6. Molecular taxonomy of a new isolated macrofungus designated as KU-Alk4

Using morphological characteristics of the new isolated strain designated as KU-Alk4 identified the mushroom up to Genus *Ganoderma*. The morphology was not clearly enough to identify to species by following the Systematic Mycology. Therefore, molecular taxonomy using ITS4 region of the 28S ribosomal DNA (Figure 54) was used in addition to the morphological approach followed by the construction of phylogenetic tree.

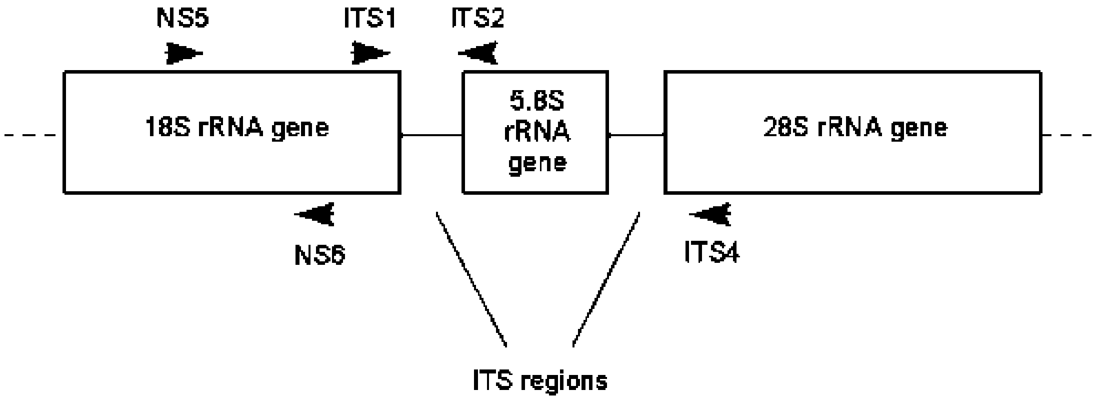

Figure 54 Structure of the rRNA gene cluster and positions of fungal PCR primers. The cluster is split into coding (18S, 5.8S and 28S genes) and non-coding (Internally Transcribed Spacer or ITS) regions. The positions of the PCR primers and their direction of synthesis are indicated by arrows.

6.1 Comparison of ITS4 region

The sequence of ITS4 region (440 nucleotides) of *Ganoderma* sp. KU-Alk4 was determined. The sequence was aligned and assembled using CLASTAL X1.8. Comparative taxonomy of the ITS4 sequence of *Ganoderma* sp. KU-Alk4 was performed by comparison to sequence in the GenBank database using BLAST N program from the NCBI website. The ITS4 of *Ganoderma* sp. KU-Alk4 was the closest similar to 18S rDNA of *Ganoderma philippii* as shown in Figure 55. Comparative taxonomy of the 2 species showed only 93% identity (414 out of 443 bases) (Figure 56). Characteristics of fruiting body of *Ganoderma* sp. KU-Alk4 and *Ganoderma philippii* were compared in Figure 57. It showed that fruiting body of *Ganoderma* sp. KU-Alk4 was not similar to that of *Ganoderma philippii*.

