



Fig 1. The Pancuran 7 hot spring (A). The filtrate of Pancuran 7 hot spring water that pass through 0,2 µm Millipore filter. The brown-yellow color was sulfur sediment (B)

We used BR-A and BR-B medium, that contain a half recipe of LB medium and NB medium respectively, and the need of trace element will be fulfilled by addition of the spring water in this medium. The using of these medium assumed that there will a lot enough kinds of microorganism that growth in.

The cells from cultivation and filtration were lysed to extract their chromosomal DNA as a template in amplification of 16S rRNA gene. Theoretically, the primer pair of P1 and P2 should amplify a 323-bp fragment of 16S rRNA genes. The PCR products of BR-F, BR-A, and BR-B samples appear align with the third band of marker pUC/*Hinf*I (396 bp), proofing that these primers has been succeed to amplify those fragments.

DGGE profiles of the bacteria community of Pancuran 7 hot spring from filtration and cultivation have been obtained. The illustration of DGGE patterns using 30-80% denaturant gradient showed only four distinct bands from all of the wells. However, more distinct bands emerged at the DGGE patterns when giving 35-70% denaturant gradient to the gel. There are five distinct bands at BR-B well, three distinct bands at BR-A well, and two bands at BR-F well. From all of the lines, at least two bands are align and presumable represent the same species and predominant at the natural habitat. Another two bands are seemed as different kinds of species.

Five bands have been chosen to be excised, re-amplified, and sequenced (B1, B2, B3, B4, B5). These bands were chosen according to assumption that the align bands could be represent the same bacteria. From all of these five bands, only three bands (B3, B4, and B5) that could be re-amplified. According to this reason, the sequencing procedure and phylogenetic analysis only performed to these bands.

A.

Sequence Name	< Pos = 1121
-	- ACGAGCGCA- CCCTCGACCTTAGTTGCCAGCA- GTTCAGTTGGGCACTCTAAGGTGACTGCCGGCTAAA
Consensus	0 1130 1140 1150 1160 1170 1180 119
Escherichia coli.SEQ	AACGAGCGCAACCCCTTATCCTTTGTTGCCAGCG- GTCCGGCCGGGAACCTCAAAGGAGACTGCCAGTGATA
Anoxybaeillus gener	AACGAGCGCAACCCCTCGACCTTAGTTGCCAGCA- TT CAGTTGGGCACTCTAAGGTGACTGCCGGCTAAA
Bacillus sp. YMY101	AACGAGCGCAACCCCTCGACCTTAGTTGCTAGCGAGTCAAGTCCGGCACTCTAAGGTGACTGCCGGCTAAA
B5-P1_DGGE band.se	- ACGAGCGCA- CCCTCGACCTTAGTTGCCAGCA- - TTCAGTTGGGCACTCTAAGGTGACTGCCGGCTAAA
B3-P1_DGGE band.se	- ACGAGCGCA- CCCTGACCTTAGTTGCCAGCAAGTCAAGTCCGGCACTCTAAGGTGACTGCCGGCTAAA
B4-P1_DGGE band.se	- ACGAGCGCA- CCCTCGACCTTAGTTGCCAGCA- - TTCAGTTGGGCACTCTAAGGTGACTGCCGGCTAAA
Mbw-P1_DGGE band.s	- ACGAGCGCA- CCCTCGCCTCTAGTTGCCAGCAG- - AAGGTGGGCACTCTAGAGGACTGCCGGCGACA