

The highly conserved Cas1 protein can be used as a marker to investigate the evolution of the CRISPR-Cas system.

The aim of this project is to understand evolutionary relationships among bacteria family containing "CRISPR associated protein Cas1", and it is important to sample as widely as possible, not only within but also between species. Closely related species will have few sequence differences, while less related species tend to have more!

I have 46 genera to compare. I wrote the procedure of collecting data and python code to fetch them during project. The data is selected from pfam. It was about 4000 sequences but after cleaning duplicates, unclassified species and so on, I have 235 sequences with 1061 amino-acid sites.

During the project, I tried programs both in command line and also online version because sometimes online version showed error because of large file!

reference <https://biopython.org/DIST/docs/api/Bio.Phylo.BaseTree.TreeMixin-class.html>
[\(https://biopython.org/DIST/docs/api/Bio.Phylo.BaseTree.TreeMixin-class.html\)](https://biopython.org/DIST/docs/api/Bio.Phylo.BaseTree.TreeMixin-class.html)
https://biopython.org/wiki/Phylo_cookbook (https://biopython.org/wiki/Phylo_cookbook)

first I tried to fetch data using Entrez but it was not enough!

In [19]:

```
from Bio import Entrez
Entrez.email = "snassirnia@outlook.com"
handle = Entrez.esearch(db='gene', term='cas1[gene] AND bacteria[orgn] AND alive[prop]', rettype="gb", retmode="text", retmax='500')
record = Entrez.read(handle)
handle.close()
print(record)
#"mobA[All Fields]"
```

```
{'Count': '71', 'RetMax': '71', 'RetStart': '0', 'IdList': ['9472228', '10565562', '35805086', '41382383', '41373254', '35832916', '34475982', '1089910', '35855886', '29731732', '34166558', '25052529', '8421934', '41482824', '34330625', '34323306', '34304247', '34291322', '31606449', '29732280', '29671049', '13177181', '41342360', '35765609', '31211663', '39648702', '31853470', '40396401', '8637323', '32076289', '31859234', '31490183', '40854312', '40836338', '39490974', '39490969', '34475242', '34138360', '32435829', '29672879', '41531810', '41072045', '40701950', '32075453', '31546458', '16761303', '41198105', '41004943', '40920164', '40889529', '35779917', '34423274', '32051924', '32050683', '9904236', '3978173', '2781520', '2741544', '35799809', '41381817', '39344944', '39300272', '39300242', '38299364', '35819833', '34755902', '34073080', '29900444', '29740956', '29257235', '12374249'], 'TranslationSet': [{"From": "bacteria[orgn]", "To": "'Bacteria'[Organism]"}], 'TranslationStack': [{"Term": "cas1[gene]", "Field": "gene", "Count": '391', "Explode": 'N'}, {"Term": "'Bacteria'[Organism]", "Field": "Organism", "Count": '12822231', "Explode": 'Y'}, {"Term": "alive[prop]", "Field": "prop", "Count": '25787020', "Explode": 'N'}, {"Term": "AND", "Field": null, "Count": null}], 'QueryTranslation': 'cas1[gene] AND "Bacteria"[Organism] AND alive[prop]'}]
```

In [20]:

```
id = record["IdList"]
id
```

Out[20]:

```
['947228', '10565562', '35805086', '41382383', '41373254', '3583291
6', '34475982', '1089910', '35855886', '29731732', '34166558', '2505
2529', '8421934', '41482824', '34330625', '34323306', '34304247', '3
4291322', '31606449', '29732280', '29671049', '13177181', '4134236
0', '35765609', '31211663', '39648702', '31853470', '40396401', '863
7323', '32076289', '31859234', '31490183', '40854312', '40836338',
'39490974', '39490969', '34475242', '34138360', '32435829', '2967287
9', '41531810', '41072045', '40701950', '32075453', '31546458', '167
61303', '41198105', '41004943', '40920164', '40889529', '35779917',
'34423274', '32051924', '32050683', '9904236', '3978173', '2781520',
'2741544', '35799809', '41381817', '39344944', '39300272', '3930024
2', '38299364', '35819833', '34755902', '34073080', '29900444', '297
40956', '29257235', '12374249']
```

In [21]:

```
#get sequences
id_str = ",".join(id)
handle = Entrez.efetch(db="gene", id=id_str, rettype="fasta", retmode="fasta",
retmax='500')
record = handle.read()
out_handle = open('cas1.fasta', 'w')
out_handle.write(record.rstrip('\n'))
```

Out[21]:

14965

Then I tried pfam database! There was 2890 species with 4329 sequences in whole bacterial family that contain cas1! but from pfam I could not get annotation. It was just IDs and sequences.

