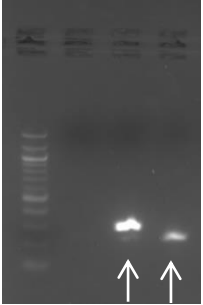

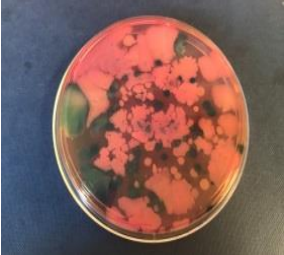


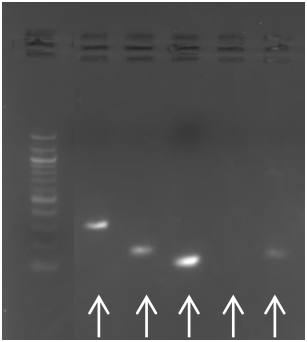
| Sample | Bacteria  |                  | LB Plate   | Gel  |
|--------|---|------------------|--|--|
| 7.     | B. subtilis<br>B. liceniformis<br>Nitrosomonas<br>Nitrobacter | ✓<br>?<br>X<br>? | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div>   |  <div style="display: flex; justify-content: space-around; margin-top: 10px;"> <div style="text-align: center;"> <p>↑</p> <p><i>Bacillus subtilis</i> (298 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>Bacillus liceniformis</i> (154 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>Lactobacillus acidophilus</i> ( no band)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>Nitrobacter</i> (138 bp)</p> </div> </div> |
| 8.     | No Data   |                  |  |  |
| 9.     | B. subtilis<br>B. megaterium<br>Thiiothrix<br>Nitrococcus     | ✓<br>?<br>?<br>? | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div> |  <div style="display: flex; justify-content: space-around; margin-top: 10px;"> <div style="text-align: center;"> <p>↑</p> <p><i>Bacillus subtilis</i> (298 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>B. megaterium</i> (111 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>Thiiothrix</i> (244 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>Nitrococcus</i> (231 bp)</p> </div> </div>                         |



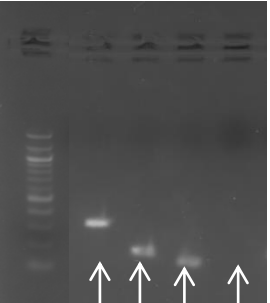

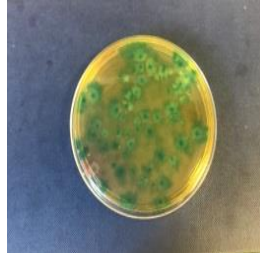
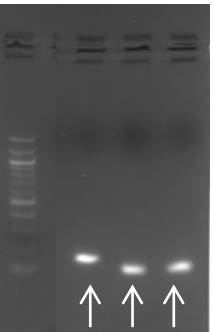
| Sample | Bacteria                       |        | LB plate   |   | Gel  |
|--------|--------------------------------|--------|--|---|--|
| 10.    | L. acidophilus<br>B. subtilis  | X<br>✓ | <p data-bbox="846 147 892 181">LB</p>   | <p data-bbox="1155 147 1240 181">BDA</p>   |  <p data-bbox="1489 475 1528 689">L. acidophilus (no band)</p> <p data-bbox="1586 475 1624 689">Bacillus subtilis (298 bp)</p>      |
| 11.    | B. subtilis<br>L. acidophillus | ✓<br>X | <p data-bbox="846 818 892 852">LB</p>  | <p data-bbox="1155 818 1240 852">BDA</p>  |  <p data-bbox="1528 1143 1566 1358">L. acidophilus (no band)</p> <p data-bbox="1624 1129 1663 1343">Bacillus subtilis (298 bp)</p> |



