The bacteria obtaining of bacillus luciferensis

Science, Biology

After culturing cells, there are multiple colonies in the 1x102X culture plate. There are not only bacterial colonies but also fungal growth in the plate. A colony was carefully chosen and picked up to be cultured in nutrient broth for further use.

From the results of the gram staining, the colour of the cells indicate gram-negative cells. However, from reports of Bacillus luciferensis, it is typically a gram-positive bacteria. It is deduced that B. luciferensis is a gram-variable microorganism that becomes gram-negative after 24 hours of culture at 30°C and above. Since it was cultured for more than 24 hours, gram-negative morphology can be explained.

After DNA purification, the sample was faintly seen on the gel. Presence of genetic material is typically indicated with a bright band. However, in this gel it is faint. This shows that there is little genetic material in the sample after purification. This can be explained by insufficient genetic material during gel electrophoresis. During the mixing of loading dye and genetic material, the mixture was left out too long and was stuck to the wall of the pipette tip leaving insufficient mixture to go into the gel, hence had to be re-done. The band might not be as bright due to too much loading dye in comparison to DNA. The DNA purification could have been done improperly, hence showing only a faint band on the gel. Genetic material taken for gel electrophoresis might not be the accurate amount, hence showing a faint band on the gel.

When making the master mix for PCR reaction, 9 sets were run for the bench for 7 samples and 1 set was for the negative control to be run. One extra set was also run so as to eliminate the carry-over effect whereby liquid is lost

when stuck to the sides of the eppendorf tube. The negative control is inclusive of everything in the master mix except for DNA, which was replaced with distilled water. This was run on the gel to ensure that there was no contamination for any components in the master mix. Should the band light up for the negative control, the master mix is contaminated. PCR was carried out to amplify the genetic material to send for sequencing. 25μl of PCR product was mixed with 5μl of loading dye, and 28μl of the mixture was used for gel electrophoresis in order to prevent a carry-over effect from occuring as much as possible. The rest of the DNA that was not used in the PCR reaction was stored at 4°C. It can be stored for safety purposes, in the case that the PCR reaction does not work as well as intended.

One of two samples that was initially cultured, purified and has undergone PCR has not shown a band in the second gel electrophoresis that was carried out after PCR. However, there was a band in the gel electrophoresis after DNA purification. This is an indication of the presence of DNA. However, there is absence of genetic material after PCR is performed. This can be explained by damaged, poor quality template, or impure DNA. There could also have been a contamination while doing the first gel, which shows the presence of DNA in the gel. However, this may not have been the DNA of the bacteria that was initially cultured. Since the genetic material was found to be an rRNA, it could have been degraded after purification and when being stored. This could have resulted in degraded rRNA running for PCR. Hence possibly explaining why there was no band in the second gel electrophoresis after PCR was performed.

There were two gels run, once after DNA purification and another after having run PCR. A gel electrophoresis was run after DNA purification to determine the presence of DNA before proceeding with PCR. The second gel electrophoresis was carried out to confirm the presence of DNA in the PCR product before proceeding with further DNA purification after the excision of the gel containing the band.

After sending the purified product for sequencing, a computer program, FinchTV, was used to view the chromatography of the sequence. On FinchTV, the first few nucleotides and the last few nucleotides were removed due to weak peaks. This was done to ensure increased accuracy of the nucleotide sequence, as the first few nucleotides were not as accurate as those in subsequent nucleotides. The first 25 and last 2 nucleotides were removed, leaving a 229 nucleotide long sequence to be cross-matched on NCBI Blast to discover that the bacteria obtained was Bacillus luciferensis.