<https://pfam.xfam.org/family/PF01867#tabview=tab7> (<https://pfam.xfam.org/family/PF01867#tabview=tab7>).

Then I got all the links in kegg with my gene cas1 sequences(different IDs) then make a soap and fetch the infoermation that was needed in each link

In []:

```
# part 1: fetching data
```

In [2]:

```
import os
import urllib
from bs4 import BeautifulSoup as bs
import sys
import requests
import re
```

In [3]:

```
os.chdir('/home/sedreh/Desktop/phyloproject_cas1/python')
```

In [1]:

```
##### EXAMPLE CODE #####
```

In [2]:

```
#example
#page = requests.get("https://www.kegg.jp/entry/mmed:Mame_02484")
# https://www.dataquest.io/blog/web-scraping-tutorial-python/
```

In [3]:

```
#page
```

In [4]:

```
#page.content
```

In [55]:

```
#soup = bs(page.content, 'html.parser')
```

In [6]:

```
#print(soup.prettify())
```

We need these two tags

from class:

316 aa



[\(/dbget-bin/www_bget?-f+-n+a+aal:EP13_09450 \)]((/dbget-bin/www_bget?-f+-n+a+aal:EP13_09450))



[\(https://www.genome.jp/tools-bin/search_sequence?prog=blast&seqid=aal:EP13_09450+aa\)](https://www.genome.jp/tools-bin/search_sequence?prog=blast&seqid=aal:EP13_09450+aa)

MKKSVVQINVGEDVGKDFLDIHIRPLGEYLVNSDKKGIGEALKRLRKLTVRIVIEAT
 GRYEMAFVSACAKAELPFSVVNPIHVKRFAAGRKAKTDLDAALIAHFSEVIQPALST
 LKPENIQKMADTVARRNQHLEMQTREKNRLQIMPQTVQSSIKAILTVLKKQIQKMDDELA
 DLIESCPEYQAKSELVQSMPGIGKVSAALISYLPELGMMNSKQVAALGVVAPMNKESGR
 YKGKQVTQGGRHQVRTVLFMAMMSAMQCNAFKATYERLVAAGKPKKVAIACVRKMVVI
 LNSMVRDGMWEEKAA

and

Organism from:

Also maybe

NCBI-ProteinID: [AIF98882](https://www.ncbi.nlm.nih.gov/protein/AIF98882) (<https://www.ncbi.nlm.nih.gov/protein/AIF98882>)

UniProt: [A0A075P6F6](https://www.uniprot.org/uniprot/A0A075P6F6) (<https://www.uniprot.org/uniprot/A0A075P6F6>)

In [7]:

```
#children= (list(soup.children)[2]).get_text()
```

In [8]:

```
#children
```

In [43]:

```
#r = re.compile("(?<=\\xa0\\xa0)(.*?)(?=\\:)")  
#less_children = list(filter(r.match, children))
```

In [44]:

```
#name = re.findall("(?<=\\xa0\\xa0\\xa0)(.*?)(?=\\:)", children)[0]
```

In [45]:

```
#genus, species=format(re.sub(r'[\\d-]', '', name)).split(" ",1)
```

In [9]:

```
#genus
```

In [10]:

```
#species.strip(" ")
```

In [11]:

```
#ids = re.findall("(?s)(?=<UniProt:\\xa0)(.*?)(?=\\nLinkDB\\n)", children)
```

In [12]:

```
#ids[0]
```

In [13]:

```
#seq = re.findall("(?s)(?=<aa \\n)(.*?)(?=\\nNT seq\\n)", children)
```

In [14]:

```
#seq = format(re.sub('\\n', '', seq[0]))
```

In [15]:

```
#seq
```

now I have all links.

