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(54) **PROBIOTIC BIFIDOBACTERIAL SPECIES**

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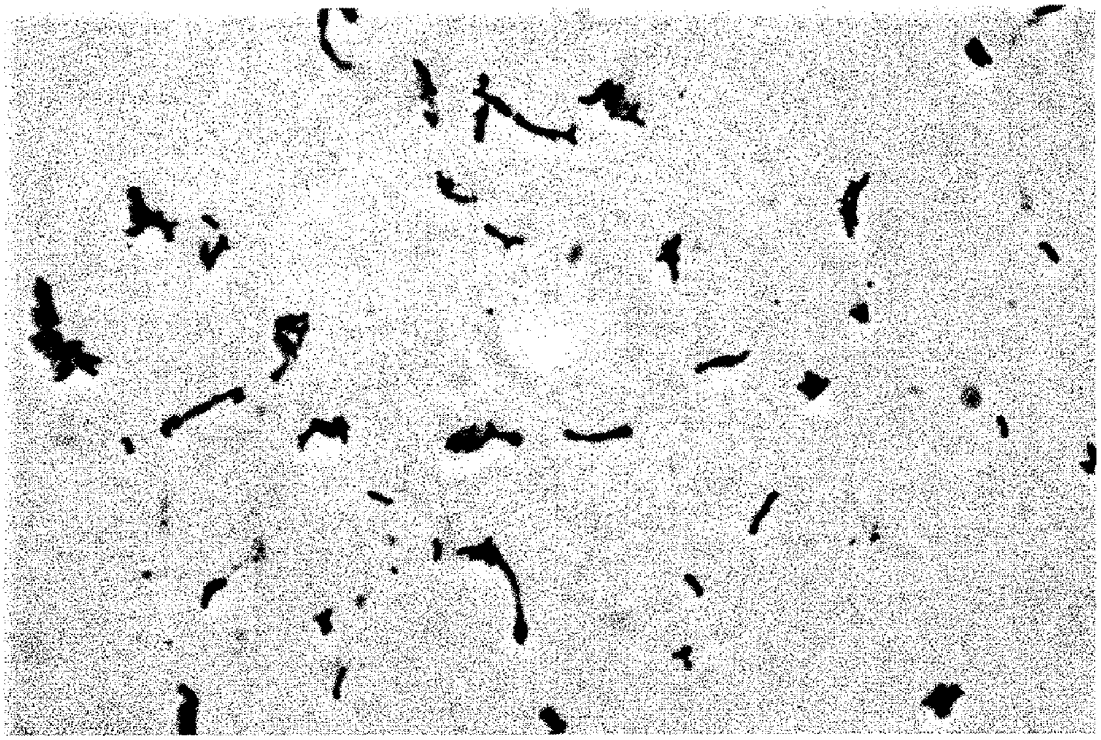
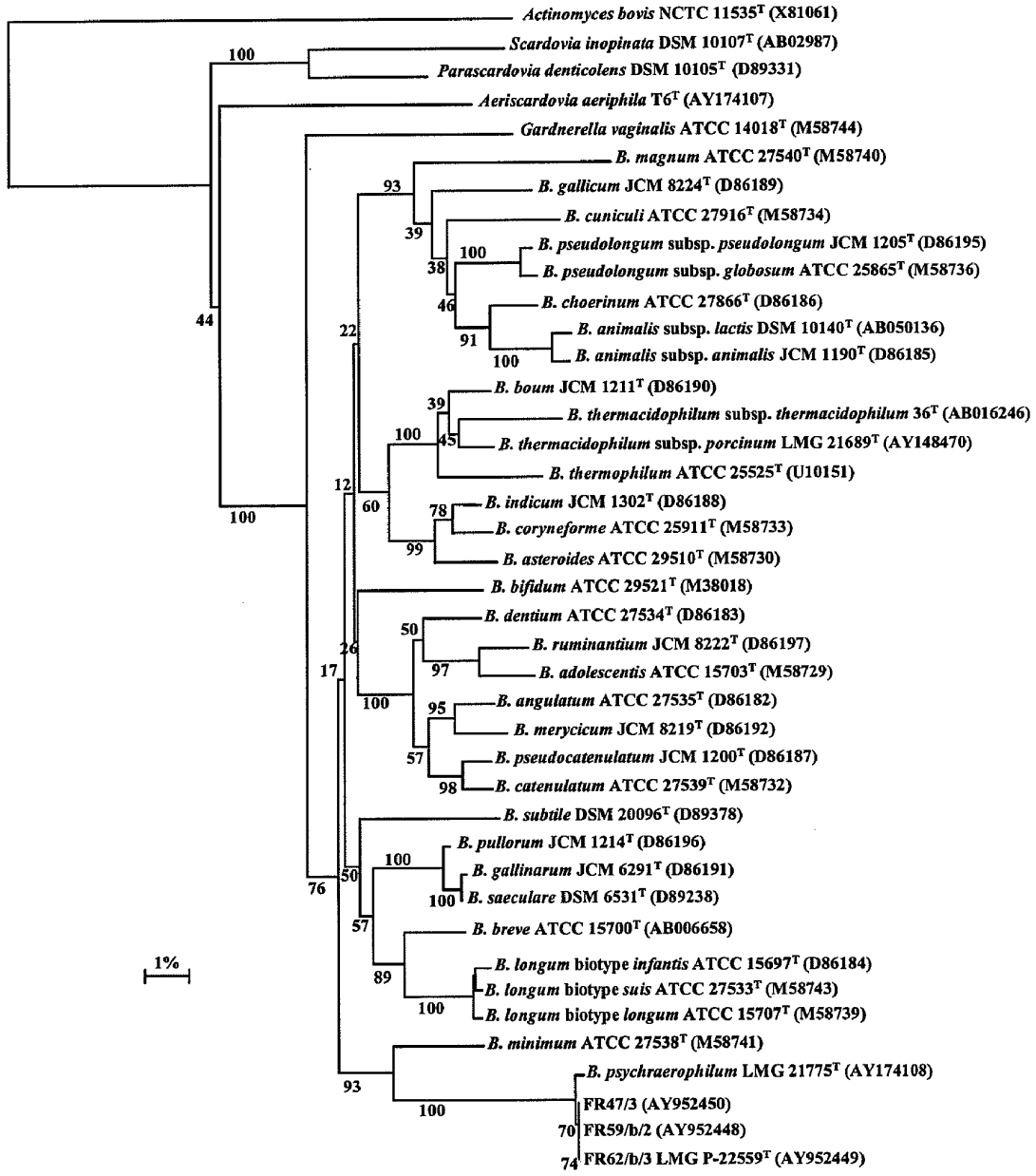
(57) **ABSTRACT**

A probiotic composition comprising a *Bifidobacterium* strain which has DNA sequence homology of greater than 40% to *Bifidobacterium* GC56, wherein *Bifidobacterium* GC56 was deposited at the Collection Nationale de Cultures de Micro-organismes (CNCM, Institut Pasteur) on 9 Dec. 2004 with accession number CNCM 1-3342.

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Scardovia inopinata DSM 10107T (AB02987)
Parascardovia denticolens DSM 10105T (D89331)
Aeriscardovia aeriphila T6T (AY174107)
Gardnerella vaginalis ATCC 14018T (M58744)
B. magnum ATCC 27540T (M58740)
B. gallicum JCM 8224T (D86189)
B. cuniculi ATCC 27916T (M58734)
B. pseudolongum subsp. pseudolongum JCM 1205T (D86195)
B. pseudolongum subsp. globosum ATCC 25865T (M58736)
B. choerinum ATCC 27866T (D86186)
B. animalis subsp. lactis DSM 10140T (AB050136)
B. animalis subsp. animalis JCM 1190T (D86185)
B. boum JCM 1211T (D86190)
B. thermacidophilum subsp. thermacidophilum 36T (AB016246)
B. thermacidophilum subsp. porcinum LMG 21689T (AY148470)
B. thermophilum ATCC 25525T (U10151)
B. indicum JCM 1302T (D86188)
B. coryneforme ATCC 25911T (M58733)
B. asteroides ATCC 29510T (M58730)
B. bifidum ATCC 29521T (M38018)
B. dentium ATCC 27534T (D86183)
B. ruminantium JCM 8222T (D86197)
B. adolescentis ATCC 15703T (M58729)
B. angulatum ATCC 27535T (D86182)
B. merycicum JCM 8219T (D86192)
B. pseudocatenulatum JCM 1200T (D86187)
B. catenulatum ATCC 27539T (M58732)
B. subtile DSM 20096T (D89378)
B. pullorum JCM 1214T (D86196)
B. gallinarum JCM 6291T (D86191)
B. saeculare DSM 6531T (D89238)
B. breve ATCC 15700T (AB006658)
B. longum biotype infantis ATCC 15697T (D86184)
B. longum biotype suis ATCC 27533T (M58743)
B. longum biotype longum ATCC 15707T (M58739)
B. minimum ATCC 27538T (M58741)
B. psychraerophilum LMG 21775T (AY174108)
FR47/3 (AY952450)
FR59/b/2 (AY952448)
FR62/b/3 LMG P-22559T (AY952449)
1%