6.2 Comparison of 18S rDNA

The sequence of 18S rDNA region (1881 nucleotides) of *Ganoderma* sp. KU-Alk4 was determined. The sequence was aligned and assembled using CLASTAL X1.8. Comparative taxonomy of the 18S rDNA sequence of *Ganoderma* sp. KU-Alk4 was performed by comparison to sequence in the GenBank database using BLAST N program from the NCBI website. Comparative taxonomy showed that *Ganoderma* sp. KU-Alk4 was the most closely to 18S rDNA of *Coriolopsis byrsina* strain CRM-46, *Hexagonia hirta* strain CBS 515.6 and *Ganoderma australe* by 99% and 98% closely similar to *Trametes versicolor* BCRC 36387 (Figure 58). Figure 59 showed the comparison of only 1769 base out of 1881 analyzed bases of 18S rDNA of *Ganoderma* sp. KU-Alk4 and that of *Coriolopsis byrsina*, 1755 bases were identical, resulted 99% similarity. The 18S rDNA of *Ganoderma* sp. KU-Alk4 was compared to that of *G. australe* as shown in Figure 60. The results showed 99% identity (1753 out of 1769 bases).

```
BLASTN 2.2.6 [Apr-09-2007]


Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs", Nucleic Acids Res. 25:3389-3402.


Query= KU-Alk4-ITS4
         (586 letters)


Database: ddbjpln1.seq; ddbjpln2.seq; ddbjpln3.seq; ddbjpln4.seq;
ddbjpln5.seq; ddbjpln6.seq; ddbjpln7.seq; ddbjpln8.seq; ddbjpln9.seq;
/db/DDBJNEW.DATA/b/new_ddbjpln.seq; ddbjuna.seq;
/db/DDBJNEW.DATA/b/new_ddbjuna.seq; ddbjgss1.seq; ddbjgss10.seq;
ddbjgss11.seq; ddbjgss12.seq; ddbjgss13.seq; ddbjgss14.seq;


Searching..................................................done


                                                                 Score    E
                                                                 
                                                                 
Sequences producing significant alignments:                      (bits) Value


AJ627584|AJ627584.1 Ganoderma philippii 18S rRNA gene (partial),...   632   e-178
AF255097|AF255097.1 Ganoderma sp. JM97/52 internal transcribed s...   632   e-178
BD082760|BD082760.1 Method for detecting a disease germ of oil p...   626   e-176
AF255096|AF255096.1 Ganoderma sp. DL008 internal transcribed spa...   624   e-175
AF255095|AF255095.1 Ganoderma sp. JM97/2 internal transcribed sp...   624   e-175
AF255094|AF255094.1 Ganoderma sp. JM97/3 internal transcribed sp...   624   e-175
AF255093|AF255093.1 Ganoderma sp. CBS187.31 internal transcribed...   624   e-175
AY456341|AY456341.1 Ganoderma lucidum NC-8349 18S ribosomal RNA ...   611   e-171
AF255092|AF255092.1 Ganoderma sp. GYONGYI-0150 internal transcri...   611   e-171
AF255183|AF255183.1 Ganoderma sp. PKB92/040 internal transcribed...   585   e-163
AY089739|AY089739.1 Perenniporia subacida isolate HHb-14877-T in...   579   e-162
AF255149|AF255149.1 Ganoderma sp. JM98/2 internal transcribed sp...   579   e-162
AY508882|AY508882.1 Ganoderma sp. TNAU-CRS-1 internal transcribe...   577   e-161
AF255177|AF255177.1 Ganoderma sp. BAFC2532 internal transcribed ...   577   e-161
AF255176|AF255176.1 Ganoderma sp. BAFC2531 internal transcribed ...   577   e-161
```