In [30]:

```
with open('/home/sedreh/ITM0/semester3/Molecular_phylogenetic/COURSE_PROJECT/phyloproject_cas1/links.txt', 'r') as links:
    for link in links:
        print(link)
```

https://www.kegg.jp/entry/azd:CDA09_21065
https://www.kegg.jp/entry/azl:AZL_008990
https://www.kegg.jp/entry/azl:AZL_010420
https://www.kegg.jp/entry/azl:AZL_027440
https://www.kegg.jp/entry/azm:DM194_03325
https://www.kegg.jp/entry/azm:DM194_12305
<https://www.kegg.jp/entry/azo:azo3662>
https://www.kegg.jp/entry/azr:CJ010_02280
https://www.kegg.jp/entry/azz:DEW08_03440
https://www.kegg.jp/entry/azz:DEW08_24135
https://www.kegg.jp/entry/bacb:0Y17_13580
https://www.kegg.jp/entry/bacc:BRDCF_p1865
https://www.kegg.jp/entry/bacl:BS34A_24750
https://www.kegg.jp/entry/bacp:SB24_18410
https://www.kegg.jp/entry/bacs:AUL54_19515
https://www.kegg.jp/entry/bact:AB656_06700
https://www.kegg.jp/entry/bacy:QF06_09425
https://www.kegg.jp/entry/bad:BAD_1180
https://www.kegg.jp/entry/badl:BAD0_1326
https://www.kegg.jp/entry/bae:BATR1942_09095
https://www.kegg.jp/entry/bag:Bcoa_1935
https://www.kegg.jp/entry/bag:Bcoa_1937
https://www.kegg.jp/entry/bagr:BA6348_02510
https://www.kegg.jp/entry/bagr:BA6348_11120
https://www.kegg.jp/entry/bagr:BA6348_23390
https://www.kegg.jp/entry/bagr:BA6348_24995
https://www.kegg.jp/entry/bah:BAMEG_3379
https://www.kegg.jp/entry/bai:BAA_1285
https://www.kegg.jp/entry/bal:BACI_c12370
https://www.kegg.jp/entry/balm:BsLM_2189
https://www.kegg.jp/entry/bama:RBAU_2200

https://www.kegg.jp/entry/bamb:BAPNAU_1526
https://www.kegg.jp/entry/bamc:U471_21260
https://www.kegg.jp/entry/bamf:U722_11260
https://www.kegg.jp/entry/bami:KS0_009115
https://www.kegg.jp/entry/baml:BAM5036_1988
https://www.kegg.jp/entry/bamn:BASU_1989
https://www.kegg.jp/entry/bamp:B938_10650
https://www.kegg.jp/entry/bamt:AJ82_11705
https://www.kegg.jp/entry/bamy:V529_23280
https://www.kegg.jp/entry/ban:BA_1210
https://www.kegg.jp/entry/bang:BBAG_1185
https://www.kegg.jp/entry/banh:HYU01_06220
https://www.kegg.jp/entry/bani:Bl12_1226
https://www.kegg.jp/entry/banl:BLAC_07130
https://www.kegg.jp/entry/banm:EN10_06625
https://www.kegg.jp/entry/banr:A16R_12780
https://www.kegg.jp/entry/bans:BAPAT_1140
https://www.kegg.jp/entry/bant:A16_12590
https://www.kegg.jp/entry/banv:DJ46_45
https://www.kegg.jp/entry/bao:BAMF_2146
https://www.kegg.jp/entry/bapi:BBC0122_017350
https://www.kegg.jp/entry/baq:BACAU_2069
https://www.kegg.jp/entry/bar:GBAA_1210
https://www.kegg.jp/entry/barc:A0A65_1875
https://www.kegg.jp/entry/bast:BAST_1429
<https://www.kegg.jp/entry/bat:BAS1117>
https://www.kegg.jp/entry/bax:H9401_1137
https://www.kegg.jp/entry/bay:RBAM_020610
https://www.kegg.jp/entry/baz:BAMTA208_05740
https://www.kegg.jp/entry/bbag:E10_30610