Figure 1

```
          10         20         30         40         50         60
1 GTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATCAAGCT   60
2 ------------------------------------GTCGAACGGGATCCATCAAGCT   22
3 ----------------------------------------------ATCCATCAAGCT   12
4 ------------------------------------------AGGGATCCATCAAGCT   16
C .....................................:::::::+++ATCCATCAAGCT
          70         80         90        100        110        120
1 TGCTTGATGGTGAGAGTGGCGAACGGGTGAGTAATACGTGACTAACCTGCCTCATACACC  120
2 TGCTTGATGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACTAACCTGCCTCATACACC   82
3 TGCTTGATGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACTAACCTGCCTCATACACC   72
4 TGCTTGATGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACTAACCTGCCTCATACACC   76
C TGCTTGATGGTGAGAGTGGCGAACGGGTGAGTAAT+CGTGACTAACCTGCCTCATACACC

         130        140        150        160        170        180
1 GGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAACATTTCACATGTTTTGTT  180
2 GGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAACATTTCACATGTTTTGTT  142
3 GGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAACATTTCACATGTTTTGTT  132
4 GGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAACATTTCACATGTTTTGTT  136
C GGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAACATTTCACATGTTTTGTT

         190        200        210        220        230        240
1 GGGAAAGCGTTAGCGGTATGAGATGGGGTCGCGTCCTATCAGCTTGTTGGTGAGGTAATG  240
2 GGGAAAGCGTTAGCGGTATGAGATGGGGTCGCGTCCTATCAGCTTGTTGGTGAGGTAATG  202
3 GGGAAAGCGTTAGCGGTATGAGATGGGGTCGCGTCCTATCAGCTTGTTGGTGAGGTAATG  192
4 GGGAAAGCGTTAGCGGTATGAGATGGGGTCGCGTCCTATCAGCTTGTTGGTGAGGTAATG  196
C GGGAAAGCGTTAGCGGTATGAGATGGGGTCGCGTCCTATCAGCTTGTTGGTGAGGTAATG

         250        260        270        280        290        300
1 GCTCACCAAGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGACTGA  300
2 GCTCACCAAGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGACTGA  262
3 GCTCACCAAGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGACTGA  252
4 GCTCACCAAGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGACTGA  256
C GCTCACCAAGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGACTGA
```

Figure 2

```
          310        320        330        340        350        360
1 GATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC  360
2 GATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC  322
3 GATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC  312
4 GATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC  316
C GATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC

          370        380        390        400        410        420
1 CTGATGCAGCGACGCCGCGTGCGGGATGAAGGCCTTCGGGTTGTAAACCGCTTTTAATTG  420
2 CTGATGCAGCGACGCCGCGTGCGGGATGAAGGCCTTCGGGTTGTAAACCGCTTTTAATTG  382
3 CTGATGCAGCGACGCCGCGTGCGGGATGAAGGCCTTCGGGTTGTAAACCGCTTTTAATTG  372
4 CTGATGCAGCGACGCCGCGTGCGGGATGAAGGCCTTCGGGTTGTAAACCGCTTTTAATTG  376
C CTGATGCAGCGACGCCGCGTGCGGGATGAAGGCCTTCGGGTTGTAAACCGCTTTTAATTG

          430        440        450        460        470        480
1 GGAGCAAGCGAGAGTGAGTGTACCTTTTGAATAAGCACCGGCTAACTACGTGCCAGCAGC  480
2 GGAGCAAGCGAGAGTGAGTGTACCTTTTGAATAAGCACCGGCTAACTACGTGCCAGCAGC  442
3 GGAGCAAGCGAGAGTGAGTGTACCTTTTGAATAAGCACCGGCTAACTACGTGCCAGCAGC  432
4 GGAGCAAGCGAGAGTGAGTGTACCTTTTGAATAAGCACCGGCTAACTACGTGCCAGCAGC  436
C GGAGCAAGCGAGAGTGAGTGTACCTTTTGAATAAGCACCGGCTAACTACGTGCCAGCAGC

          490        500        510        520        530        540
1 CGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGG  540
2 CGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGG  502
3 CGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGG  492
4 CGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGG  496
C CGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGG

          550        560        570        580        590        600
1 CGGTTTGTCACGCCTGGTGTGAAAGTCCATCGCTTAACGGTGGATCTGCGCCGGGTACGG  600
2 CGGTTTGTCACGCCTGGTGTGAAAGTCCATCGCTTAACGGTGGATCTGCGCCGGGTACGG  562
3 CGGTTTGTCACGCCTGGTGTGAAAGTCCATCGCTTAACGGTGGATCTGCGCCGGGTACGG  552
4 CGGTTTGTCACGCCTGGTGTGAAAGTCCATCGCTTAACGGTGGATCTGCGCCGGGTACGG  556
C CGGTTTGTCACGCCTGGTGTGAAAGTCCATCGCTTAACGGTGGATCTGCGCCGGGTACGG
```

Figure 2 continued

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          610        620        630        640        650        660
1 GCAGGCTAGAGTGCAGTAGGGGAGATTGGAATTCCCGGTGTAACGGTGGAATGTGTAGAT  660
2 GCAGGCTAGAGTGCAGTAGGGGAGATTGGAATTCCCGGTGTAACGGTGGAATGTGTAGAT  622
3 GCAGGCTAGAGTGCAGTAGGGGAGATTGGAATTCCCGGTGTAACGGTGGAATGTGTAGAT  612
4 GCAGGCTAGAGTGCAGTAGGGGAGATTGGAATTCCCGGTGTAACGGTGGAATGTGTAGAT  616
C GCAGGCTAGAGTGCAGTAGGGGAGATTGGAATTCCCGGTGTAACGGTGGAATGTGTAGAT

          670        680        690        700        710        720
1 ATCGGGAAGAACACCAATGGCGAAGGCAGATCTCTGGGCTGTTACTGACGCTGAGGAGCG  720
2 ATCGGGAAGAACACCAATGGCGAAGGCAGATCTCTGGGCTGTTACTGACGCTGAGGAGCG  682
3 ATCGGGAAGAACACCAATGGCGAAGGCAGATCTCTGGGCTGTTACTGACGCTGAGGAGCG  672
4 ATCGGGAAGAACACCAATGGCGAAGGCAGATCTCTGGGCTGTTACTGACGCTGAGGAGCG  676
C ATCGGGAAGAACACCAATGGCGAAGGCAGATCTCTGGGCTGTTACTGACGCTGAGGAGCG

          730        740        750        760        770        780
1 AAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGGTGGATG  780
2 AAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGGTGGATG  742
3 AAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGGTGGATG  732
4 AAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGGTGGATG  736
C AAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGGTGGATG

          790        800        810        820        830        840
1 CTGGATGTGGGGCCCTTCCACGGGCTCCGTGTCGGAGCTAACGCGTTAAGCATCCCGCCT  840
2 CTGGATGTGGGACCCTTCCACGGGTTCCGTGTCGGAGCTAACGCGTTAAGCATCCCGCCT  802
3 CTGGATGTGGGACCCTTCCACGGGTTCCGTGTCGGAGCTAACGCGTTAAGCATCCCGCCT  792
4 CTGGATGTGGGACCCTTCCACGGGTTCCGTGTCGGAGCTAACGCGTTAAGCATCCCGCCT  796
C CTGGATGTGGG+CCCTTCCACGGG+TCCGTGTCGGAGCTAACGCGTTAAGCATCCCGCCT

          850        860        870        880        890        900
1 GGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCG  900
2 GGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCG  862
3 GGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCG  852
4 GGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCG  856
C GGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCG
```

Figure 2 continued

```
          910        920        930        940        950        960
1 GAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTAGGCTTGACATGTTTCGG  960
2 GAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTAGGCTTGACATGTTTCGG  922
3 GAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTAGGCTTGACATGTTTCGG  912
4 GAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTAGGCTTGACATGTTTCGG  916
C GAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTAGGCTTGACATGTTTCGG

          970        980        990       1000       1010       1020
1 ACAGCCCCAGAGATGGGGTCTCCCTTCGGGGCCGATTCACAGGTGGTGCATGGTCGTCGT 1020
2 ACAGCCCCAGAGATGGGGTCTCCCTTCGGGGCCGATTCACAGGTGGTGCATGGTCGTCGT  982
3 ACAGCCCCAGAGATGGGGTCTCCCTTCGGGGCCGATTCACAGGTGGTGCATGGTCGTCGT  972
4 ACAGCCCCAGAGATGGGGTCTCCCTTCGGGGCCGATTCACAGGTGGTGCATGGTCGTCGT  976
C ACAGCCCCAGAGATGGGGTCTCCCTTCGGGGCCGATTCACAGGTGGTGCATGGTCGTCGT

         1030       1040       1050       1060       1070       1080
1 CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTGTGT 1080
2 CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTGTGT 1042
3 CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTGTGT 1032
4 CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTGTGT 1036
C CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTGTGT

