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### ◆ 주요경력

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사무차장 (General Secretary)  
1999. 01. - 현 재 "Journal of Microbiology and Biotechnology", Editor)  
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# Genome Analysis and Comparative Genomics of a Commensal Thermophile, *Symbiobacterium toebii*

Biocatalysis Research Lab., KRIBB, Korea  
Bioleaders Corp. ([www.bioleaders.co.kr](http://www.bioleaders.co.kr))



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# Isolation and Characterization of *Symbiobacterium toebii*

*Symbiobacterium toebii*

*Geobacillus toebii*



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## Isolation of *Symbiobacterium* from Korean compost

### Detection of enzyme activity

**Tyrosine Phenol-Lyase (TPL) activity**

$$\text{Tyrosine} + \text{H}_2\text{O} \xrightleftharpoons{\text{TPL}} \text{Phenol} + \text{Pyruvate} + \text{NH}_4^+$$

**Tryptophan Indole-Lyase (TNA) activity**

$$\text{Tryptophan} + \text{H}_2\text{O} \xrightleftharpoons{\text{TNA}} \text{Indole} + \text{Pyruvate} + \text{NH}_4^+$$

**Composition of BM broth (per liter)**


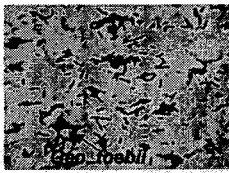
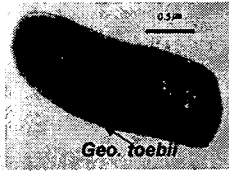
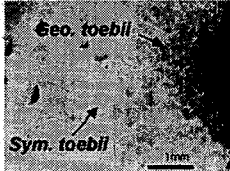
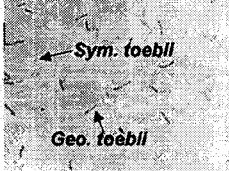
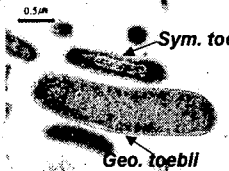
5g, polypeptone; 1g, yeast extract; 6g  $\text{K}_2\text{HPO}_4$ ; 2g,  $\text{KH}_2\text{PO}_4$ ; 0.5g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .  
Substrate : 0.5g, L-tyrosine