Figure 55 Score of ITS4 sequences producing significant alignments compare between *Ganoderma* sp. KU-Alk4 and 15 other strains that had the closest relation.

```
>AJ627584|AJ627584.1 Ganoderma philippii 18S rRNA gene (partial,
          5.8S rRNA gene, 26S  rRNA gene (partial), ITS1 and ITS2,
          isolate FRIM 589.
          Length = 656

 Score = 632 bits (319), Expect = e-178
 Identities = 414/443 (93%), Gaps = 4/443 (0%)
 Strand = Plus / Minus


Query: 1   tacctgatttgaggtcagaggtcaatgtgttgtct-cataagagacgattagaagctcgc 59
           ||||||||||||||||||||||||    | ||||| ||   |||||||||||||||||||
Sbjct: 628 tacctgatttgaggtcagaggtcataaagctgtcttcaactgagacgattagaagctcgc 569


Query: 60  caaacgcttcacggtcgcggcgtagacaattatcacaccgagagccgatccgcaaggaat 119
           |||||||||||||||||||||||||||  ||||||||||||||||||||||||||||||
Sbjct: 568 caaacgcttcacggtcgcggcgtagacr-ttatcacaccgagagccgatccgcaaggaac 510


Query: 120 caagctaatgcatttaagaggagccgaccntta--ggccgacaagcctccaagtccaagc 177
           |||||||||||||||||||||||||||||   |  |||||||||||||||||||||||||
Sbjct: 509 caagctaatgcatttaagaggagccgaccgagaayggccgacaagcctccaagtccaagc 450


Query: 178 ctacaaacccgcaaaggtttataggttgaagatttcatgacactcaaacaggcatgctcc 237
           ||||||| || |||| | || |||||||||||||||||||||||||||||||||||||||
Sbjct: 449 ctacaaaaccacaaaagcttgtaggttgaagatttcatgacactcaaacaggcatgctcc 390


Query: 238 tcggaataccaaggagcgcaaggtgcgttcaaagattcgatgattcactgaattctgcaa 297
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 389 tcggaataccaaggagcgcaaggtgcgttcaaagattcgatgattcactgaattctgcaa 330


Query: 298 ttcacattacttatcgcatttcgctgcgttcttcatcgatgcgagagccaagagatccgt 357
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 329 ttcacattacttatcgcatttcgctgcgttcttcatcgatgcgagagccaagagatccgt 270


Query: 358 tgctgaaagttgtatatagatgcgttatatcgcaatacacattcatttacttgtaagagt 417
           ||||||||||||||||||||||||||| ||||||||||||||||   |||||   |||||
Sbjct: 269 tgctgaaagttgtatatagatgcgttacatcgcaatacacattctgatactttatagagt 210


Query: 418 ttgtaataaacgcaggcacagac 440
           |||||||||||||||||||||||
Sbjct: 209 ttgtaataaacgcaggcacagac 187
```

Figure 56 Comparison of ITS4 sequences from *Ganoderma* sp. KU-Alk4 (Query) and *Ganoderma philippii* (Sbjct). BLAST analysis indicated 414 identities from 443 bases (93%), with 4 gaps.

Figure 57 Fruiting body of *Ganoderma* sp. KU-Alk4 (A) and *Ganoderma philippii* (B). Figure 57(B) from the book named “mushroom and macrofungi in Thailand” (Soytong, 1994).

```
BLASTN 2.2.15 [Oct-15-2006]


Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs",  Nucleic Acids Res. 25:3389-3402.

Query= query
         (1911 letters)

Database: /a/DNA.DATA/ddbjpln1.seq;
/a/DNA.DATA/ddbjpln2.seq;
/a/DNA.DATA/ddbjpln3.seq;
/a/DNA.DATA/ddbjpln4.seq;
/a/DNA.DATA/ddbjpln5.seq;
/a/DDBJNEW.DATA/new_ddbjpln.seq;
/a/DNA.DATA/ddbjphg.seq;
/a/DDBJNEW.DATA/new_ddbjphg.seq
           908,299 sequences; 3,034,693,674 total letters

Searching..................................................done


                                                                 Score    E
Sequences producing significant alignments:                      (bits) Value

AY336773|AY336773.1 Coriolopsis byrsina strain CRM-46 18S riboso...  3420  0.0
AY336760|AY336760.1 Hexagonia hirta strain CBS 515.96 18S riboso...  3420  0.0
AY309019|AY309019.1 Trametes versicolor strain BCRC 36387 18S ri...  3416  0.0
AY309018|AY309018.1 Trametes versicolor strain ATCC 11235 18S ri...  3416  0.0
AY309017|AY309017.1 Trametes versicolor strain BCRC 36450 18S ri...  3416  0.0
AY309015|AY309015.1 Trametes versicolor strain BCRC 36089 18S ri...  3416  0.0
AY309016|AY309016.1 Trametes versicolor strain BCRC 36093 18S ri...  3408  0.0
AY336763|AY336763.1 Ganoderma australe strain Wu 9302-56 18S rib...  3404  0.0
AY946269|AY946269.1 Lentinus tigrinus 18S ribosomal RNA gene, pa...  3398  0.0
AY336751|AY336751.1 Trametes versicolor strain Wu 9507-7 18S rib...  3396  0.0
AY336759|AY336759.1 Hexagonia hirta strain Wu 9906-35 18S riboso...  3392  0.0
AY336766|AY336766.1 Earliella scabrosa strain Wu 9704-83 18S rib...  3390  0.0
AY665780|AY665780.1 Grifola sordulenta isolate AFTOL-ID 562 18S ...  3388  0.0
AY336758|AY336758.1 Hexagonia pobeguinii strain CBS 510.96 18S r...  3388  0.0
AY705969|AY705969.1 Ganoderma tsugae isolate AFTOL-ID 771 18S ri...  3380  0.0
```