         1090       1100       1110       1120       1130       1140
1 TGCCAGCACGTTATGGTGGGAACTCACAAGGGACCGCCGGGGTTAACTCGGAGGAAGGTG 1140
2 TGCCAGCACGTTATGGTGGGAACTCACAAGGGACCGCCGGGGTTAACTCGGAGGAAGGTG 1102
3 TGCCAGCACGTTATGGTGGGAACTCACAAGGGACCGCCGGGGTTAACTCGGAGGAAGGTG 1092
4 TGCCAGCACGTTATGGTGGGAACTCACAAGGGACCGCCGGGGTTAACTCGGAGGAAGGTG 1096
C TGCCAGCACGTTATGGTGGGAACTCACAAGGGACCGCCGGGGTTAACTCGGAGGAAGGTG

         1150       1160       1170       1180       1190       1200
1 GGGATGACGTCAGATCATCATGCCCCTTACGTCTAGGGCTTCACGCATGCTACAATGGCC 1200
2 GGGATGACGTCAGATCATCATGCCCCTTACGTCTAGGGCTTCACGCATGCTACAATGGCC 1162
3 GGGATGACGTCAGATCATCATGCCCCTTACGTCTAGGGCTTCACGCATGCTACAATGGCC 1152
4 GGGATGACGTCAGATCATCATGCCCCTTACGTCTAGGGCTTCACGCATGCTACAATGGCC 1156
C GGGATGACGTCAGATCATCATGCCCCTTACGTCTAGGGCTTCACGCATGCTACAATGGCC
```

Figure 2 continued

```
          1210       1220       1230       1240       1250       1260
1 GGTACAACGAGATGCGACATGGCGACATGAAGCGAATCTCTTAAAACCGGTCTCAGTTCG 1260
2 GGTACAACGAGATGCGACATGGCGACATGAAGCGAATCTCTTAAAACCGGTCTCAGTTCG 1222
3 GGTACAACGAGATGCGACATGGCGACATGAAGCGAATCTCTTAAAACCGGTCTCAGTTCG 1212
4 GGTACAACGAGATGCGACATGGCGACATGAAGCGAATCTCTTAAAACCGGTCTCAGTTCG 1216
C GGTACAACGAGATGCGACATGGCGACATGAAGCGAATCTCTTAAAACCGGTCTCAGTTCG

          1270       1280       1290       1300       1310       1320
1 GATTGGAGCCTGCAACTCGGCTCCATGAAGGCGGAGTCGCTAGTAATCGCGAATCAGCAA 1320
2 GATTGGAGCCTGCAACTCGGCTCCATGAAGGCGGAGTCGCTAGTAATCGCGAATCAGCAA 1282
3 GATTGGAGCCTGCAACTCGGCTCCATGAAGGCGGAGTCGCTAGTAATCGCGAATCAGCAA 1272
4 GATTGGAGCCTGCAACTCGGCTCCATGAAGGCGGAGTCGCTAGTAATCGCGAATCAGCAA 1276
C GATTGGAGCCTGCAACTCGGCTCCATGAAGGCGGAGTCGCTAGTAATCGCGAATCAGCAA
            1330       1340       1350       1360       1370       1380
1 CGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCATGAAAGTGGG 1380
2 CGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCATGAAAGTGGG 1342
3 CGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCATGAAAGTGGG 1332
4 CGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCATGAAAGTGGG 1336
C CGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCATGAAAGTGGG

          1390       1400       1410       1420
1 TAGCACCCGAAGCCGGTGGCCTAACCTTTTGGAGGGAGCCGTCTAAGG              1428
2 TAGCACCCGAAGCCGGTGGCCTAACCTTTT------------------              1372
3 TAGCACCCGAAGCCGGTGGCCTAA------------------------              1356
4 TAGCACCCGAAGCCGGTGGCCTAACCTTTTGGAGGGAGCCGTCTAAGG              1384
C TAGCACCCGAAGCCGGTGGCCTAA++++++::::::::::::::::::
```

Figure 2 continued

```
Seq ID no
AY004274        CGTCACCGCCGGCTCCAACCCGATCGCTTTGCGTCGTGGTATCGAGAAGG 112      7
FR47/3          CGTCGTTGCGGGCTCCAACCCCATCGCTCTTCGTCGCGGCATCGAGAAGG 65       8
FR54/e/1        CGTCGTTGCGGGCTCCAACCCCATCGCTCTTCGTCGCGGCATCGAGAAGG 70       9
AF210319        CGTCACCGCCGGTTCCAACCCGATCGCGCTGCGTCGTGGCATCGAGAAGG 550     10
AF240571        CGTCACCGCCGGCTCCAACCCGATCGCGCTGCGTCGCGGCATCGAAAAGG 112     11
AY004277        CGTGGTCGCCGGCTCCAACCCGATCGCTCTGCGTCGCGGTATCGAGAAGG 112     12
AF240568        CGTGGTCGCCGGCTCCAACCCGATTGCGCTGCGCCGCGGCATTGAAAAGG 113     13
FR51/h/1        ---CGTTTGTGGCNTTAGCC--ATCGCTCTTCGTCGCGGCATCGAGAAGG 45      14
FR59/b/2        ----------------GCC--ATCGCTCTTCGTCGCGGCATCGAGAAGG 31       15
                              *   ** **  * ** ** ** ** ** ****

AY004274        CTTCCGAAGCCATCGTCAAGGAGCTTATCGCAGCTGCCAAGGACGTTGAG 162     16
FR47/3          CCTCAGACGCCATCGTCAAGGAGCTGATTTCCGCAGCCAAGGACGTTGAG 115     17
FR54/e/1        CCTCAGACGCCATCGTCAAGGAGCTGATTTCCGCAGCCAAGGACGTTGAG 120     18
AF210319        CCGCCGACGCCATCGTCAAGGAACTCGTCGCAGCGGCCAAGGACGTTGAG 600     19
AF240571        CTTCCGACGCCATTGTCAAGGAACTTGTCGCGGCCGCCAAGGATGTCGAG 162     20
AY004277        CCACCGAAGTCATCGTCAAGGAACTCGTCGCCGCCGCCAAGGACGTCGAG 162     21
AF240568        CCGCCGACGCCATCGTCAAGGAACTCGTCGCAGCCGCCAAGGACGTCGAG 163     22
FR51/h/1        C-TCAGACGCCATCGTCAAGGAGCTGATTTCCGCAGCCAAGGACGTTGAG 94      23
FR59/b/2        CCTCAGACGCCATCGTCAAGGAGCTGATTTCCGCAGCCAAGGACGTTGAG 81      24
                *  * ** * *** ******** **  *  * ** ******** ** ***

AY004274        ACCAAGGATCAGATCGCCGCTACCGCAACGATTTCCGCCGCCGATCCGGA 212     25
FR47/3          ACCAAGGATCAGATCGCCGCGACGGCAACGATCTCCGCGGCCGATCCCGA 165     26
FR54/e/1        ACCAAGGATCAGATCGCCGCGACGGCAACGATCTCCGCGGCCGATCCCGA 170     27
AF210319        ACCAAGGATCAGATCGCTGCCACCGCAACGATCTCCGCCGCTGATCCGGA 650     28
AF240571        ACCAAGGACCAGATCGCTGCCACCGCAACGATCTCCGCCGCTGATCCGGA 212     29
AY004277        ACCAAGGATCAGATCGCTGCCACTGCTACGATCTCCGCCGCCGATCCTGA 212     30
AF240568        ACCAAGGATCAGATCGCTGCCACCGCAACGATTTCCGCCGCCGATCCCGA 213     31
FR51/h/1        ACCAAGGATCAGATCGCCGCGACGGCAACGATCTCCGCGGCCGATCCCGA 144     32
FR59/b/2        ACCAAGGATCAGATCGCCGCGACGGCAACGATCTCCGCGGCCGATCCCGA 131     33
                ******** ******** ** ** ** ***** ***** ** ***** **