https://www.kegg.jp/entry/bbau:AEM51_07200
https://www.kegg.jp/entry/bbay:A4V04_05205
https://www.kegg.jp/entry/bbb:BIF_01985
https://www.kegg.jp/entry/bbc:BLC1_1266
https://www.kegg.jp/entry/bbd:Belba_3199
https://www.kegg.jp/entry/bbev:BBEV_2838
https://www.kegg.jp/entry/bbi:BBIF_0206
https://www.kegg.jp/entry/bbo:BBOV_III006720
https://www.kegg.jp/entry/bbrc:B7019_1593
https://www.kegg.jp/entry/bbrj:B7017_1610
https://www.kegg.jp/entry/bbrn:B2258_1385
https://www.kegg.jp/entry/bbrs:BS27_1429
https://www.kegg.jp/entry/bbru:Bbr_1406
<https://www.kegg.jp/entry/bbub:102401479>
https://www.kegg.jp/entry/bbv:HMPREF9228_1445
https://www.kegg.jp/entry/bcat:BBCT_1280
https://www.kegg.jp/entry/bcew:DM40_5470
https://www.kegg.jp/entry/bcf:bcf_06025
https://www.kegg.jp/entry/bcg:BCG9842_B4090
https://www.kegg.jp/entry/bch:Bcen2424_2374
https://www.kegg.jp/entry/bcho:BcFMB_00640
https://www.kegg.jp/entry/bck:BC026_2451
<https://www.kegg.jp/entry/bcl:ABC3592>
https://www.kegg.jp/entry/bcn:Bcen_1762
https://www.kegg.jp/entry/bcoa:BF29_1596
https://www.kegg.jp/entry/bcoa:BF29_585
https://www.kegg.jp/entry/bcu:BCAH820_1280
https://www.kegg.jp/entry/bcx:BCA_1241
<https://www.kegg.jp/entry/bcz:BCE33L1093>
https://www.kegg.jp/entry/bde:BDP_1253
https://www.kegg.jp/entry/bde:BDP_1640

https://www.kegg.jp/entry/bdh:GV66_02000
https://www.kegg.jp/entry/bdl:AK34_5353
https://www.kegg.jp/entry/bdn:BBDE_1195
https://www.kegg.jp/entry/bdn:BBDE_1555
https://www.kegg.jp/entry/beb:AEM42_05595
https://www.kegg.jp/entry/beb:AEM42_05800
https://www.kegg.jp/entry/beb:AEM42_13385
https://www.kegg.jp/entry/beo:BEH_13400
https://www.kegg.jp/entry/beq:BEGA_007440
https://www.kegg.jp/entry/bex:A11Q_911
https://www.kegg.jp/entry/bfg:BF638R_3988
https://www.kegg.jp/entry/bfi:CIY_03630
https://www.kegg.jp/entry/bfo:BRAFLDRAFT_113716
https://www.kegg.jp/entry/bfo:BRAFLDRAFT_121762
https://www.kegg.jp/entry/bfo:BRAFLDRAFT_66940
<https://www.kegg.jp/entry/bfr:BF2544>
https://www.kegg.jp/entry/bfs:BF9343_1064
https://www.kegg.jp/entry/bfs:BF9343_3847
https://www.kegg.jp/entry/bfu:BCIN_05g05790
https://www.kegg.jp/entry/bfu:BCIN_12g05370
https://www.kegg.jp/entry/bfw:B5J99_06775
https://www.kegg.jp/entry/bgj:AWC36_10010
https://www.kegg.jp/entry/bgm:CAL15_21600
https://www.kegg.jp/entry/bgp:BGL_1c18810
https://www.kegg.jp/entry/bgy:BGLY_0117
<https://www.kegg.jp/entry/bha:BH0341>
https://www.kegg.jp/entry/bhan:CGC63_13430
https://www.kegg.jp/entry/bhf:C3V43_07410
https://www.kegg.jp/entry/bhf:C3V43_11265
https://www.kegg.jp/entry/bhk:B4U37_00630