Figure 58 Score of 18S rDNA sequences producing significant alignments compare between *Ganoderma* sp. KU-Alk4 and 15 other strains that had the closest relation.

```
>AY336773|AY336773.1 Coriolopsis byrsina strain CRM-46 18S ribosomal
                     RNA gene, complete sequence.
                     Length = 1776

 Score = 3420 bits (1724), Expect = 0.0
 Identities = 1755/1769 (99%)
 Strand = Plus / Plus


Query: 97   gtagtcatatgcttgtctcaaagattaagccatgcatgtctaagtataaacaagtttgta 156
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1    gtagtcatatgcttgtctcaaagattaagccatgcatgtctaagtataaacaagtttgta 60


Query: 157  ctgtgaaactgcgaatggctcattaaatcagttatagtttatttgatggtaccttgctac 216
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 61   ctgtgaaactgcgaatggctcattaaatcagttatagtttatttgatggtaccttgctac 120


Query: 217  atggataactgtggtaattctagagctaatacatgcaatcaagccccgacttctggaagg 276
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 121  atggataactgtggtaattctagagctaatacatgcaatcaagccccgacttctggaagg 180


Query: 277  ggtgtatttattagataaaaaaccaacgcggttcgccgctccattggtgattcataataa 336
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 181  ggtgtatttattagataaaaaaccaacgcggttcgccgctccattggtgattcataataa 240


Query: 337  cttctcgaatcgcatggccttgcgccggcgatgcttcattcaaatatctgccctatcaac 396
            |||||||||||||||||||||| |||||||||||||||||||||||||||||||||||||
Sbjct: 241  cttctcgaatcgcatggccttgtgccggcgatgcttcattcaaatatctgccctatcaac 300


Query: 397  tttcgatggtaggatagaggcctaccatggtttcaacgggtaacggggaataagggttcg 456
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 301  tttcgatggtaggatagaggcctaccatggtttcaacgggtaacggggaataagggttcg 360


Query: 457  attccggagagggagcctgagaaacggctaccacatccaaggaaggcagcaggcgcgcaa 516
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 361  attccggagagggagcctgagaaacggctaccacatccaaggaaggcagcaggcgcgcaa 420


Query: 517  attacccaatcccgacacggggaggtagtgacaataaataacaatatggggctctttcgg 576
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 421  attacccaatcccgacacggggaggtagtgacaataaataacaatatggggctctttcgg 480
```