AY004274        AGTCGGCGAGAAGATCGCCGAAGCTCTTGACAAGGTTGGCCAGGACGGCG 262     34
FR47/3          GGTTGGCGAGAAGATCGCCGAAGCTCTGGACAAGGTCGGCCAGGACGG-- 193     35
FR54/e/1        GGTTGGCGAGAAGATCGCCGAAGCTCTGGACAAGGTCGGCCAGGACGGT- 176     36
AF210319        AGTCGGCGAGAAGATCGCCGAAGCTCTGGACAAGGTCGGCCAGGATGGCG 700     37
AF240571        AGTCGGCGAGAAGATCGCCGAAGCTCTGGACAAGGTCGGCCAGGACGGTG 262     38
AY004277        GGTTGGTGAGAAGATCGCCGAAGCCCTGGACAAGGTCGGCCAGGATGGCG 262     39
AF240568        GGTTGGCGAGAAGATCGCCGAAGCCCTGGACAAGGTTGGCCAGGACGGCG 263     40
FR51/h/1        GGTTGGCGAGAAGATCGCCGAAGCTCTGGACAAGGTCGGCCAGGACGG-- 192     41
FR59/b/2        GGTTGGCGAGAAGATCGCCGAAGCTCTGGACAAGGTCGGCCAGGACGGT- 180     42
```

Figure 3

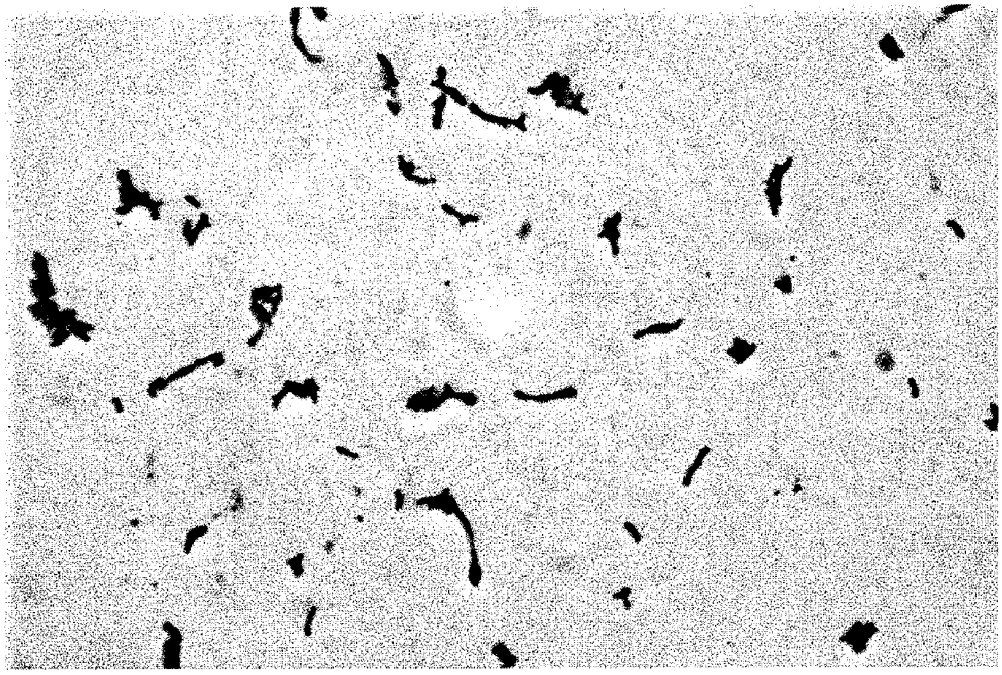

Figure 4

PROBIOTIC BIFIDOBACTERIAL SPECIES

[0001] The invention relates to a bacterium belonging to the genus *Bifidobacterium*, to probiotic compositions comprising said bacterium, particularly food products, and to their use in the treatment of gastrointestinal diseases.

BACKGROUND TO THE INVENTION

[0002] Bifidobacteria (or bacteria belonging to the Bifidobacterium genus) constitute one of the most important populations of human and animal faecal flora. It is generally considered an indication of good health when these bacteria are present at a high rate in faecal flora. For this reason, they are known as probiotic bacteria (beneficial microorganisms which improve the natural balance of intestinal flora when ingested alive). Examples of known Bifidobacteria include *B. adolescentis, B. animalis, B. bifidum, B. breve, B. catenulatum* and *B. longum* and these bacteria have been shown to have beneficial technological, organoleptic and probiotic effects.

[0003] Bifidobacteria are most commonly found as an additive in fermented milks (yoghurts with "active Bifidus") and thus constitute an economically important commodity. The strains chosen by the milk industry must meet numerous strict requirements, such as resistance to the process of manufacture and survival within the foodstuff. The most commonly used species in France are *B. animalis* subsp. lactis and *B. animalis* subsp. *animalis*, which is a subspecies from animal origin, never isolated from humans. In view of the importance of bifidobacteria, there is a great need to identify novel species within this genus having properties optimally matched to the requirements of the food industry. For example, in 2004, a group identified and isolated *Bifidobacterium psychraerophilum* from a porcine caecum (Simpson, P. J. et al. (2004) Int J Syst Evol Microbiol 54: 401-6). Previously known *Bifidobacterium* species had only been able to grow at temperatures between 20° C. and 46-49.5° C. (Biavati, B. et al., (2000), Annals of Microbiology 50: 117-131; Dong et al., (2000) Int J Syst Evol Microbiol 50 Pt 1: 119-25), however, this bacterium demonstrated an advantage over all previous species by growing at between 4 and 10° C. This is beneficial for probiotic compositions as the bacteria are more likely to survive the low storage temperatures and would therefore prolong shelf-life. There is thus a great need for the identification of further bifidobacterial species, which not only possess unique advantages but also retain the benefits of previously identified bifidobacterial species.

SUMMARY OF THE INVENTION

[0004] Thus, according to a first aspect of the invention there is provided *Bifidobacterium* GC56 deposited at the Collection Nationale de Cultures de Micro-organismes (CNCM, Institut Pasteur) on 9 Dec. 2004 with accession number CNCM I-3342 or a homolog, descendant or mutant thereof.

[0005] It will be appreciated that a homolog of *Bifidobacterium* GC56 will be understood to refer to any bifidobacteria strain having DNA sequence homology of greater than 40% with *Bifidobacterium* GC56 deposited at the Collection Nationale de Cultures de Micro-organismes (CNCM, Institut Pasteur) on 9 Dec. 2004, with accession number CNCM I-3342 (hereinafter referred to as 'GC56'). Preferably, a GC56 homolog is one having greater than 50% homology with GC56, more preferably greater than 60%, most preferably greater than 70%, especially preferably greater than 80% or most especially preferably greater than 90% (or any range between any of the above values). It will also be appreciated that sequence homology can be tested as described herein with reference to DNA-DNA reassociation experiments. Such experiments may include detection of specific sequences of 16S rDNA or of the hsp60 gene and these sequences and linked endonuclease restriction sites allow the detection of GC56. Other experiments, which may also identify GC56, include ELISA-PCR and PCR-RFLP.

[0006] GC56 represents a new species of *Bifidobacterium* called *Bifidobacterium crudilactis*. The terms GC56, GC56 group, *Bifidobacterium* GC56 and *Bifidobacterium crudilactis* are used interchangeably to refer to this new species of *Bifidobacterium*.

[0007] Examples of strains of GC56 discussed herein include FR62/b/3, FR59/b/2, and FR47/3. The 16S rDNA gene sequences of these strains have been deposited in GenBank and have the accession numbers: AY952449 (Sequence ID No: 3), AY952448 (Sequence ID No: 4) and AY952450 (Sequence ID No: 2) respectively. The 16S rDNA gene sequence for AY952448 deposited at GenBank has an error at the application date of the present application. The inventors have applied to correct the error in the sequence.

[0008] GC56 was identified during the cheese making of "L'étoile du Vercors" which is a traditional and manual process. GC56 is present throughout the cheese production process (i.e. from raw milk to the end of maturing), with a statistically significant increase during the process. These bacteria belong to a natural microbial population which takes part in the development of organoleptic properties of the end product.

[0009] GC56 has the key advantage of being the first bifidobacterial species isolated from a food production process whereas previous bifidobacteria have been extracted from the digestive tracts of humans or animals, thus GC56 is easier to integrate into the manufacturing process and is also easier to stabilise in food and fermented products than other bifidobacteria.

[0010] GC56 has also been found to be psychotrophic and to be able to grow at temperatures as low as 10-12° C., the maturing temperature of the "L'étoile du Vercors" process, whereas most others need a temperature of more than 20° C. GC56 is thought to constitute a milk subdominant population selected by the low temperature of the L'étoile du Vercors process (milk at 4° C., warmed to 22° C. until the removal from the mould at Day 2, then maintained at 12° C. from the maturing at Day 8 until Day 28). The key advantage of growth at low temperatures is that GC56 bacteria are more likely to survive low storage temperatures than most other probiotic bacterial compositions, which would therefore prolong the shelf-life.

[0011] A further advantage of GC56 is that it is aero-tolerant, whereas most others need strict anaerobiotic conditions to multiply and survive.

[0012] Further advantages of *Bifidobacterium* GC56 are that they provide a good fermentation of milk (alone or in a mix), a good resistance to lactic acid in the end product (more than 10^6 cfu/g), a sufficient growth rate, a good resistance to stomach acidity, the biliary salts and to the intestinal enzymes and a lower need for growth factors (yeast extract, hydrolysed protein, vitamins and other elements).

[0013] As a second aspect of the invention there is provided a probiotic composition comprising Bifidobacterium GC56 as hereinbefore defined and one or more acceptable excipients.

[0014] It will be appreciated that an acceptable excipient will be well known to the person skilled in the art of probiotic composition preparation. Examples of such acceptable excipients include: sugars such as sucrose, isomerized sugar, glucose, fructose, palatinose, trehalose, lactose and xylose; sugar alcohols such as sorbitol, xylitol, erythritol, lactitol, palatinol, reduced glutinous starch syrup and reduced glutinous maltose syrup; emulsifiers such as sucrose esters of fatty acid, glycerin esters of fatty acid and lecithin; thickeners (stabilizers) such as carrageenan, xanthan gum, guar gum, pectin and locust bean gum; acidifiers such as citric acid, lactic acid and malic acid; fruit juices such as lemon juice, orange juice and berry juice; vitamins such as vitamin A, vitamin B, vitamin C, vitamin D and vitamin E; and minerals such as calcium, iron, manganese and zinc.