### Isolation Scheme

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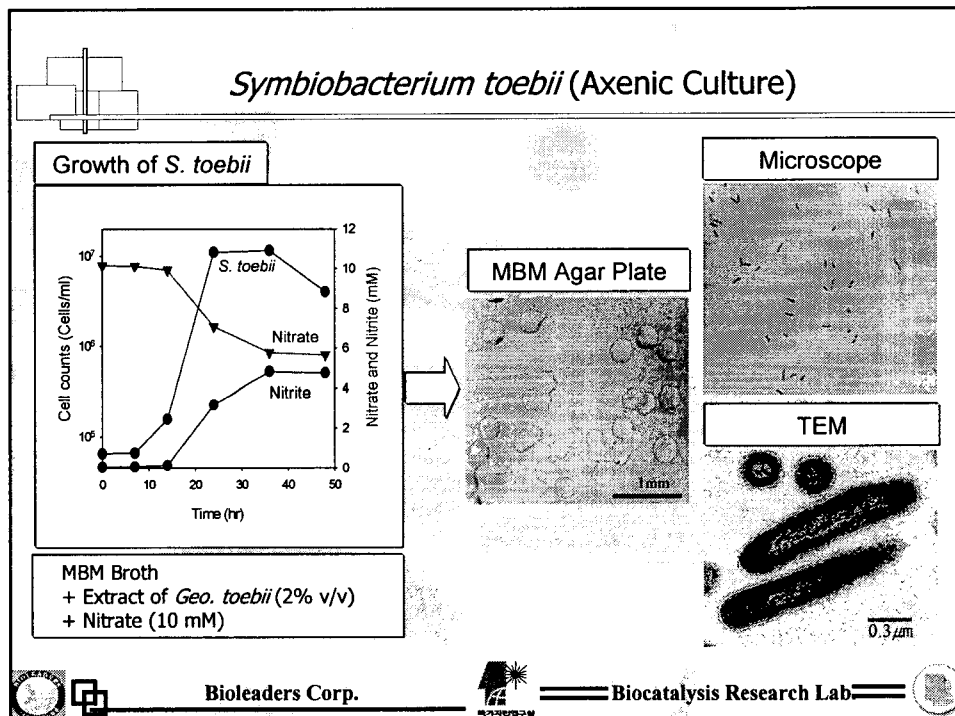
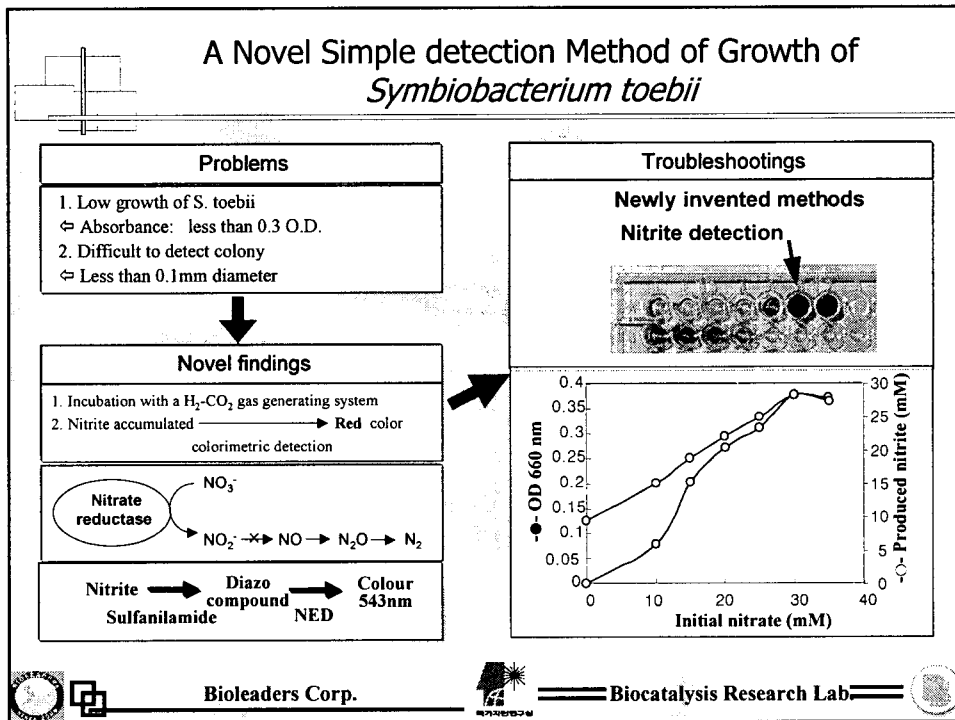
## Commensal thermophile *Symbiobacterium toebii* & Commensal host *Geobacillus toebii*

	MBM Agar Plate	Microscope	Electroscope (TEM)
Axenic Culture of Commensal host			
Co-culture of two commensal microorganisms			

*Sym. toebii* : *Symbiobacterium toebii*, *Geo. toebii*: *Geobacillus toebii*

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## Characteristics of *Symbiobacterium toebii*

- Anoxic, Micro-colony (0.3 mm) forming bacterium
- Cell growth ; Less than OD 0.3 at 600 nm
- Gram staining ; Negative
- Nonmotile without flagella
- Optimum growth temperature ; 60°C
- Optimum growth pH ; 7.4
- Quinone ; Menaquinone-6 (MK-6), MK-7
- G+C content ; 68.77 mol %
- The fatty acid profile ; 39% iso-C<sub>15:0</sub>, 28% iso-C<sub>17:0</sub>, 10% iso-C<sub>16:0</sub>,  
7% C<sub>16:0</sub>, 7% anteiso-C<sub>17:0</sub>, 2% anteiso-C<sub>15:0</sub>, 2% C<sub>18:0</sub>

### References

*Extremophiles* (2000) 4: 131-136

*Extremophiles* (2002) 6: 57-64



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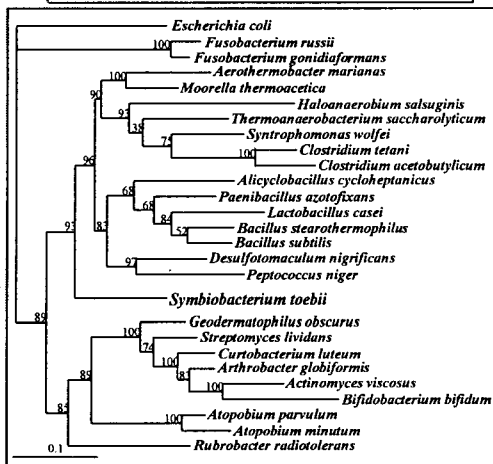