Figure 59 Comparison of 18S rDNA sequences from *Ganoderma* sp. KU-Alk4 (Query) and *Trametes versicolor* (Sbjct). BLAST analysis indicated 1773 identities from 1787 bases (99%), with 2 gaps.

```
Query: 577  gtctcataattggaatgagtacaatttaaatctcttaacgaggaacaattggagggcaag 636
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 481  gtctcataattggaatgagtacaatttaaatctcttaacgaggaacaattggagggcaag 540


Query: 637  tctggtgccagcagccgcggtaattccagctccaatagcgtatattaaagttgttgcagt 696
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 541  tctggtgccagcagccgcggtaattccagctccaatagcgtatattaaagttgttgcagt 600


Query: 697  taaaaagctcgtagttgaacttcagacctggccgggcggtctgcctaacggtatgtactg 756
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 601  taaaaagctcgtagttgaacttcagacctggccgggcggtctgcctaacggtatgtactg 660


Query: 757  tctggctgggtcttacctcttggtgagccggcatgcccttttrstgggtgtgtcggggaac 816
            ||||||||||||||||||||||||||||||||||||||||   |||||||||||||||||
Sbjct: 661  tctggctgggtcttacctcttggtgagccggcatgcccttcactgggtgtgtcggggaac 720


Query: 817  caggacttttaccttgagaaaattagagtgttcaaagcaggcctatgcccggatacatta 876
            ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct: 721  caggacttttaccttgagaaaattagagtgttcaaagcaggcctatgcccgaatacatta 780


Query: 877  gcatggaataataaaataggacgtgcggttctatttgttggtttctagagtcgccgtaa 936
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 781  gcatggaataataaaataggacgtgcggttctatttgttggtttctagagtcgccgtaa 840


Query: 937  tgattaatagggatagttgggggcattagtattcagttgctagaggcgaaattcttggat 996
            |||||||||||||||||||||||||||||||||||||||||||||| |||||||||||||
Sbjct: 841  tgattaatagggatagttgggggcattagtattcagttgctagaggtgaaattcttggat 900


Query: 997  ttactgaagactaactactgcgaaagcatttgccaaggatgttttcattaatcaagaacg 1056
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 901  ttactgaagactaactactgcgaaagcatttgccaaggatgttttcattaatcaagaacg 960


Query: 1057 aaggttaggggatcgaaaacgatcagataccgttgtagtcttaacagtaaactatgccga 1116
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 961  aaggttaggggatcgaaaacgatcagataccgttgtagtcttaacagtaaactatgccga 1020


Query: 1117 ctagggatcgggcgatctcaatcttatgtgtcgctcggcaccttacgagaaatcaaagtc 1176
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1021 ctagggatcgggcgatctcaatcttatgtgtcgctcggcaccttacgagaaatcaaagtc 1080


Query: 1177 tttgggttctgggggagtatggtcgcaaggctgaaacttaaaggaattgacggaagggc 1236
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1081 tttgggttctgggggagtatggtcgcaaggctgaaacttaaaggaattgacggaagggc 1140
```

Figure 59 (Continued)

```
Query: 1237 accaccaggagtggagcctgcggcttaatttgactcaacacggggaaactcaccaggtcc 1296
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1141 accaccaggagtggagcctgcggcttaatttgactcaacacggggaaactcaccaggtcc 1200


Query: 1297 agacatgactaggattgacagattgatagctctttcatgattttatgggtggtggtgcat 1356
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1201 agacatgactaggattgacagattgatagctctttcatgattttatgggtggtggtgcat 1260


Query: 1357 ggccgttcttagttggtggagtgatttgtctggttaattccgataacgaacgagacctta 1416
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1261 ggccgttcttagttggtggagtgatttgtctggttaattccgataacgaacgagacctta 1320


Query: 1417 acctgcttaatagccaggccggcttttgctggtcgccggcttcttagagggactgtctgc 1476
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1321 acctgcttaatagccaggccggcttttgctggtcgccggcttcttagagggactgtctgc 1380


Query: 1477 gtctagcagacggaagtttgaggcaataacaggtctgtgatgcccttagatgttctgggc 1536
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1381 gtctagcagacggaagtttgaggcaataacaggtctgtgatgcccttagatgttctgggc 1440


Query: 1537 cgcacgcgcgctacactgacagagccagcgagnnnnnnnccttggccggaaggtctgggt 1596
            ||||||||||||||||||||||||||||||||       |||||||||||||||||||||
Sbjct: 1441 cgcacgcgcgctacactgacagagccagcgagtttttttccttggccggaaggtctgggt 1500


Query: 1597 aatcttgtgaaactctgtcgtgctggggatagagcattgcaattattgctcttcaacgag 1656
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1501 aatcttgtgaaactctgtcgtgctggggatagagcattgcaattattgctcttcaacgag 1560


Query: 1657 gaattcctagtaagcgtgagtcatcagctcgcgttgattacgtccctgccctttgtacac 1716
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1561 gaattcctagtaagcgtgagtcatcagctcgcgttgattacgtccctgccctttgtacac 1620


Query: 1717 accgcccgtcgctactaccgattgaatggcttagtgaggtcttgggattggcttcgggga 1776
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1621 accgcccgtcgctactaccgattgaatggcttagtgaggtcttgggattggcttcgggga 1680


Query: 1777 gccggcaacggcaccctgtcgctgagaacttgatcaaacttggtcatttagaggaagtaa 1836
            ||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct: 1681 gccggcaacggcaccctgtcgctgaaaacttgatcaaacttggtcatttagaggaagtaa 1740


Query: 1837 aagtcgtaacaaggtttccgtaggtgaac 1865
            |||||||||||||||||||||||||||||
Sbjct: 1741 aagtcgtaacaaggtttccgtaggtgaac 1769
```