https://www.kegg.jp/entry/bhl:Bache_3082
https://www.kegg.jp/entry/bhp:BHAMNSH16_04760
https://www.kegg.jp/entry/bhp:BHAMNSH16_05320
https://www.kegg.jp/entry/bih:BIP78_0924
https://www.kegg.jp/entry/bit:BIS30_00875
<https://www.kegg.jp/entry/biu:109564186>
<https://www.kegg.jp/entry/bja:bsr8214>
https://www.kegg.jp/entry/bjs:MY9_2251
https://www.kegg.jp/entry/bka:AH68_07800
https://www.kegg.jp/entry/bkw:BkAM31D_24940
https://www.kegg.jp/entry/bla:BLA_0514
https://www.kegg.jp/entry/blag:BLTE_26260
https://www.kegg.jp/entry/blas:BSY18_3176
https://www.kegg.jp/entry/blat:WK25_18015
https://www.kegg.jp/entry/blb:BBMN68_1822
https://www.kegg.jp/entry/blc:Balac_1308
https://www.kegg.jp/entry/blh:BaLi_c01280
https://www.kegg.jp/entry/blh:BaLi_c24700
https://www.kegg.jp/entry/blj:BLD_1903
https://www.kegg.jp/entry/blk:BLNIAS_00548
https://www.kegg.jp/entry/blq:L21SP5_00928
https://www.kegg.jp/entry/blq:L21SP5_00994
https://www.kegg.jp/entry/blq:L21SP5_03450
https://www.kegg.jp/entry/bls:W91_1345
https://www.kegg.jp/entry/blt:Balat_1308
https://www.kegg.jp/entry/blv:BalV_1266
https://www.kegg.jp/entry/blw:W7Y_1313
https://www.kegg.jp/entry/bmec:WJ16_16080
https://www.kegg.jp/entry/bmp:NG74_02160
<https://www.kegg.jp/entry/bna:106350895>
https://www.kegg.jp/entry/bng:EH206_02250

https://www.kegg.jp/entry/bni:BANAN_06850
https://www.kegg.jp/entry/bnm:BALAC2494_01821
https://www.kegg.jp/entry/boi:BLM15_23835
https://www.kegg.jp/entry/boj:CBF45_13540
<https://www.kegg.jp/entry/bom:102268823>
https://www.kegg.jp/entry/bos:BSY19_4790
https://www.kegg.jp/entry/bpdz:BBN53_16780
<https://www.kegg.jp/entry/bpec:110153249>
<https://www.kegg.jp/entry/bpec:110170297>
https://www.kegg.jp/entry/bpj:B2904_orf475
https://www.kegg.jp/entry/bpo:BP951000_0891
https://www.kegg.jp/entry/bpr:GBP346_A3093
https://www.kegg.jp/entry/bpsc:BBPC_1358
https://www.kegg.jp/entry/bpsi:IX83_03035
https://www.kegg.jp/entry/bpw:WESB_2214
https://www.kegg.jp/entry/bpyr:ABD05_34485
https://www.kegg.jp/entry/bql:LL3_02429
https://www.kegg.jp/entry/bqy:MUS_2490
https://www.kegg.jp/entry/brg:A4249_14970
https://www.kegg.jp/entry/brk:CWS35_37705
https://www.kegg.jp/entry/brm:Bmur_1230
<https://www.kegg.jp/entry/brp:103842476>
<https://www.kegg.jp/entry/brp:103851677>
https://www.kegg.jp/entry/brz:CFK38_03535
https://www.kegg.jp/entry/bsa:Bacsa_1111
https://www.kegg.jp/entry/bsca:BBSC_1927
https://www.kegg.jp/entry/bsed:DN745_02420
https://www.kegg.jp/entry/bsh:BSU6051_22460
https://www.kegg.jp/entry/bsl:A7A1_2935
https://www.kegg.jp/entry/bsm:BSM4216_3208