[0015] Compositions of the invention may be prepared by admixture, suitably at ambient temperature and atmospheric pressure, usually adapted for oral administration. Such compositions may be in the form of tablets, capsules, oral liquid preparations, conventional food products, powders, granules, lozenges, reconstitutable powders or suspensions.

[0016] Tablets and capsules for oral administration may be in unit dose form, and may contain one or more conventional excipients, such as binding agents, fillers, tabletting lubricants, disintegrants, and acceptable wetting agents. The tablets may be coated according to methods well known in pharmaceutical practice.

[0017] Oral liquid preparations may be in the form of, for example, aqueous or oily suspension, solutions, emulsions, syrups or elixirs, or may be in the form of a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), preservatives, and if desired, conventional flavourings or colourants.

[0018] In one preferred embodiment, the composition of the invention is formulated as a conventional food product, more preferably, a dairy based product (e.g. fermented milk, vegetable milk, soybean milk, butter, cheese or yoghurt) or fruit juice. The composition is preferably formulated as a food or drink for adult and infant humans and animals. In an alternatively preferred embodiment, the composition is formulated as a lyophilised or spray-dried powder.

[0019] As well as exhibiting a probiotic effect (i.e. maintaining the balance of intestinal flora), bifidobacteria are also generally believed to be of potential use in the treatment and/or prophylaxis of a variety of disorders, such as gastrointestinal diseases (e.g. diarrhoea), cancer, cholesterol excesses, allergies and infection.

[0020] Thus, as a further aspect of the invention, there is provided *Bifidobacterium* GC56 for use as a therapeutic substance, in particular in the treatment and/or prophylaxis of the above disorders.

[0021] The invention further provides a use of *Bifidobacterium* GC56 in the preparation of a medicament for the treatment and/or prophylaxis of the above disorders.

[0022] The invention further provides a method of treatment and/or prophylaxis of the above disorders, in a human or animal subject, which comprises administering to the subject a therapeutically effective amount of *Bifidobacterium* GC56.

[0023] *Bifidobacterium* GC56 may be used in combination with other therapeutic agents, for example, other medicaments known to be useful in the treatment and/or prophylaxis of gastrointestinal diseases (e.g. diarrhoea), cancer, cholesterol excesses, allergies and infection.

[0024] Thus, as a further aspect of the invention, there is provided a combination comprising *Bifidobacterium* GC56 together with a further therapeutic agent or agents.

[0025] The combinations referred to above may conveniently be presented for use in the form of a probiotic composition and thus probiotic compositions comprising a combination as defined above together with one or more excipients comprise a further aspect of the invention. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined probiotic compositions.

[0026] In a preferred embodiment, *Bifidobacterium* GC56 is combined with other bifidobacteria or other probiotic bacteria such as: bacteria belonging to the genus *Lactobacillus* such as *Lactobacillus acidophilus, Lactobacillus gasseri, Lactobacillus plantarum, Lactobacillus buchneri, Lactobacillus casei, Lactobacillus johnsonii, Lactobacillus gallinarum, Lactobacillus amylovorus, Lactobacillus brevis, Lactobacillus rhamnosus, Lactobacillus kefir, Lactobacillus paracasei, Lactobacillus crispatus, Lactobacillus delbrueckii* subsp. *delbrueckii, Lactobacillus delbrueckii* subsp. *bulgaricul, Lactobacillus helveticus, Lactobacillus zeae* and *Lactobacillus salivalius*; bacteria belonging to the genus *Streptococcus* such as *Streptococcus thermophilus*; bacteria belonging to genus *Lactococcus* such as *Lactococcus lactis* subsp. cremoris and *Lactococcus lactis* subsp. *lactis*; bacteria belonging to the genus *Bacillus* such as *Bacillus subtilis*; and yeast belonging to the genus *Saccharomyces, Torulaspora* and *Candida* such as *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Candida kefyr.*

DETAILED DESCRIPTION OF THE INVENTION

[0027] Preferred embodiments of the invention will now be described merely by way of example with reference to the accompanying drawings in which:

[0028] FIG. **1** shows the phylogenetic tree of *Bifidobacterium* 16S rDNA sequences (1419 nucleotides) including FR62/b/3 strain (Genbank Accession No. AY952449), FR47/3 strain (Genbank Accession No. AY952450), FR59/b/2 strain (Genbank Accession No. AY952448). FR62/b/3, FR47/3 and FR59/b/2 are all strains of *Bifidobacterium crudilactis*. The sequences were aligned with ClustalX. The tree was rooted with *Actinomyces bovis* and constructed using a neighbour-joining algorithm. Bootstrap values, calculated from 1000 trees, are given at each node. Numbers in parenthesis correspond to the GenBank accession numbers;

[0029] FIG. **2** shows sequence alignments of 16S rDNA of GC56 FR62/b/3, Fr47/3 and Fr59/b/2 strains with *B. psychraerophilum*. The sequences were aligned using the program ClustalW from the European Bioinformatics Institute.

[0030] Sequence 1 (Sequence ID No: 1) is *B. psychraerophilum* (AY174108), Sequence 2 (Sequence ID No: 2) is GC56 FR47/3 (AY952450), Sequence 3 (Sequence ID No: 3) is GC56 FR62/b/3 (AY952449) and Sequence 4 (Sequence ID No: 4) is GC56 FR59/b/2

(AY952448). Sequence C is the consensus sequence. In the consensus sequence the following symbols are used "." if majority character has a representation>=20% in the sequences; ":" if majority character has a representation>=40% in the sequences; "+" if majority character has a representation>=60% in the sequences; "*" if majority character has a representation>=80% in the sequences; and the character itself, if this is present in all the sequences.

[0031] The restriction map between 16S rDNAup (5'-aatagctcctggaaacgggt-3' (Sequence ID No: 5)) and 16S rDNAdwn (5'-cgtaagggcatgatgatct-3' (Sequence ID No: 6)) includes: AluI: AGCT cut positions 5, 100, 411, 696, 902 (underlined) and TaqI: TCGA cut positions 132, 796 (italics);

[0032] FIG. **3** shows an hsp60 gene sequence alignment of several strains of the GC56 group with closely identified sequences. More specifically, FIG. **3** show the alignment of partial sequences of the hsp60 gene of 4 of the GC56 group (FR47/3, FR51/h/1, FR54/e/1, 59/b/2) with closely identified sequences found on Genbank (PubMed-BLAST), namely: *B. pseudocatenulatum* (AY004274), *B. adolescentis* (AF210319), *B. ruminantium* (AF240571), *B. merycicum* (AY004277), *B. angulatum* (AF240568). FAM-ATTTCCGCAGCCAAGGACGTTGA-DQ (Sequence ID No: 43): is a probe used for targeting the hsp60 gene and is specific of the GC56 group. 5'-ATTTCCGCAGC-CAAGGACGT-3' (Sequence ID No: 44) and 5'TCCA-GAGCTTCGGCGATCTTC-3' (Sequence ID No: 45): are forward and reverse primers specific to the GC56 group used for targeting the hsp60 gene; and

[0033] FIG. **4** shows the morphological appearance of GC56.

Isolation of Bifidobacterium GC56

[0034] GC56 was isolated from a raw milk cheese process in the industry "L'étoile du Vercors" (France). Cultural methods (Delcenserie et al., (2005) J Microbiol Methods 61: 55-67), but particularly molecular methods, allowed detection of these bacteria throughout the production chain of the product and the checking of the composition of the end products. 95 strains were isolated from 31/31 studied cheeses "L'Etoile du Vercors" at different stages of the production, in 3/7 cheeses from trade raw milk cheeses other than those from "L'Etoile du Vercors" and in raw milk samples. Bifidobacterium GC56 strain FR62/b/3 (=CUETM 04/3) has been deposited at the Collection Nationale de Cultures de Micro-organismes (CNCM, Institut Pasteur) on 9 Dec. 2004 with accession number CNCM I-3342 and the sequence has been deposited on Genbank with accession number AY952449.

Detection of Bifidobacterium GC56 by PCR

1. Preparation of DNA Targets