Figure 59 (Continued)

```
>AY336763|AY336763.1 Ganoderma australe strain Wu 9302-56 18S
                     ribosomal RNA gene, complete sequence.
                     Length = 1774

 Score = 3404 bits (1716), Expect = 0.0
 Identities = 1753/1769 (99%)
 Strand = Plus / Plus


Query: 97   gtagtcatatgcttgtctcaaagattaagccatgcatgtctaagtataaacaagtttgta 156
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1    gtagtcatatgcttgtctcaaagattaagccatgcatgtctaagtataaacaagtttgta 60


Query: 157  ctgtgaaactgcgaatggctcattaaatcagttatagtttatttgatggtaccttgctac 216
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 61   ctgtgaaactgcgaatggctcattaaatcagttatagtttatttgatggtaccttgctac 120


Query: 217  atggataactgtggtaattctagagctaatacatgcaatcaagccccgacttctggaagg 276
            ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct: 121  atggataactgtggtaattctagagctaatacatgcaatcaagccccgacttccggaagg 180


Query: 277  ggtgtatttattagataaaaaaccaacgcggttcgccgctccattggtgattcataataa 336
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 181  ggtgtatttattagataaaaaaccaacgcggttcgccgctccattggtgattcataataa 240


Query: 337  cttctcgaatcgcatggccttgcgccggcgatgcttcattcaaatatctgccctatcaac 396
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 241  cttctcgaatcgcatggccttgcgccggcgatgcttcattcaaatatctgccctatcaac 300


Query: 397  tttcgatggtaggatagaggcctaccatggtttcaacgggtaacggggaataagggttcg 456
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 301  tttcgatggtaggatagaggcctaccatggtttcaacgggtaacggggaataagggttcg 360


Query: 457  attccggagagggagcctgagaaacggctaccacatccaaggaaggcagcaggcgcgcaa 516
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 361  attccggagagggagcctgagaaacggctaccacatccaaggaaggcagcaggcgcgcaa 420


Query: 517  attacccaatcccgacacggggaggtagtgacaataaataacaatatggggctctttcgg 576
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 421  attacccaatcccgacacggggaggtagtgacaataaataacaatatggggctctttcgg 480
```