https://www.kegg.jp/entry/bso:BSNT_08640
https://www.kegg.jp/entry/bsol:FSW04_13845
https://www.kegg.jp/entry/bsp:U712_10880
https://www.kegg.jp/entry/bsq:B657_22460
https://www.kegg.jp/entry/bsr:I33_2306
https://www.kegg.jp/entry/bss:BSUW23_11015
https://www.kegg.jp/entry/bst:GY0_2473
https://www.kegg.jp/entry/bstl:BBJ41_34395
<https://www.kegg.jp/entry/bsu:BSU22460>
https://www.kegg.jp/entry/bsul:BSUA_02413
https://www.kegg.jp/entry/bsus:Q433_12240
https://www.kegg.jp/entry/bsut:BSUB_02413
https://www.kegg.jp/entry/bsx:C663_2119
https://www.kegg.jp/entry/bsy:I653_10710
https://www.kegg.jp/entry/btc:CT43_P127052
https://www.kegg.jp/entry/bthi:BTK_32926
https://www.kegg.jp/entry/bthm:BTRA_1534
https://www.kegg.jp/entry/bthr:YBT1520_32841
https://www.kegg.jp/entry/bths:CNY62_00585
https://www.kegg.jp/entry/bths:CNY62_08555
https://www.kegg.jp/entry/bths:CNY62_12590
https://www.kegg.jp/entry/btht:H175_107p039
https://www.kegg.jp/entry/bthy:AQ980_31575
https://www.kegg.jp/entry/btj:BTJ_12
https://www.kegg.jp/entry/btk:BT9727_1099
https://www.kegg.jp/entry/btl:BALH_1058
https://www.kegg.jp/entry/btm:MC28_3022
[https://www.kegg.jp/entry\(btn:BTF1_03725](https://www.kegg.jp/entry(btn:BTF1_03725)
https://www.kegg.jp/entry/bto:WQG_14130
https://www.kegg.jp/entry/bto:WQG_7830
https://www.kegg.jp/entry/btp:D805_0641

https://www.kegg.jp/entry/btra:F544_14460
https://www.kegg.jp/entry/btra:F544_8250
https://www.kegg.jp/entry/btre:F542_14220
https://www.kegg.jp/entry/btre:F542_7910
https://www.kegg.jp/entry/btrh:F543_15870
https://www.kegg.jp/entry/btrh:F543_9160
https://www.kegg.jp/entry/bts:Btus_2679
https://www.kegg.jp/entry/bts:Btus_2723
https://www.kegg.jp/entry/btt:HD73_7015
https://www.kegg.jp/entry/btw:BF38_2430
https://www.kegg.jp/entry/bue:BRPE67_BCDS16480
https://www.kegg.jp/entry/bui:AX768_08535
https://www.kegg.jp/entry/bum:AXG89_06290
https://www.kegg.jp/entry/bvc:CEP68_10290
<https://www.kegg.jp/entry/bvg:104897093>
https://www.kegg.jp/entry/bvm:B9C48_10480
https://www.kegg.jp/entry/bvr:BVIR_1623
https://www.kegg.jp/entry/bvr:BVIR_2086
https://www.kegg.jp/entry/bvr:BVIR_2220
https://www.kegg.jp/entry/bvs:BARVI_02495
https://www.kegg.jp/entry/bxb:DR64_7834
https://www.kegg.jp/entry/bxe:Bxe_C0612
https://www.kegg.jp/entry/bxh:BAXH7_01200
https://www.kegg.jp/entry/bxi:BK049_15380
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In [37]:

```
print(">{} {} {}\\n{}".format(genus, species, ids, seq))
```

>Martelella mediterranea ['A0A1U9Z296']

M

In [38]:

```
def write_fasta(genus, species, ids, seq):
    with open('/home/sedreh/ITMO/semester3/Molecular_phylogenetic/COURSE_PROJECT/phyloproject_cas1/cas1_pfam.fasta', 'a') as fasta:
        fasta.write(">{} {} {}\\n{}".format(genus, species, ids, seq))
    fasta.close()
```

Final function

In [57]:

```
def write_fasta(genus, species, ids, seq):
    with open('/home/sedreh/ITMO/semester3/Molecular_phylogenetic/COURSE_PROJECT/phyloproject_cas1/cas1_pfam.fasta', 'a') as fasta:
        fasta.write(">{} {} {}\\n{}\\n".format(genus, species, ids, seq))

def fetch_fasta(link):
    page = requests.get(link)
    soup = bs(page.content, 'html.parser')
    children= (list(soup.children)[2]).get_text()
    name = re.findall("(?=<\xa0\x09\x09)(.*?)(?=:\")", children)[0]
    genus, species=format(re.sub(r'[\d-]', '', name)).split(" ",1)
    species.strip(" ")
    ids = re.findall("(?s)(?=<UniProt:\xa0)(.*?)(?=\\n)", children)
    seq = re.findall("(?s)(?=<aa \\n)(.*?)(?=\\nNT seq\\n)", children)
    seq = format(re.sub('\\n', '', seq[0]))

    return write_fasta(genus, species, ids, seq)

def main():
    list_of_links = list()
    with open('/home/sedreh/ITMO/semester3/Molecular_phylogenetic/COURSE_PROJECT/phyloproject_cas1/links.txt', 'r') as links:
        for link in links:
            list_of_links.append(link.strip('\\n'))

    for link in list_of_links:
        fetch_fasta(link)
```