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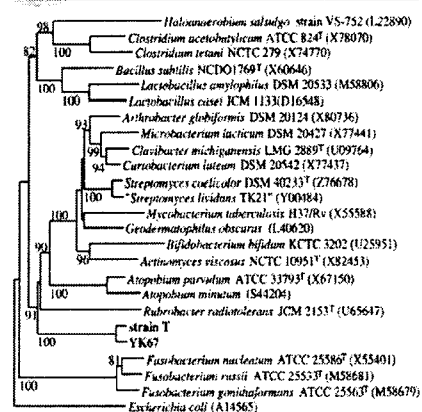


## Phylogenetic Tree for *S. toebii* and Related Bacteria by Using 16S rDNA Sequence

### *S. toebii* (KRIBB, Korea)



### *S. thermophilum* (Beppu's Lab., Japan)



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## Similarity Matrix for 16S rDNA of *S. toebii* and Related Bacteria

Strains	1	2	3	4	5	6	7	8	9	
<i>S. toebii</i>	1									
<i>S. thermophilum</i>	2	98.6								
<i>G. toebii</i>	3	85.4	84.1							
<i>G. thermoglucosidasius</i>	4	85.1	84.5	98.4						
<i>G. stearothermophilus</i>	5	85.5	84.4	96.2	96.3					
<i>B. halodurans</i>	6	83.9	82.8	92.4	92.6	91.6				
<i>B. subtilis</i>	7	83.3	82.4	92.4	92.0	91.3	94.2			
<i>D. radiodurans</i>	8	77.8	77.9	78.7	79.7	78.7	79.0	78.9		
<i>T. thermophilus</i>	9	79.3	79.2	81.2	81.2	81.1	79.0	79.0	80.9	
<i>E. coli</i>	10	77.5	77.6	78.2	79.3	78.5	78.2	78.7	77.0	76.6



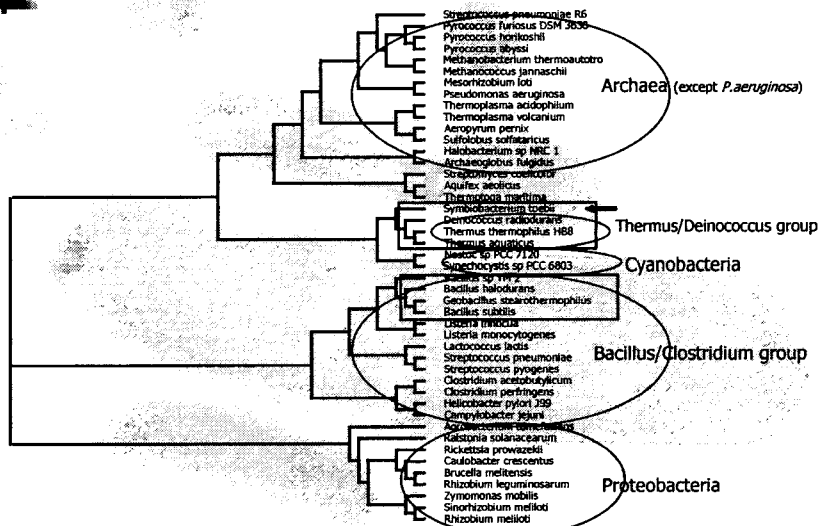
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## Phylogenetic Tree for *S. toebii* and Related Bacteria Deduced from Aspartate Aminotransferase Sequence



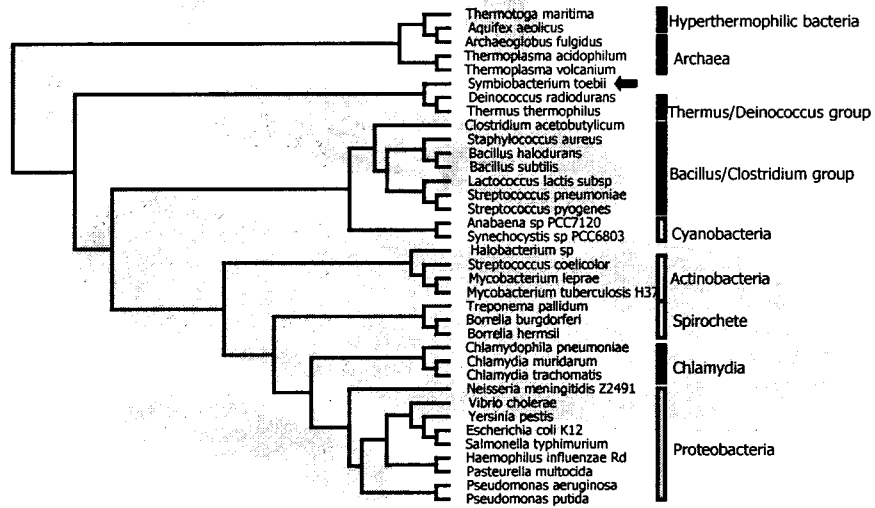
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## Phylogenetic Tree for *S. toebii* and Related Bacteria Deduced from Gyrase B Amino Acid Sequence