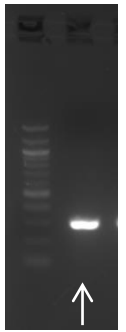

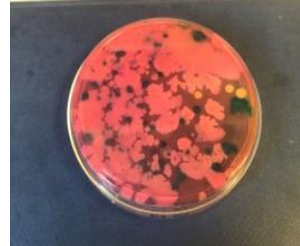
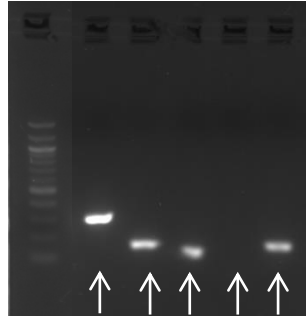

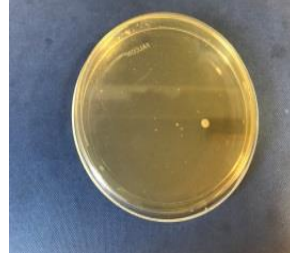
| Sample | Bacteria  |                       | LB plate  |  | Gel  |
|--------|---|-----------------------|---|--|--|
| 12.    | B. subtilis<br>B. liceniformis<br>B. megaterium<br>B. pumilus<br>B. amyloliquefaciens | ✓<br>?<br>?<br>?<br>? | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div>  |  |  <p style="text-align: center;"> <i>Bacillus subtilis</i> (298 bp)<br/> <i>Bacillus liceniformes</i> (154 bp)<br/> <i>Bacillus megaterium</i> (111 bp)<br/> <i>Bacillus pumilus</i> (227 bp)<br/> <i>Bacillus amyloliquefaciens</i> (298 bp)         </p> |
| 13.    | No data   |                       |   |  |  |
| 14.    | L. acidophilus<br>B. subtilis   | X<br>✓                | <div style="display: grid; grid-template-columns: 1fr 1fr; gap: 10px;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> <div style="text-align: center;"> <p>YMA</p>  </div> <div style="text-align: center;"> <p>MRS</p>  </div> </div> |  |  <p style="text-align: center;"> <i>L. acidophilus</i> ( no band)<br/> <i>Bacillus subtilis</i> (298 bp)         </p>  |

| Sample | Bacteria   |                            | LB plate  |   | Gel  |
|--------|--|----------------------------|---|---|--|
| 15.    | B. mesentericus<br>B. subtilis<br>B. liceniformis<br>L. acidophilus<br>Nitrobacter<br>Nitrosomonas | ?<br>✓<br>?<br>X<br>?<br>X | LB<br>   | BDA<br>  |  <p data-bbox="1437 482 1649 694">             Bacillus sp. (154 bp)<br/>             Bacillus subtilis (298 bp)<br/>             B. liceniformis (154 bp)<br/>             L. acidophilus (no band)<br/>             Nitrobacter (138 bp)           </p> |
| 16.    | Bacillus sp.<br>L. Sporogenesis  | ✓<br>?                     | LB<br> | BDA<br> |  <p data-bbox="1586 1143 1657 1350">             Bacillus sp. (154 bp)<br/>             L. sporogenesis (227 bp)           </p>  |