In []:

main()

In []:

```
list_of_links = list()
with open('/home/sedreh/ITMO/semester3/Molecular_phylogenetic/COURSE_PROJECT/CAS1/data/links.txt', 'r') as links:
    for link in links:
        list_of_links.append(link.strip('\n'))
```

after cleaning data in R, deleting duplicates and unclassified data, I did alignment in UGENE for saving time and start to make nj tree.

choosig best alignment

Muscle has a better theoretical basis than Clustal. Clustal is highly sensitive to the order in which you list the sequences, because its search algorithm always depends on the order, especially the first sequence. Muscle takes an iterative approach, which is a plus. The advantage of an iterative approach is that it can search through a variety of alignments and phylogenies, make changes to them, test if it's an improvement or not, and continue onward until it has a more optimal solution. and then we can use use GBLOCKS (http://molevol.cmima.csic.es/castresana/Gblocks/Gblocks_documentation.html). Gblocks (http://molevol.cmima.csic.es/castresana/Gblocks/Gblocks_documentation.html) will just remove a badly aligned column across the entire sequences. It will eliminates poorly aligned positions and divergent regions of protein sequences. it is better to do the alignment in MUSCLE and after that edit the sequences aligned in BioEdit to delete in both side the mismatches due mainly to the primers and allow the sequence to start and end in the same position without mismatches (intermediary mismatches are ok). But i skkipped this step! I had too many gaps(around 50%) but if remove all gap-containing positions then a lot of "information will lose! Also we can establish the relationships between species with WinClada software, which is one of the most common phylogenetic program used. free download WinClada from internet (<http://www.cladistics.com/wincDownload.htm> (<http://www.cladistics.com/wincDownload.htm>));

In [4]:

```
import Bio
from Bio import AlignIO
from Bio import SeqIO
from Bio import Entrez
from Bio import Phylo
import matplotlib
import matplotlib.pyplot as plt
```

In [5]:

```
#Read alignment file
aln = AlignIO.read('/home/sedreh/Desktop/phyloproject_cas1/python/cas1_clean_aln', 'fasta')
```

removing white spaces in species name in fasta file:

```
sed 's, ,_,g' -i /home/sedreh/Desktop/phyloproject_cas1/python/cas1_clean_aln
```

In [6]:

```
from Bio.Phylo.TreeConstruction import DistanceCalculator
calculator = DistanceCalculator('identity')
dm = calculator.get_distance(aln)
dm
```