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## Genome Analysis of *Symbiobacterium toebii*



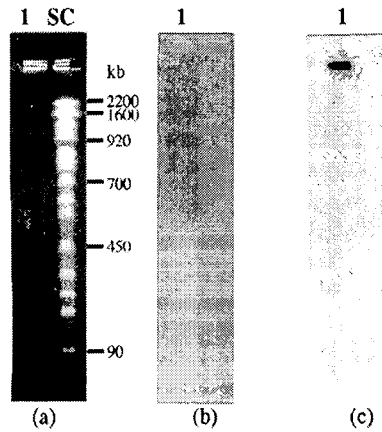
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## Purification of Pure Chromosomal DNA of *Symbiobacterium toebii*



### Undigested DNA from a pure culture of *S. toebii* separated by PFGE.

- (a) Ethidium bromide-stained gel, containing undigested *S. toebii* DNA (lane 1) and size standard *S. cerevisiae* chromosomes (lane 2). Electrophoretic condition of 200 V, 25–75s switch rate, and a 23h run time were used.
- (b) Autoradiograph of a Southern blot hybridization of this gel probed with <sup>32</sup>P-labelled D-amino acid aminotransferase gene from *G. toebii*.
- (c) The same blot, stripped and then probed with <sup>32</sup>P-labelled tyrosine phenol-lyase gene from *S. toebii*.



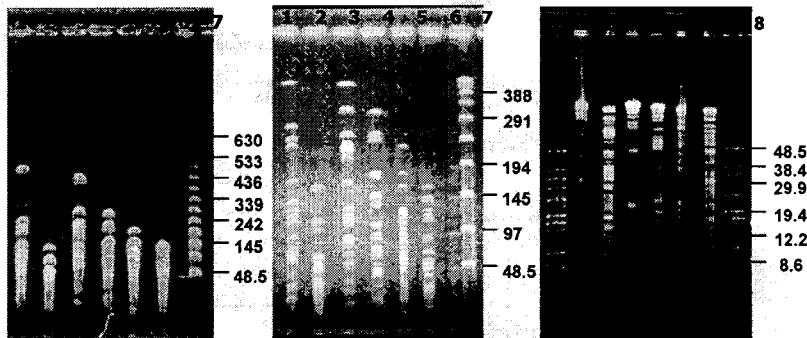
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## Genome Analysis of *Symbiobacterium toebii* by Pulsed-Field Gel Electrophoresis



*SspI* (lane 1), *EcoRI* (lane 2), *SpeI* (lane 3), *XbaI* (lane 4), *HpaI* (lane 5), and *HindIII* (lane 8)



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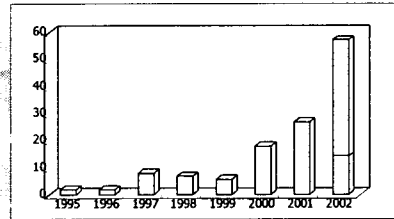




# The Trends of Genome Analysis

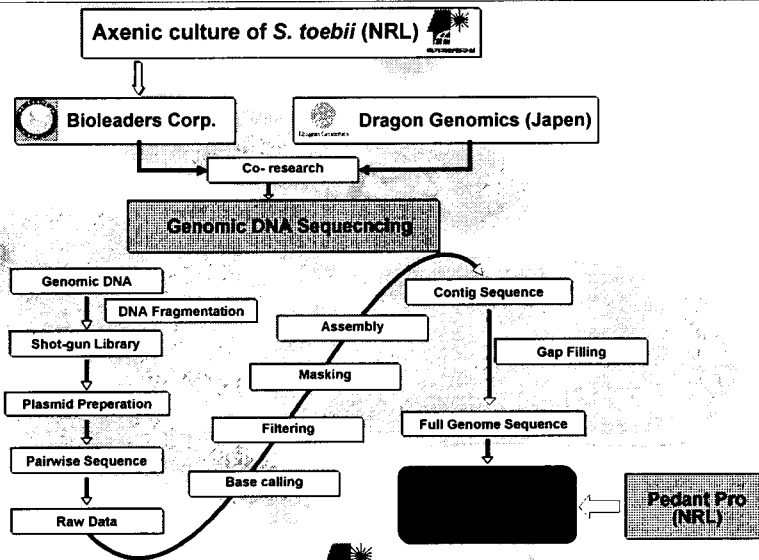
(06-01-2002)