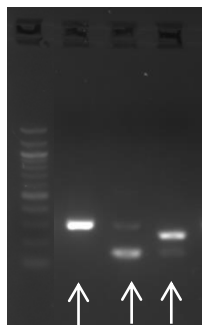

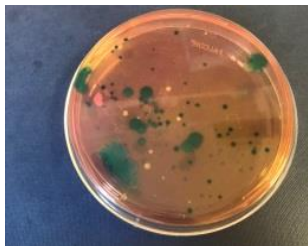

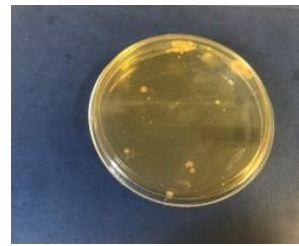
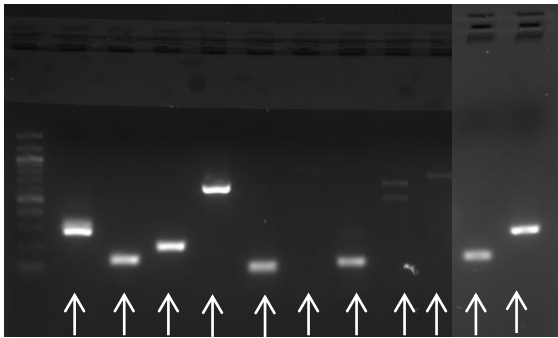
|     | Bacteria   |   | LB plate   | Gel  |
|-----|--|---|--|--|
| 17. | B. subtilis<br>b. liceniformis<br>B. polymyxa<br>B. megaterium<br>B. pumilus<br>P. putida<br>P. denitrificans<br>Nitrosomonas<br>Nitrobacter<br>L. lactis<br>L. Helveticus | ✓<br>?<br>?<br>?<br>?<br>X<br>?<br>X<br>?<br>?<br>X | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div style="text-align: center;"> <p>YMA</p>  </div> <div style="text-align: center;"> <p>MRS</p>  </div> </div> |  <p style="text-align: center;">       ↑<br/> <i>Bacillus subtilis</i> (298 bp)<br/>       ↑<br/> <i>Bacillus liceniformes</i> (154 bp)<br/>       ↑<br/> <i>Bacillus polymyxa</i> (219 bp)<br/>       ↑<br/> <i>Bacillus megaterium</i> (111 bp)<br/>       ↑<br/> <i>P. putida</i> (170 bp) – no band<br/>       ↑<br/> <i>Pseudomonas denitrificans</i>(118 bp)<br/>       ↑<br/> <i>Nitrosomonas</i> ( no band)<br/>       ↑<br/> <i>Nitrobacter</i> (138 bp)<br/>       ↑<br/> <i>Lactobacillus lactis</i> (no band - 108 bp)<br/>       ↑<br/> <i>L. helveticus</i> (no band – 118bp)     </p> |
| 18  | B. subtilis<br>B. polymyxa<br>B. liceniformis<br>Nitrosomonas<br>Nitrobacter<br>P. Denitrificans   | ✓<br>?<br>?<br>X<br>?<br>?                          | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div>   |  <p style="text-align: center;">       ↑<br/> <i>Bacillus subtilis</i> (298 bp)<br/>       ↑<br/> <i>B. polymyxa</i> (219 bp)<br/>       ↑<br/> <i>B.liceniformes</i> (154 bp)<br/>       ↑<br/> <i>Nitrosomonas</i> ( no band)<br/>       ↑<br/> <i>Nitrobacter</i> (138 bp)<br/>       ↑<br/> <i>P. denitrificans</i>(118 bp)     </p>   |



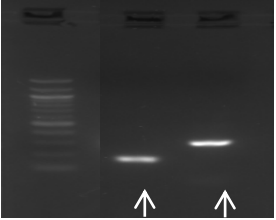
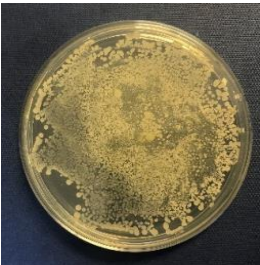

