B.

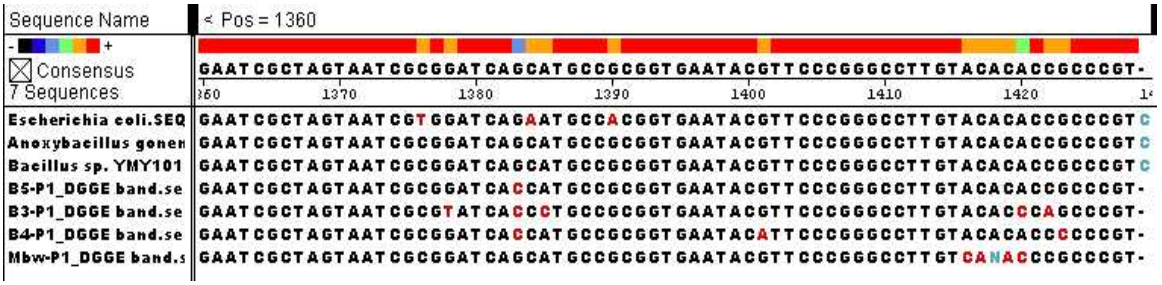

Fig 2. Comparison of nucleotide differences in DGGE band sequences (designated by band number (sequence type)) with sequences of their closest phylogenetic relatives. (A) upstream sequences, (B) downstream sequences

According to BLAST study, these DGGE bands and also one of single colony culture that growth at ½ LB medium (Mbw) are closely related to *Bacillus* group. B4 and B5 have a high similarity sequence with *Anoxybacillus sp* and Mbw similar with *Geobacillus lituanicus.* Figure 2 displays differences among bacterial sequence types. All DGGE bands showed a deletion of a nucleotide at position 1121, comparing their relatives and E coli that have nucleotide A at this position. The nucleotide sequence of B4 and B5 are quite similar, there are only four nucleotides that different. Both of them are bands that excised from BR-B culture and their positions are much closed. Probably means that these bands represent the same species but different strain. Besides these, B4 and B5 sequences showed another two deletion at position 1155 and 1156 comparing with B3 and Mbw. B3 and Mbw are species that growth at BR-A medium, but their nucleotide sequence is quite different (almost 10%). Mbw has a close relation with Geobacillus sp., while B3 perhaps represent a novel species of Bacillus.

All of DGGE bands from this study are in place in the near branches of *Bacillus* group. This evident could be explained from the culture medium that used. The ½ LB medium that used is generally a suitable medium for *Bacillus sp*. From this point, it could be suggest using more varied medium in order to explore more species. Especially there is evidence that the Pancuran 7 spring is rich with sulfuric sediment, proposed that sulfuric bacteria are present.

CONCLUSIONS

Microbial communities study of Baturraden hotspring has been performed using PCR-DGGE analysis of 16S rRNA gene fragments. Some bands have been explore both from culture-dependent and uncultured-independent method. Unfortunately, the bands from uncultured procedure that represent the predominant bacteria that live in this spring could not be analyzed. The phylogenetic study of three DGGE bands from culture-dependent procedure and single colony culture revealed that these bacteria belongs to *Bacillus sp*.

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