Year	Number	Complete genome				
		Pathogen	Industrial microorganism	Eukaryotes	Extremophile	Native microorganism
1995	2	•				
1996	2	•				
1997	8	•	•	•		
1998	7	•	•	•	•	
1999	6	•	•		•	
2000	18	•	•	•	•	•
2001	27	•	•		•	•
2002	15(42)	•	•	•	•	•



**Complete Genome**  
 Eukaryotes: 5  
 Bacteria: 63  
 Archaea: 17

# Strategy for Genome Analysis of *Symbiobacterium toebii*



## The Results of Genome Sequencing of *Symbiobacterium toebii*

### General features

Genome size	3.47Mb
Redundancy	7.3X
Total no. of reads	53,176 clone
G+C ratio	68.8%
Open Reading Frames	3,942
Extrachromosomal Elements :	None

### Sequencing Result

Island	No. of ORFs
Island 1 : 2,604,074 base	2,960
Island 2 : 217,078 base	261
Island 3 : 466,176 base	524
Island 4 : 186,721 base	197
Total : 3,474,049 base	3,942



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## Genome Features of *Symbiobacterium toebii*

### Information for chromosomal coding sequence

	ORFs
Total ORFs	<b>3,942</b>
No. of similar to known proteins	1,546
No. of conserved hypotheticals	431
No. of similar to proteins of unknown function	1,746
No. without database match	219

	Hypothetical	Annotated	Total
Strong similarity; Blastp Expect ( <input type="checkbox"/> 1.00E-20)	306	1,247	1,553
Weak similarity; Blastp Expect ( <input type="checkbox"/> 1.00E-10)	125	299	424
Sum	431	1,546	1,977
Unknown	No hit (219)		1,965



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# Comparative Genomics of *Symbiobacterium toebii*



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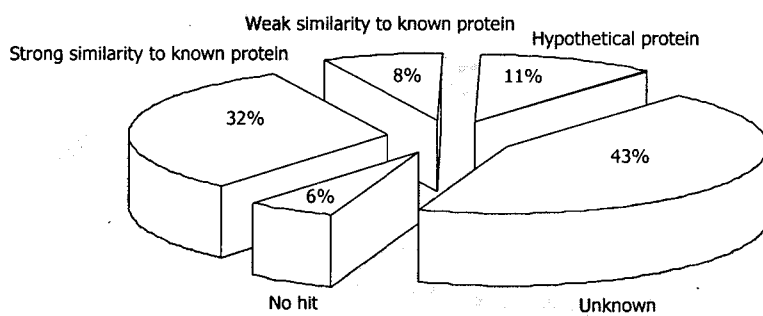
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## General Feature of Predicted ORFs in Genome of *Symbiobacterium toebii*

(SwissPlot Blastp)

### *Symbiobacterium toebii*



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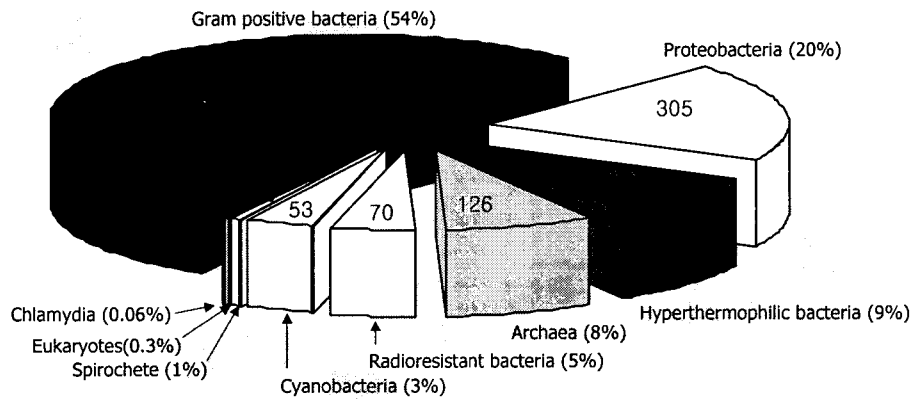