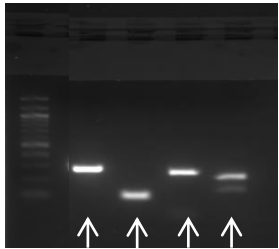
| Sample | Bacteria   |                       | LB plate  | Gel  |
|--------|--|-----------------------|---|--|
| 19.    | <i>B. subtilis</i><br><i>Nitrococcus</i>   | ✓<br>?                | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div>   |  <p style="text-align: center;"> <i>B. subtilis</i> (298 bp)<br/> <i>Nitrococcus</i> (231 bp)         </p>  |
| 20.    | <i>Spirulina</i>   |                       |   |  |
| 21.    | <i>B. subtilis</i><br><i>B. liceniformis</i><br><i>B. megaterium</i><br><i>L. acidophilus</i><br><i>L. plantarum</i> | ✓<br>?<br>?<br>X<br>X | <div style="display: grid; grid-template-columns: 1fr 1fr; gap: 10px;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> <div style="text-align: center;"> <p>YMA</p>  </div> <div style="text-align: center;"> <p>MRS</p>  </div> </div> |  <p style="text-align: center;"> <i>Bacillus subtilis</i> (298 bp)<br/> <i>B. liceniformis</i> (154 bp)<br/> <i>B. megaterium</i> (111 bp)<br/> <i>L. acidophilus</i> ( no band)<br/> <i>L. plantarum</i> ( 138 bp)         </p> |

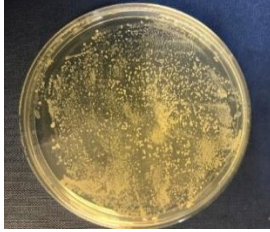


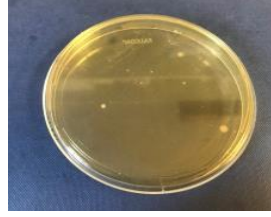
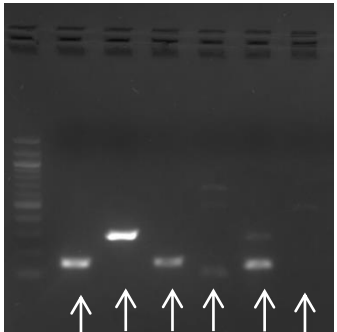



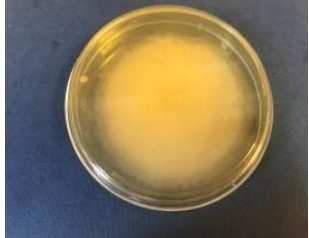
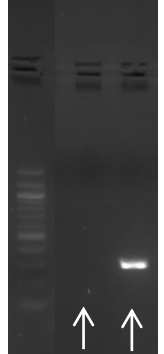
| Sample | Bacteria   |                  | LB plate  |   | Gel   |
|--------|--|------------------|---|---|---|
| 22.    | B. subtilis<br>B. liceniformis<br>B. megaterium<br>L. acidophilus                      | ✓<br>?<br>?<br>X | <p style="text-align: center;">LB</p>   | <p style="text-align: center;">BDA</p>   |  <p style="text-align: center;"> <i>B. subtilis</i> (298 bp)<br/> <i>B. liceniformis</i> (154 bp)<br/> <i>B. megaterium</i> (111 bp)<br/> <i>L. acidophilus</i> (no band)         </p> |
| 23.    | B. mesentericus<br>(Bacillus sp.)<br>Enterococcus faecalis<br>Clostridium<br>butyricum | ✓<br>?<br>?      | <p style="text-align: center;">LB</p>  | <p style="text-align: center;">BDA</p>  |  <p style="text-align: center;"> <i>Bacillus sp.</i> (154 bp)<br/> <i>Enterococcus faecalis</i> (110 bp)<br/> <i>Clostridium butyricum</i> (104 bp)         </p>                      |