[0035] DNA-extraction (Wizard® genomic DNA purification kit of Promega): One millilitre broth was centrifuged for 2 min. at 13000 g. The pellet was suspended in 480 µl EDTA, 60 µl of lysozyme, and 120 µl of cellular lysis solution and incubated for 45 min at 37° C. After centrifugation, 600 µl of nuclei lysis solution was added to the pellet followed by incubation for 5 min at 80° C. When cooled, 200 µl of protein lysis solution was added followed by vortexing. The resultant suspension was then incubated for 5 min on ice and was centrifuged for 5 min at 13000 g. The supernatant was transferred to a clean tube containing 600 µl of isopropanol and the tube was then centrifuged. The supernatant was decanted and 600 µl ethanol 70% was added and the tube centrifuged. The ethanol was aspirated and pellet air-dried for 10 min. Finally, the DNA pellet was rehydrated in 100 µl of rehydration solution overnight at 4° C.

2. PCR Protocols

[0036] GC56 can be detected within a complex microbial population by using two PCR methodologies based on the hsp60 gene:

(a) PCR Using Species Specific Primers

[0037] Four microlitres DNA (50-100 ng), 0.2 µl (400 pmoles/l) of the upstream primer (5'-ATTTCCGCAGC-CAAGGACGT-3' (Sequence ID No: 44); Table 1) and 0.2 µl (400 pmoles/l) of the downstream primer (5'-TCCAGAGCT-TCGGCGATCTTC-3' (Sequence ID No: 45); Table 1), 0.2 mol/l dNTP, 0.2 µl (1U) Taq polymerase enzyme, 2 µl enzyme buffer, 1.6 µl $MgCl_2$ and 11.6 µl ultra-pure water were mixed to achieve a total volume of 20 µl.

[0038] The thermal-cycler was programmed as follows: one 10 minute cycle at 95° C. followed by 30 cycles composed of 30 seconds at 95° C., 30 seconds at 64.2° C. (annealing temperature) and 30 seconds at 72° C. and ended by 5 minutes at 72° C.

[0039] Twenty micro-litres of each PCR-product were added to 2 µl of colouring agent and transferred to a well of a 2% agarose-agar gel. The size evaluation was made using a Smart Ladder®. The agar was immersed in TAE 1× buffer and migration conditions were of 60 min at 120V at 400 mA. After migration, the agar was incubated for 10 min in ethidium bromide and washed for 10 min under water. The amplification products were observed under ultra-violet trans-illumination. The hsp60 gene sequence was identified by correlating the position with the Smart Ladder®. A blank control well was also made to evaluate contamination in each PCR.

(b) Real-Time PCR Using Species Specific Probe

[0040] A pair of degenerate primers specific to the *Bifidobacterium* genus (Table 1) were used for the PCR on the hsp60 gene. One probe was chosen from hsp60 sequences of the GC56 group (Table 1) after DNA sequencing of 4 strains of the GC56 group (FR51/h/1, FR47/3b, FR59/b/2, FR54/e/1). The bifidobacteria sequences were aligned using the program ClustalW from the European Bioinformatics Institute. The alignments revealed specific sequences for the GC56 group.

[0041] From these sequences, probes were derived using the primers and probes design guidelines provided by Applied Biosystems (Applied Biosystems, Foster city, USA). To check for specificity, the selected probes were compared to all available hsp60 gene sequences using the BLAST database search program. The GC56 probe was labelled with FAM (a reporter dye) and DQ.

TABLE 1

Primers and probes used for amplification of *bifidobacteria* and identification of the GC56 group.

Target organism(s)	Primers/ probes	Targeted gene	Sequence (5'-3')	Amplicon size	Reference
Bifidobacterium spp.	Forward primer	hsp60 gene	GTSCAYGARGGYC TSAAGAA (Seq ID No: 46)	217 bp	Delcenserie et al. (2004)
	Reverse primer		CGTAAGGGGCATG ATGATCT (Seq ID No: 47)		
GC56 group	Forward primer	hsp60 gene	5'-ATTTCCGCAGCCA AGGACGT-3' (Seq ID No: 44)	105 bp	This study
	Reverse primer		5'-TCCAGAGCTTCGG CGATCTTC-3' (Seq ID No: 45)		
GC56 group	Probe	hsp60 gene	FAM-ATTTCCGCAGCCA AGGACGTTGA-DQ (Seq ID No: 43)		This study

Sensitivity and Specificity of the Assays

[0042] To check the specificity of the probe, PCR was performed on 55 strains belonging to 13 different Bifidobacterium species and 9 Bifidobacterium strains belonging to the GC56 group. The results observed with the GC56 probe revealed a specificity of 98% (only one *B. adolescentis* strain (5031e), was positive) and a sensitivity of 100% (the 9 tested strains from the GC56 group were positive).

Real-Time PCR Conditions

[0043] Amplification reaction mixtures contained 10 to 50 ng of DNA, 12.5 μl of qPCR™ Mastermix (Eurogentec, Seraing, Belgium), 960 nM of each primer, 50 to 150 nM of fluorogenic probe and 5 mM $MgCl_2$ in a total volume of 25 μl. In each microwell plate, one well was used as non-template control, which contained all the reagents except the DNA sample. The amplification, 50° C. for 2 min, 95° C. for 10 min, and then 40 cycles of two-temperature PCR (95° C. for 30 s and 60° C. for 90 s) and detection was carried out on an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster city, USA). The PCR results for the samples were expressed as deltaRn (relative sensitivity) fluorescence signal.

[0044] A sample was considered as positive when the Ct value was lower than 25 for a relative fluorescence value higher than 500.

[0045] Other variations of these methods such as PCR-multiplex, Real-time PCR could be used as well as other techniques such as hybridization techniques, dot-blot hybridization, fluorescent in situ hybridization, colony hybridization, restriction fragment length polymorphism analysis.

Characterisation of *Bifidobacterium* GC56

(a) Temperature

[0046] In a preliminary study, strain FR62/b/3 of GC56 was incubated in 10 ml of full-cream milk, full-cream milk with milk powder (to reach 15% protein in dry matter), half-skimmed milk, half-skimmed milk with milk powder and sweetened concentrated milk (Nestlé®). UHT milk and Nestlé® milk powder were used. Inoculated milk samples were incubated in anaerobiotic jars at 30 and 37° C. for 72 hours and bacteria were then counted. Growth rate was found to be better at 30° C. than at 37° C. so 30° C. was chosen for further studies.

(b) Growth

[0047] Bifidobacteria were subcultured in BHI (double concentration) broth at 30° C. during 3 days in anaerobiotic conditions. Initial concentration in broth was of 10^7 cfu/ml. The broth was diluted in peptone-saline water from ten to ten. One hundred micro-litres of broth and dilutions −2, −4, −6 and −8 were harvested and suspended in 10 ml of full-cream milk or full-cream milk with milk powder (to reach 15% protein in dry matter). The milks were incubated in anaerobic jars for 6, 12, 24 and 48 hours at 30° C. At each incubation time, pH was measured and the bacteria counted with a spiral sowing.

[0048] Results showed better growth in complemented milk: The bacterial concentration arose 10^{10} cfu/ml after 48 hours with an initial inoculation of 10^5, 10^3 and 10^1 cfu/ml and 10^9 with an initial inoculation of 10^{-1} cfu/ml. The pH values tended to 4.6-5.3 in all the complemented milk.

[0049] In full-cream milk only 10^9 cfu/ml were produced from the initial rate of 10^5 cfu/ml and 10^6 from 10^3 cfu/ml. The pH decrease reached 5.7-6.4.

[0050] The aim of this preliminary study was to know the needed initial concentration and to study the evolution of the fermentation process.

[0051] Then, an innoculum of strain FR62/b/3 (9.5 log cfu/ml from a based milk medium) was cultured at 30° C. during 24 hours in a 20 L fermentation vat containing milk based medium (0.3% Peptone-casein, 0.5% Yeast extract and 8% Skimmed milk powder). Eight 50 ml samples were harvested at the following times: inoculation time and after 2, 4, 6, 8, 10, 12 and 24 incubation hours. Spiral sowing was straight made for each sample. The pH was followed automatically in the fermentation vat.

[0052] The results showed a growth from 8.4 log cfu/ml (inoculation time) to 9.2 after 6 hours and this rate was maintained until 24 hours.

[0053] When inoculated with a *Lactobacillus acidophilus* strain, the FR62/b/3 strain grew from 8.1 log cfu/ml (inoculation time) to 9.2 after 10 hours and we observed a rate of 8.9 log cfu/ml after 24 hours although the pH decreased more than when it was alone (see section (c) below).

[0054] GC56 is also able to multiply in TPY broth (in bain-marie) up to a maximum temperature of 41.5° C. and a minimum temperature of 7° C., the optimum temperature for growth is 39° C. In TPY, the minimum initial pH for growth is 4.7. GC56 is also able to multiply on TPY agar under aerobic conditions at 37° C. and 39° C.

[0055] Colonies of GC56 on TPY agar at 39° C. under anaerobic conditions are cream, circular, and convex with entire edges. They reach a diameter of up 1 mm. They reach a reduced diameter (less than 1 mm) under aerobic conditions.

(c) pH

[0056] The milk media pH was initially 6.6.

[0057] When cultured alone in the fermentation vat, strain FR62/b/3 of GC56 produced a pH decrease from 5.9 (at inoculation time) to 5.0 after 6 hours, 4.2 after 12 hours and 3.9 after 24 hours.