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## Classification of Annotated ORFs in Genome of *Symbiobacterium toebii*

(1,553 ORFs □ 1.00E-20)



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## The Result of COG Database Search

Strain Name	No. of ORFs		Percentage	
	Total ORFs	In COGs	No hit	Hit
<i>Symbiobacterium toebii</i>	3,942	1,786	55%	45%
<i>Bacillus halodurans</i>	4,066	2,878	29%	71%
<i>Bacillus subtilis</i>	4,118	2,870	30%	70%
<i>Deinococcus radiodurans</i>	3,187	2,226	30%	70%
<i>Mycobacterium leprae</i>	1,605	1,134	29%	71%
<i>Mycobacterium tuberculosis</i>	3,927	2,585	34%	66%
<i>Escherichia coli K12</i>	4,275	3,414	20%	80%
<i>Escherichia coli O-157</i>	5,315	3,662	31%	69%
<i>Pseudomonas aeruginosa</i>	5,567	4,392	21%	79%
<i>Archaeoglobus fulgidus</i>	2,420	1,872	23%	77%
<i>Aquifex aeolicus</i>	1,560	1,329	15%	85%
<i>Aeropyrum pernix</i>	1,841	1,178	36%	64%
<i>Methanococcus jannaschii</i>	1,786	1,330	26%	74%
<i>Thermoplasma acidophilum</i>	1,482	1,230	17%	83%
<i>Xylella fastidiosa</i>	2,831	1,589	44%	56%
<i>Streptomyces coelicolor</i>	7,825	N.D	-	-



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## Conclusions

- Axenic culture of *S. toebii* was successfully carried out by addition of cell free extract of *G. toebii*.
- During the anoxic culture of *S. toebii*, the growth was easily monitored by newly developed method, the measurement of nitrite.
- The genome consisted of 3,474,049 base pairs and had 3,942 ORFs.
- The average G+C content of the *S. toebii* genome were 68.77%.
- The total predicted ORFs were compared against a non-redundant protein database and their biological roles were assigned.
- The 1,546 (40 %) predicted coding sequences matched known protein and 2,396 (60%) had no database match.
- Comparisons with bacteria- and archaea-specific database reveal that 847 ORFs (54 %) of the annotated gene products are strongly similar to amino acid sequence of Gram-positive bacteria.



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## Biocatalysis Research Laboratory (NRL)

### Investigation of Bioconversion



Dr. J.J. Song



M.H. Lee

### Novel Biocatalyst Investigation



Dr. J.W. Bae



J.J. Kim

### NRL



Dr. Moon-Hee Sung

### Biocatalyst Engineering



Dr. T.G. Oh



Dr. S.C. Lee

### Microbial Genome Analysis



Dr. K. Kim



J.S. Ha (M.S.)



G.T. Kang

### Detached Abroad



Dr. S.G. Lee  
USA



M.S. Kwak  
Japan



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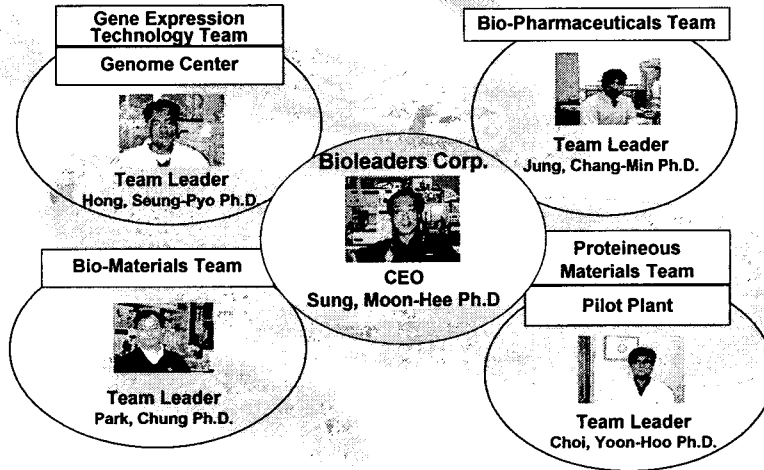


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