Figure 60 Comparison of 18S rDNA sequences from *Ganoderma* sp. KU-Alk4 (Query) and *Ganoderma australe* (Sbjct). BLAST analysis indicated 1773 identities from 1787 bases (99%), with 2 gaps.

```
Query: 577  gtctcataattggaatgagtacaatttaaatctcttaacgaggaacaattggagggcaag 636
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Figure 60 (Continued)

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Figure 60 (Continued)

6.3 Phylogenetic relationship

Multiple-sequence alignment of the nucleotide sequences of *Ganoderma* sp. KU-Alk4 ITS4 and 18S rDNA and those of other fungi was made using both the CLUSTAL X1.8 and MEGA2 programs. Then, phylogenetic trees were constructed by either the Neighbor-Joining, UPGMA or Minimum Evolution (ME) and Maximum Parsimony (MP) methods. All of the methods showed that *Ganoderma* sp. KU-Alk4 was not most closely related to any fungi, including *Ganoderma philippii*. The phylogenetic relationship of *Ganoderma* sp. KU-Alk4 ITS4 and 18S rDNA to that of other fungi is shown in Figure 61.

From morphological characteristics and molecular taxonomy of ITS4 and 18S rDNA, it was concluded that *Ganoderma* sp. KU-Alk4 would be a new fungal species.

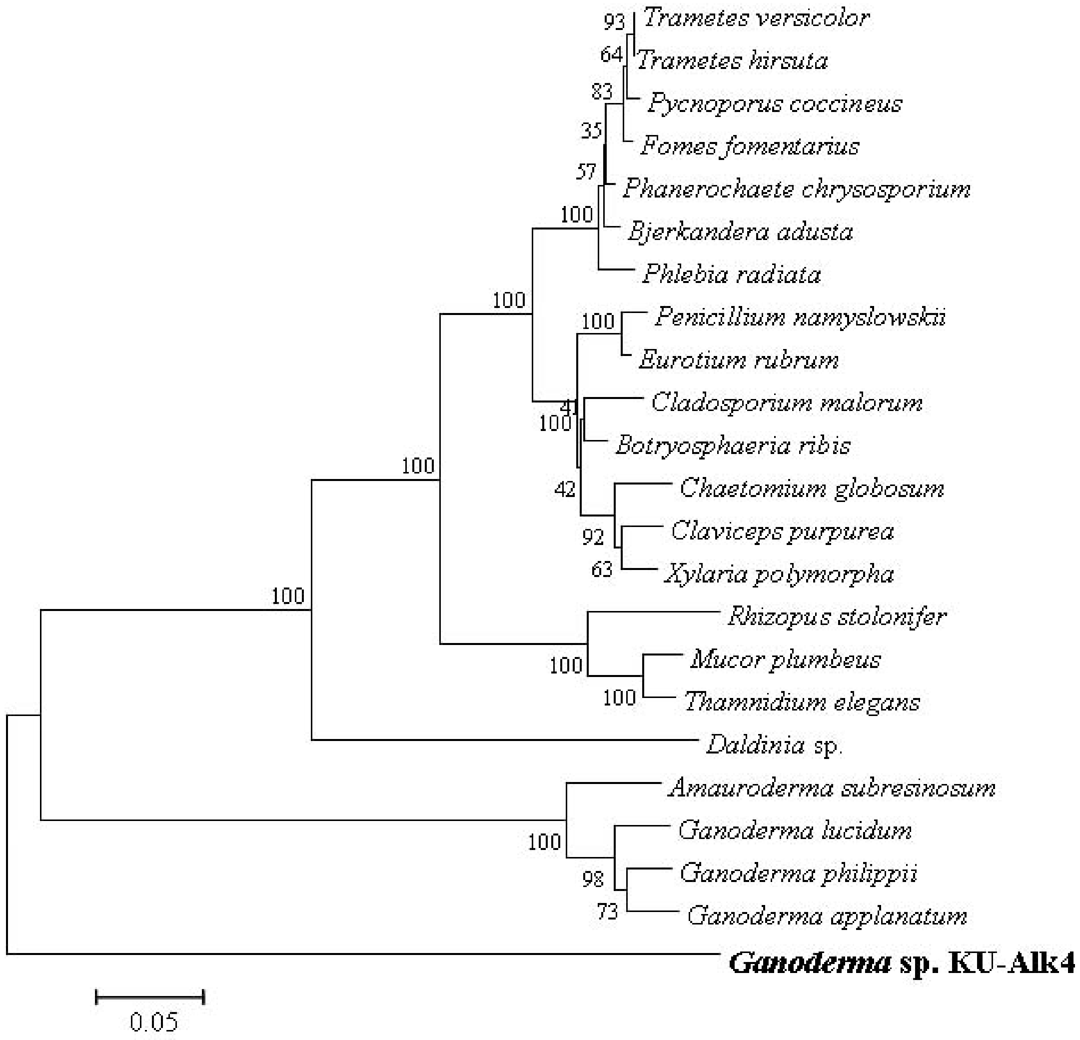

Figure 61 Phylogenetic relation of *Ganoderma* sp. KU-Alk4 and other fungi constructed by neighbor-joining method from the MEGA 2 program.