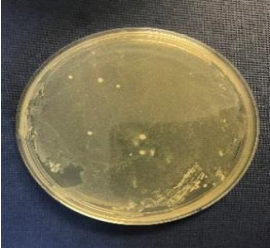

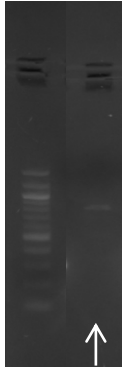


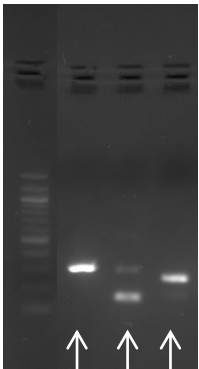
| Sample | Bacteria   |                       | LB plate   |  | Gel  |
|--------|--|-----------------------|--|--|--|
| 24.    | <i>B. subtilis</i>   | ✓                     | <p style="text-align: center;">LB</p>     | <p style="text-align: center;">BDA</p>    |  <p style="text-align: center;">↑<br/><i>B. subtilis</i> (298 bp)</p>   |
| 25.    | <i>B. subtilis</i><br><i>B. liceniformis</i><br><i>B. megaterium</i><br><i>L. acidophilus</i><br><i>L. plantarum</i> | ✓<br>?<br>?<br>X<br>? | <p style="text-align: center;">LB</p>     | <p style="text-align: center;">BDA</p>    |  <p style="text-align: center;"> ↑<br/><i>Bacillus subtilis</i> (298 bp)<br/> ↑<br/><i>Bacillus liceniformis</i> (154 bp)<br/> ↑<br/><i>Bacillus megaterium</i> (111 bp)<br/> ↑<br/><i>L. acidophilus</i> (no band)<br/> ↑<br/><i>L. plantarum</i> (138 bp) </p> |
|        |  |                       | <p style="text-align: center;">YMA</p>  | <p style="text-align: center;">MRS</p>  |  |




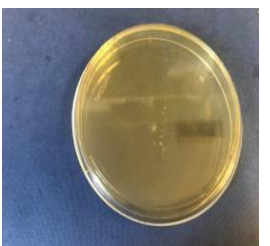
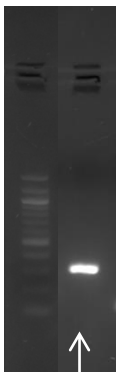
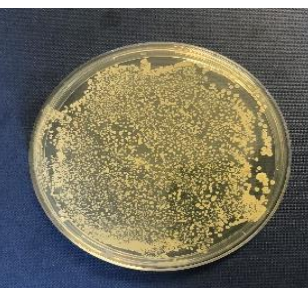

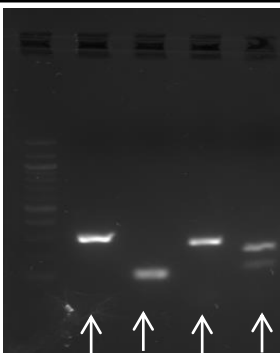


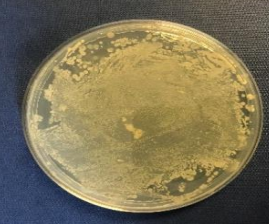

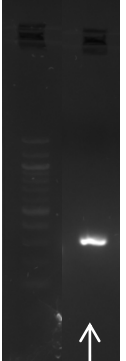
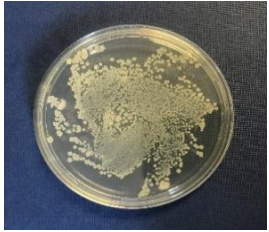
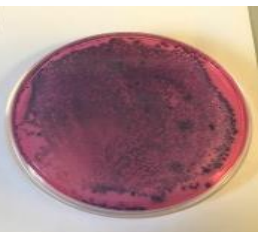
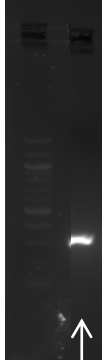
| Sample | Bacteria  |   | LB plate   | Gel   |
|--------|---|---|--|---|
| 26.    | <i>B. subtilis</i><br>Nitrobacter<br>Nitrococcus  | ✓<br>?<br>?   | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div>  |  <div style="display: flex; justify-content: space-around; margin-top: 10px;"> <div style="text-align: center;"> <p>↑</p> <p><i>B. subtilis</i> (298 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p>Nitrobacter (138 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p>Nitrococcus (231 bp)</p> </div> </div>   |
| 27.    | None  |   |  |   |
| 28.    | <i>B. subtilis</i><br><i>b. liceniformis</i><br><i>B. polymyxa</i><br><i>B. megaterium</i><br><i>B. pumilus</i><br><i>P. putida</i><br><i>P. denitrificans</i><br>Nitrosomonas<br>Nitrobacter<br><i>L. lactis</i><br><i>L. Helveticus</i> | ✓<br>?<br>?<br>?<br>?<br>X<br>?<br>X<br>?<br>?<br>? | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div style="text-align: center;"> <p>YMA</p>  </div> <div style="text-align: center;"> <p>MRS</p>  </div> </div> |  <div style="display: flex; justify-content: space-around; margin-top: 10px;"> <div style="text-align: center;"> <p>↑</p> <p><i>B. subtilis</i> (298 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>B. liceniformes</i> (154 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>B. polymyxa</i> (219 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>P. putida</i> (170 bp) – no band</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>P. denitrificans</i> (118 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p>Nitrosomonas (no band)</p> </div> <div style="text-align: center;"> <p>↑</p> <p>Nitrobacter (138 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>L. lactis</i> (no band - 108 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>L. helveticus</i> (no band – 118bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>B. megaterium</i> (111 bp)</p> </div> <div style="text-align: center;"> <p>↑</p> <p><i>B. pumilus</i> (227 bp)</p> </div> </div> |