[0058] In the presence of the *Lactobacillus acidophilus* strain, which further acidifies the medium, the pH values decreased from 5.9 to 4.4 after 6 hours, 3.8 after 12 hours and 3.5 after 24 hours. The bacterial counts of the 2 species increased to 8 log cfu/ml after 24 hours.

(d) Survival

[0059] After incubation in a fermentation vat, the survival in refrigerator conditions (aerobic conditions, temperature between 4-8° C.) was studied. From an initial rate of 9.2 log cfu/ml, strain FR62/b/3 of GC56 reached 8.5 log cfu/ml after 18 days and 7.3 log cfu/ml after 29 days. According to the literature, a rate greater than 10^6 or 10^7 cfu/ml or /g of probiotics in the product is required to allow observation of positive effects.

(e) Biochemical Analysis

[0060] The GC56 group is well phenotypically individualized by numerical analysis (classification based on unweighted average linkage and Hartigan's clustering methods). No type or reference strains belonging to another species of the *Bifidobacterium* genus join the group.

[0061] Biochemical characteristics which differentiate the GC56 group and the species *B. pseudolongum* the most frequently isolated species in raw milk and in raw milk cheeses are presented in Table 2 below which shows the percentage of positives for certain defined characteristics:

TABLE 2

Characteristics	GC56 group (138 strains)	*B. pseudolongum* (98 strains)
Fermentation:		
Ribose	96	43
Alpha-methyl-D-glucoside	66	10
Esculine	99	51
Starch	0	94
Glycogen	0	90

TABLE 2-continued

Characteristics	GC56 group (138 strains)	*B. pseudolongum* (98 strains)
Enzymatic tests:		
Beta-glucosidase	93	47
Glycine arylamidase	86	47

[0062] Phenotypic characteristics that differentiate the new strain isolates of *Bifidobacterium crudilactis* from *B. psychraerophilum*, the closest phylogenetically species, are presented in Table 3. The data in Table 3 is based on 141 different isolates of *B. crudilactis*. The results are given as the percentage of positive responses observed in the 141 isolates, FR62/b/3T (a strain of *B. crudilactis*), and *B. psychraerophilum* LMG 21775T.

TABLE 3

Characteristics	*B. crudilactis* (141 strains)	*B. crudilactis* FR62/b/3^T (LMG P-22559^T)	*B. psychraerophilum* LMG 21775^T (Simpson et al., 2004)
Acidification of:			
L-arabinose	1	−	+
Amygdalin	13	−	+
Arbutin	1	−	+
Salicin	2	−	+
Lactose	100	+	−
Melezitose	1	−	+
Enzymatic test:			
Alpha-arabinosidase	1	−	+
Maximum growth temperature*	—	41.5° C. (6 days)	42° C.
Minimum growth temperature†	—	7° C. (6 weeks)	4° C.
Minimum growth pH		4.7	4.5
DNA G + C content (mol %)	55.2 (9 strains) (SD = 0.83)	56.4 (4 experiments) (SD = 0.60)	59.2 (HPLC, Simpson et al., 2004 Int J Syst Evol Microbiol 54, 401-6) 55.7 (T_m‡)

Legend of Table 3:
*growth within 8 days;
†within 4 weeks;
‡mean of 2 experiments performed in the laboratory

[0063] All strains of *B. crudilactis* (≧98% of the strains) ferment galactose, glucose, fructose, maltose, lactose, melibiose, sucrose, raffinose, and D-turanose. None ferment (≦2% of the strains) glycerol, erythritol, D-arabinose, L-arabinose, L-xylose, adonitol, β-methyl-xyloside, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methyl-D-glucoside, N-acetyl-glucosamine, arbutin, trehalose, inulin, melezitose, starch, glygogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-keto-gluconate, 5-keto-gluconate. All strains (≧98% of the strains) were positive for α-galactosidase, β-galactosidase, α-glucosidase, arginine arylamidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, and histidine arylamidase. All were negative (≦2% of the strains) for urease, indole production, nitrate reduction, arginine dihy-

drolase, β-galactosidase-6-phosphate, α-arabinosidase, β-glucuronidase, β-N-acetylglucosaminidase, acide glutamique decarboxylase, α-fucosidase, acide pyro-glutamique arylamidase, glutamyl arylamidase.

(f) G+C Content

[0064] The mean GC content (Tm method) of the FR62/b/3 strain of GC56 is 56% and of 9 strains in the group is 55.2% (SD=0.83).

(g) DNA-DNA Hybridization

[0065] DNA-DNA reassociation levels were between 4 and 36% with the type strains of all the *Bifidobacterium* species and of *Aeriscardovia aeriphila*, results shown in Table 4 below, and between 76 and 100% within the *Bifidobacterium* GC56 group (13 experiments). The DNA-DNA homology between *B. psychraerophilum* and the GC56 group reference strain (FR62/b/3) is equal to 31% (2 measurements) confirming that the GC56 group does not belong to that species (definition threshold of bacterial species upper or equal to 70%; Goebel and Stackebrandt, (1994), Appl Environ Microbiol, 60: 1614-1621; Rossello-Mora and Amann (2001), FEMS Microbiol Rev, 25: 39-67).

[0066] DNA-DNA reassociation levels were determined using the spectrophotometric method from renaturation rates described by De Ley et al. (*J. Biochem*. (1970) 12, 133-142), slightly modified in hybridization temperature (Gavini et al. *Ecology in Health and Disease* (2001) 13, 40-45). The determinations were performed at 67.3° C. (T_m-25° C. according to the G+C content of the strain FR62/b/3), using a spectrophotometer Cary **100** (Varian) related to a temperature controller (Peltier system, Varian).

TABLE 4

Species	DNA Homology with FR62/b/3 (%)
B. crudilactis FR47/3, FR55/d/2, FR59/b/2, FR98/a/11	100
B. crudilactis FR50/f/4	94
B. crudilactis Brie/9	91
B. crudilactis PicV/10	86
B. crudilactis FR35/5	85
B. crudilactis Reb/13	83
B. adolescentis	31
B. angulatum	36
B. animalis	31
B. asteroides	24
B. bifidum	21
B. boum	21
B. breve	21
B. catenulatum	34
B. choerinum	23
B. coryneforme	29
B. cuniculi	23
B. dentium	17
B. gallicum	19
B. gallinarum	6
B. indicum	21
B. longum	29
B. magnum	20
B. merycicum	16
B. minimum	22
B. pseudocatenulatum	26
B. pseudolonum subsp. *globosum*	16
B. pseudolonum subsp. *pseudolongum*	28
B. psychraerophilum	31
B. pullorum	21
B. ruminantium	15
B. saeculare	5
B. scardovii	35
B. subtile	13
B. suis	32
B. thermacidophilum subsp. *thermacidophilum*	21
B. thermacidophilum subsp. *porcinum*	26
B. thermophilum	4
Aeriscardovia aeriphila	28

[0067] Table 4 shows DNA-DNA reassociation of DNA from FR62/b/3 (GC56 group) with DNAs from type strains of the *Bifidobacterium* genus, including strains of *B. crudilactis* and of *Aeriscardovia aeriphila.*

[0068] *B. crudilactis* FR62/b/3 (GC56), *B. crudilactis* FR55/d/2, FR59/b/2, FR98/a/11, *B. crudilactis* FR50/f/4, *B. crudilactis* Brie/9, *B. crudilactis* PicV/10, *B. crudilactis* FR35/5 and *B. crudilactis* Reb/13 are all strains of *B. crudilactis* which have more than 80% DNA homology with *B. crudilactis* FR62/b/3.

(h) 16S rRNA Sequencing

[0069] The sequencing of the 16S rDNA (about 1400 bp) has been realized on 3 GC56 group representative strains (FR/62/b/3, FR/47/b/3, FR/59/b/2 strains) and was compared with other close *Bifidobacterium* sequences available on Genbank (FIG. **1**). It appeared that this group presented 99.8% of similarities (3 differences) with *B. psychraerophilum* considering the FR62/b/3, FR/59/b/2 and the FR/47/b/3 strains.

[0070] A 16S rDNA sequence alignment of three GC56 strains with *B. psychraerophilum* was performed. Specific restriction enzyme areas were identified using AluI and TaqI (FIG. **2**). Restriction fragment length polymorphism could be used to detect or to identify this group in a sample. No difference was observed with *B. psychraerophilum*. However, there is a low probability of finding *B. psychraerophilum* in these kind of samples.

(i) hsp60 Gene Partial Sequencing

[0071] FIG. **3** shows an hsp60 gene sequence alignment of several strains of the GC56 group (FR47/3, FR51/h/1, FR54/e/1, 59/b/2) with closely identified sequences found on Genbank (PubMed-BLAST). No difference was observed between the GC56 group strains while differences were observed with other bifidobacterial species. These differences were used for chosen specific PCR primers and a specific probe for real-time PCR (Table 1).

(j) Appearance

[0072] GC56 cells are Gram-positive, non-spore-forming bacilli, and irregularly shaped rods. The morphology of the FR62/b/3 strain is shown in FIG. **4**.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 47

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<212> TYPE: DNA
<213> ORGANISM: Bifidobacterium psychraerophilum

<400> SEQUENCE: 1

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ggaatagctc ctggaaacgg gtggtaatgc cggatgctcc aacatttcac atgttttgtt 180

gggaaagcgt tagcggtatg agatggggtc gcgtcctatc agcttgttgg tgaggtaatg 240

gctcaccaag gcttcgacgg gtagccggcc tgagagggcg accggccaca ttgggactga 300

gatacggccc agactcctac gggaggcagc agtggggaat attgcacaat gggcgaaagc 360

ctgatgcagc gacgccgcgt gcgggatgaa ggccttcggg ttgtaaaccg cttttaattg 420

ggagcaagcg agagtgagtg tacctttga ataagcaccg gctaactacg tgccagcagc 480

cgcggtaata cgtagggtgc aagcgttatc cggaattatt gggcgtaaag agctcgtagg 540

cggtttgtca cgcctggtgt gaaagtccat cgcttaacgg tggatctgcg ccgggtacgg 600

gcaggctaga gtgcagtagg ggagattgga attcccggtg taacggtgga atgtgtagat 660

atcgggaaga acaccaatgg cgaaggcaga tctctgggct gttactgacg ctgaggagcg 720

aaagcatggg gagcgaacag gattagatac cctggtagtc catgccgtaa acggtggatg 780

ctggatgtgg ggcccttcca cgggctccgt gtcggagcta acgcgttaag catcccgcct 840

ggggagtacg gccgcaaggc taaaactcaa agaaattgac gggggcccgc acaagcggcg 900

gagcatgcgg attaattcga tgcaacgcga agaaccttac ctaggcttga catgtttcgg 960

acagccccag agatggggtc tcccttcggg gccgattcac aggtggtgca tggtcgtcgt 1020

cagctcgtgt cgtgagatgt tgggttaagt cccgcaacga gcgcaaccct cgccttgtgt 1080

tgccagcacg ttatggtggg aactcacaag ggaccgccgg ggttaactcg gaggaaggtg 1140

gggatgacgt cagatcatca tgccccttac gtctagggct tcacgcatgc tacaatggcc 1200

ggtacaacga gatgcgacat ggcgacatga agcgaatctc ttaaaaccgg tctcagttcg 1260

gattggagcc tgcaactcgg ctccatgaag gcggagtcgc tagtaatcgc gaatcagcaa 1320

cgtcgcggtg aatgcgttcc cgggccttgt acacaccgcc cgtcaagtca tgaaagtggg 1380

tagcacccga agccggtggc ctaacctttt ggagggagcc gtctaagg 1428

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ccaacatttc acatgttttg ttgggaaagc gttagcggta tgagatgggg tcgcgtccta 180

tcagcttgtt ggtgaggtaa tggctcacca aggcttcgac gggtagccgg cctgagaggg 240

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cgaccggcca cattgggact gagatacggc ccagactcct acgggaggca gcagtgggga 300

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ggttgtaaac cgcttttaat tgggagcaag cgagagtgag tgtacctttt gaataagcac 420

cggctaacta cgtgccagca gccgcggtaa tacgtagggt gcaagcgtta tccggaatta 480

ttgggcgtaa agagctcgta ggcggtttgt cacgcctggt gtgaaagtcc atcgcttaac 540

ggtggatctg cgccgggtac gggcaggcta gagtgcagta ggggagattg gaattcccgg 600

tgtaacggtg gaatgtgtag atatcgggaa gaacaccaat ggcgaaggca gatctctggg 660

ctgttactga cgctgaggag cgaaagcatg gggagcgaac aggattagat accctggtag 720

tccatgccgt aaacggtgga tgctggatgt gggacccttc cacgggttcc gtgtcggagc 780

taacgcgtta agcatcccgc ctggggagta cggccgcaag gctaaaactc aaagaaattg 840

acgggggccc gcacaagcgg cggagcatgc ggattaattc gatgcaacgc gaagaacctt 900

acctaggctt gacatgtttc ggacagcccc agagatgggg tctcccttcg gggccgattc 960

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ggggttaact cggaggaagg tggggatgac gtcagatcat catgcccctt acgtctaggg 1140

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tcttaaaacc ggtctcagtt cggattggag cctgcaactc ggctccatga aggcggagtc 1260

gctagtaatc gcgaatcagc aacgtcgcgg tgaatgcgtt cccgggcctt gtacacaccg 1320

cccgtcaagt catgaaagtg ggtagcaccc gaagccggtg gcctaacctt tt 1372

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<213> ORGANISM: Bifidobacterium GC56

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acatgttttg ttgggaaagc gttagcggta tgagatgggg tcgcgtccta tcagcttgtt 180

ggtgaggtaa tggctcacca aggcttcgac gggtagccgg cctgagaggg cgaccggcca 240

cattgggact gagatacggc ccagactcct acgggaggca gcagtgggga atattgcaca 300

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cgcttttaat tgggagcaag cgagagtgag tgtacctttt gaataagcac cggctaacta 420

cgtgccagca gccgcggtaa tacgtagggt gcaagcgtta tccggaatta ttgggcgtaa 480

agagctcgta ggcggtttgt cacgcctggt gtgaaagtcc atcgcttaac ggtggatctg 540

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ggtctcagtt cggattggag cctgcaactc ggctccatga aggcggagtc gctagtaatc 1260

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catgaaagtg ggtagcaccc gaagccggtg gcctaa 1356

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<212> TYPE: DNA
<213> ORGANISM: Bifidobacterium GC56

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tttcacatgt tttgttggga aagcgttagc ggtatgagat ggggtcgcgt cctatcagct 180

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gccacattgg gactgagata cggcccagac tcctacggga ggcagcagtg gggaatattg 300

cacaatgggc gaaagcctga tgcagcgacg ccgcgtgcgg gatgaaggcc ttcgggttgt 360

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actacgtgcc agcagccgcg gtaatacgta gggtgcaagc gttatccgga attattgggc 480

gtaaagagct cgtaggcggt ttgtcacgcc tggtgtgaaa gtccatcgct taacggtgga 540

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ccgtaaacgg tggatgctgg atgtgggacc cttccacggg ttccgtgtcg gagctaacgc 780

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aaccggtctc agttcggatt ggagcctgca actcggctcc atgaaggcgg agtcgctagt 1260

aatcgcgaat cagcaacgtc gcggtgaatg cgttcccggg ccttgtacac accgcccgtc 1320

aagtcatgaa agtgggtagc acccgaagcc ggtggcctaa ccttttggag ggagccgtct 1380

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<400> SEQUENCE: 6

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<210> SEQ ID NO 7
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<213> ORGANISM: Bifidobacterium pseudocatenulatum

<400> SEQUENCE: 7

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<210> SEQ ID NO 8
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<213> ORGANISM: Bifidobacterium adolescentis

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<210> SEQ ID NO 11
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<213> ORGANISM: Bifidobacterium ruminantium

<400> SEQUENCE: 11

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<210> SEQ ID NO 12
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<212> TYPE: DNA
<213> ORGANISM: Bifidobacterium merycicum

<400> SEQUENCE: 12

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<210> SEQ ID NO 13
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<210> SEQ ID NO 15
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<213> ORGANISM: Bifidobacterium GC56

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<213> ORGANISM: Bifidobacterium angulatum

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<210> SEQ ID NO 45
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<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: s = g or c; y = c or t; r=g or a

<400> SEQUENCE: 46

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<210> SEQ ID NO 47
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<213> ORGANISM: Bifidobacterium

<400> SEQUENCE: 47

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1. (canceled)

2. A *Bifidobacterium* strain, which has DNA sequence homology of greater than 40% over its entire genome to *Bifidobacterium* GC56, wherein *Bifidobacterium* GC56 was deposited at the Collection Nationale de Cultures de Micro-organismes (CNCM, Institut Pasteur) on 9 Dec. 2004 with accession number CNCM I-3342.

3-9. (canceled)

10. The *Bifidobacterium* strain of claim **2**, wherein the strain is *Bifidobacterium* GC56 deposited at the Collection Nationale de Cultures de Micro-organismes (CNCM, Institut Pasteur) on 9 Dec. 2004 with accession number CNCM I-3342.

11. A probiotic composition comprising a Bifidobacterium strain of claim **2**, and optionally one or more acceptable excipients.

12. The probiotic composition comprising a Bifidobacterium strain of claim **12**, and a further therapeutic agents or agents.

13. The probiotic composition of claim **12**, wherein said composition is a food product.

14. The probiotic composition of claim **13**, wherein said food product is a dairy based product selected from fermented milk, vegetable milk, soybean milk, butter, cheese, and yoghurt.

15. A method for the treatment of gastrointestinal diseases, diarrhea, cancer, excess cholesterol, allergies, or infection comprising administering to a subject in need thereof a Bifidobacterium strain which has DNA sequence homology of greater than 40% over its entire genome to Bifidobacterium GC56, wherein Bifidobacterium GC56 was deposited at the Collection Nationale de Cultures de Micro-organismes (CNCM, Institut Pasteur) on 9 Dec. 2004 with accession number CNCM I-3342.

16. The Bifidobacterium strain of claim **2**, wherein the strain possesses biochemical characteristics of ribose fermentation and not starch fermentation.

17. The Bifidobacterium strain of claim **2**, wherein the strain has phenotypic characteristic of lactose acidification.

18. The Bifidobacterium strain of claim **2**, wherein the strain is selected from the species of B-crudilactis FR47/3, B-crudilactis FR55/d/2, B-crudilactis FR59/b/2, B-crudilactis FR98/a/11, B-crudilactis FR50/V4, B-crudilactis Brie/9, B-crudilactis PicB/10, B-crudilactis FR35/5, and B-crudilactis Reb/13.

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