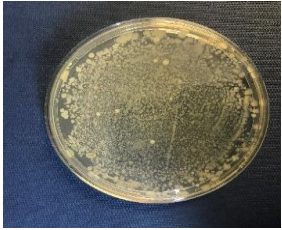

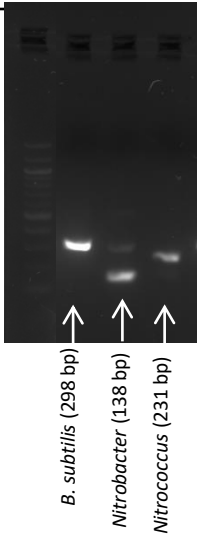
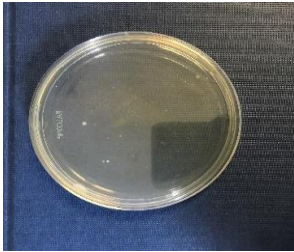
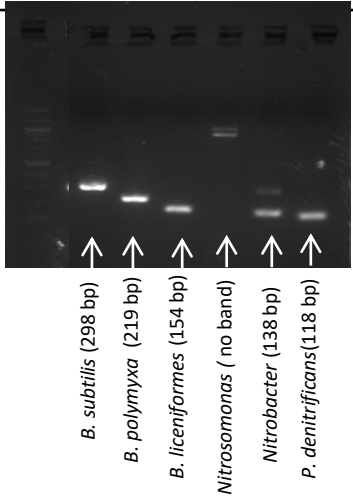
| Sample | Bacteria   |                  | LB plate  | Gel  |
|--------|--|------------------|---|--|
| 29.    | None   |                  |   |  |
| 30.    | Bacillus sp.<br><i>B. thermodenitrificans</i>  | ✓<br>?           | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div>   |  <p style="text-align: center;"> <i>Bacillus</i> sp. (154 bp)<br/> <i>B. thermodenitrificans</i><br/> (291 bp) </p>   |
| 31.    | <i>B. subtilis</i><br><i>B. megaterium</i><br><i>Thiothrix</i><br><i>Nitrococcus</i> | ✓<br>?<br>?<br>? | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div> |  <p style="text-align: center;"> <i>B. subtilis</i> (298 bp)<br/> <i>B. megaterium</i> (111 bp)<br/> <i>Thiothrix</i> (244 bp)<br/> <i>Nitrococcus</i> (231 bp) </p> |


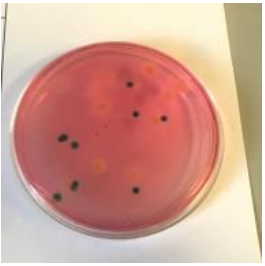
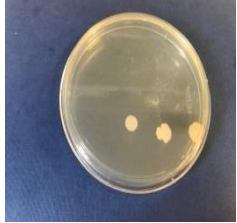
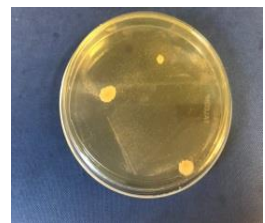
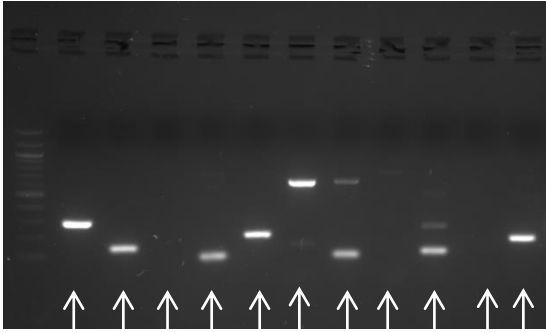


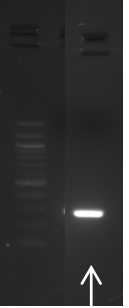
| Sample | Bacteria  | LB plate   | Gel   |
|--------|---|--|---|
| 32.    | No data   |  |   |
| 33.    | <i>B. mesentericus</i> ?<br><i>B. subtilis</i> ✓<br><i>B. licheniformis</i> ?<br><i>L. acidophilus</i> X<br><i>Nitrobacter</i> sp. ?<br><i>Nitrosomonas</i> sp. X | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div style="text-align: center;"> <p>YMA</p>  </div> <div style="text-align: center;"> <p>MRS</p>  </div> </div>       |  <p style="text-align: center;">       ↑ <i>Bacillus</i> sp. (154 bp)<br/>       ↑ <i>B. subtilis</i> (298 bp)<br/>       ↑ <i>B. licheniformis</i> (154 bp)<br/>       ↑ <i>L. acidophilus</i> (no band)<br/>       ↑ <i>Nitrobacter</i> (138 bp)<br/>       ↑ <i>Nitrosomonas</i> (no band)     </p> |
| 34.    | <i>L. acidophilus</i> X<br><i>B. subtilis</i> ✓   | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div style="text-align: center;"> <p>YMA</p>  </div> <div style="text-align: center;"> <p>MRS</p>  </div> </div> |  <p style="text-align: center;">       ↑ <i>L. acidophilus</i> (no band)<br/>       ↑ <i>Bacillus subtilis</i> (298 bp)     </p>  |

| Sample | Bacteria                                      |             | LB plate  |   | Gel   |
|--------|---|-------------|---|---|---|
| 35.    | Lactobacillus<br>B. subtilis                  | X<br>✓      | <p style="text-align: center;">LB</p>   | <p style="text-align: center;">BDA</p>   |  <p style="text-align: center;">↑<br/><i>L. acidophilus</i> (no band)</p>  |
| 36.    | B. subtilis<br>Nitrobacter sp.<br>Nitrococcus | ✓<br>?<br>? | <p style="text-align: center;">LB</p>  | <p style="text-align: center;">BDA</p>  |  <p style="text-align: center;">↑     ↑     ↑<br/><i>B. subtilis</i> (298 bp)<br/>Nitrobacter (138 bp)<br/>Nitrococcus (231 bp)</p> |

| Sample | Bacteria   |                  | LB plate   | Gel  |
|--------|--|------------------|--|--|
| 37.    | No data  |                  |  |  |
| 38.    | <i>B. subtilis</i><br><i>Saccharomyces cerevisiae</i>                                | ✓                | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div style="text-align: center;"> <p>YMA</p>  </div> <div style="text-align: center;"> <p>MRS</p>  </div> </div> |  <p style="text-align: center;">B. subtilis (298 bp)</p>  |
| 39.    | No data  |                  |  |  |
| 40.    | <i>B. subtilis</i><br><i>B. megaterium</i><br><i>Thiothrix</i><br><i>Nitrococcus</i> | ✓<br>?<br>?<br>? | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div>   |  <p style="text-align: center;"> <i>B. subtilis</i> (298 bp)<br/> <i>B. megaterium</i> (111 bp)<br/> <i>Thiothrix</i> (244 bp)<br/> <i>Nitrococcus</i> (231 bp) </p> |

| Sample | Bacteria                     |        | LB plate   |  | Gel  |
|--------|------------------------------|--------|--|--|--|
| 41.    | Lactobacillus<br>B. subtilis | X<br>✓ | <p data-bbox="666 164 724 199">LB</p>   | <p data-bbox="1033 164 1110 199">BDA</p>   |  <p data-bbox="1477 521 1516 678">B. subtilis (298 bp)</p>    |
| 42.    | B. subtilis                  | ✓      | <p data-bbox="685 749 743 785">LB</p>  | <p data-bbox="1033 749 1110 785">BDA</p>  |  <p data-bbox="1497 1106 1535 1263">B. subtilis (298 bp)</p> |
| 43.    | No data                      |        |  |  |  |

| Sample | Bacteria   |                            | LB plate  | Gel  |
|--------|--|----------------------------|---|--|
| 44.    | No data  |                            |   |  |
| 45.    | No data  |                            |   |  |
| 46.    | B. subtilis<br>Nitrobacter<br>Nitrococcus  | ✓<br>?<br>?                | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div> |   |
| 47.    | No data  |                            |   |  |
| 48.    | No data  |                            |   |  |
| 49.    | B. subtilis<br>B. polymyxa<br>B. liceniformis<br>Nitrosomonas<br>Nitrobacter<br>P. denitrificans | ✓<br>?<br>?<br>X<br>?<br>? | <div style="text-align: center;"> <p>LB</p>  </div>  |  |

| Sample | Bacteria   |  | LB plate  | Gel  |
|--------|--|--|---|--|
| 50.    | <i>B. subtilis</i><br><i>B. licheniformis</i><br><i>B. polymyxa</i><br><i>B. megaterium</i><br><i>B. pumilus</i> ,<br><i>P. putida</i><br><i>P. denitrificans</i><br><i>Nitrosomonas</i><br><i>Nitrobacter</i><br><i>L. lactis</i><br><i>L. helveticus</i> | ✓<br>?<br>?<br>?<br>?<br>?<br>?<br>?<br>X<br>?<br>?<br>? | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div style="text-align: center;"> <p>YMA</p>  </div> <div style="text-align: center;"> <p>MRS</p>  </div> </div> |  <p style="text-align: center;"> <i>B. subtilis</i> (298 bp)<br/> <i>B. licheniformis</i> (154 bp)<br/> <i>B. polymyxa</i> (219 bp)<br/> <i>B. megaterium</i> (111 bp)<br/> <i>B. pumilus</i> (227 bp)<br/> <i>P. putida</i> (170 bp) – no band<br/> <i>P. denitrificans</i> (118 bp)<br/> <i>Nitrosomonas</i> (no band)<br/> <i>Nitrobacter</i> (138 bp)<br/> <i>L. lactis</i> (no band - 108 bp)<br/> <i>L. helveticus</i> (118bp) </p> |
| 51.    | <i>B. subtilis</i>   | ✓  | <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>LB</p>  </div> <div style="text-align: center;"> <p>BDA</p>  </div> </div>  |  <p style="text-align: center;"><i>B. subtilis</i> (298 bp)</p>  |
| 52.    | No data  |  |   |  |



# Summary

We were able to identify *B. subtilis* and *Saccharomyces cerevisiae* using species-specific primers.

Cross-reaction of primers – Either the primers made were not specific or there has been contamination in the sampling procedure or during the production of these PHPs.

New species-specific primers to be tested.