Out[6]:

```
DistanceMatrix(names=['Bacillus_BSA', 'Bacillus_Pc', 'Bacillus SDL
I', 'Bifidobacterium_actinocoloniiforme', 'Bacillus_YP', 'Bifidobact
erium_adolescentis', 'Bacillus_atrophaeus', 'Bacillus_coagulans', 'B
acillus_anthracis', 'Bacillus_cereus', 'Bacillus_LM', 'Bacillus_vele
zensis', 'Bacillus_amyloliquefaciens', 'Bifidobacterium_angulatum',
'Bifidobacterium_animalis', 'Candidatus_Bathyarchaeota', 'Bifidobact
erium_asteroides', 'Bacillus_beveridgei', 'Bifidobacterium_bifidum',
'Bifidobacterium_breve', 'Bifidobacterium_catenulatum', 'Burkholderi
a_cenocepacia', 'Bifidobacterium_choerinum', 'Bacillus_clausii', 'Bi
fidobacterium_dentium', 'Bacteroides_dorei', 'Burkholderia_dolosa',
'Bacillus_filamentosus', 'Bacteroides_fragilis', 'Burkholderia_plant
arii', 'Bacillus_glycinifermentans', 'Bacillus_halodurans', 'Bactero
ides_heparinolyticus', 'Bacillus_horikoshii', 'Bacteroides_helcogene
s', 'Candidatus_Bipolaricaulis', 'Bacillus_intestinalis', 'Bacillus_
JS', 'Bifidobacterium_kashiwanohense', 'Bacillus_krulwichiae', 'Burk
holderia_latens', 'Bifidobacterium_longum', 'Bacillus_paralichenifor
mis', 'Burkholderia_metallica', 'Burkholderia_pseudomallei', 'Bifido
bacterium_pseudocatenulatum', 'Burkholderia_pyrrocinia', 'Bacteroide
s_salanitronis', 'Bifidobacterium_scardovii', 'Bacillus_subtilis',
'Bacillus_smithii', 'Burkholderia_stabilis', 'Bacillus_thuringiensi
s', 'Burkholderia_thailandensis', 'Bifidobacterium_thermophilum', 'B
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illus_xiamenensis', 'Burkholderia_YI', 'Bacteroides_zoogleoformans',
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In [11]:

```
#MAking NJ tree

from Bio.Phylo.TreeConstruction import DistanceCalculator, DistanceTreeConstructor
constructor = DistanceTreeConstructor()
tree_nj = constructor.nj(dm)
print(tree_nj)
Phylo.write(tree_nj, 'NJ_tree.nwk', 'newick')
```

```

Tree(rooted=False)
    Clade(branch_length=0, name='Inner233')
        Clade(branch_length=0.002740514659543114, name='Inner230')
            Clade(branch_length=0.0011418711878605145, name='Inner229')
                Clade(branch_length=0.0033301918434714903, name='Inner227')
                    Clade(branch_length=0.15895669427713507, name='Inner17')
                        Clade(branch_length=0.006582347754704898, name='Inner16')
                            Clade(branch_length=0.0, name='Citrobacter_CRE')
                            Clade(branch_length=0.0, name='Citrobacter_freundii')
                            Clade(branch_length=0.006612751208537326, name='Citrobacter_braakii')
                            Clade(branch_length=0.1305107892289912, name='Inner26')
                                Clade(branch_length=0.03423229287500923, name='Burkholderia_pyrrocinia')
                                Clade(branch_length=0.021139997417167947, name='Inner23')
                                    Clade(branch_length=0.002377206069566873, name='Inner22')
                                    Clade(branch_length=0.0048987027190438015, name='Inner21')
                                        Clade(branch_length=0.016760474881965304, name='Burkholderia_stabilis')
                                        Clade(branch_length=0.013399751319731257, name='Burkholderia_metallica')
                                        Clade(branch_length=0.01489394572581948, name='Burkholderia_dolosa')
                                        Clade(branch_length=0.010817892893675352, name='Inner19')
                                            Clade(branch_length=0.00026302522850318866, name='Burkholderia_latens')
                                            Clade(branch_length=0.000679481840299867, name='Burkholderia_cenocepacia')
                                            Clade(branch_length=0.005402725215906491, name='Inner225')
                                                Clade(branch_length=0.15679430569612687, name='Clostridium_butyricum')
                                                Clade(branch_length=0.15894556235288346, name='Bacillus_beveridgei')
                                                Clade(branch_length=0.007686769245692747, name='Inner221')
                                                    Clade(branch_length=0.004490812612324896, name='Inner216')
                                                        Clade(branch_length=0.17766780266026225, name='Clostridium_sporogenes')
                                                        Clade(branch_length=0.14796838961117978, name='Inner18')
                                                            Clade(branch_length=0.029644144947550882, name='Inner4')
                                                                Clade(branch_length=0.02385311232225537, name='Corynebacterium_vitaeruminis')
                                                                Clade(branch_length=0.030812297668319527, name='Corynebacterium_pseudotuberculosis')
                                                                Clade(branch_length=0.05706650538232663, name='Corynebacterium_humireducens')

```

```

Clade(branch_length=0.007467706031643068, name='Inne
r202')
    Clade(branch_length=0.008562599455377712, name
='Inner179')
        Clade(branch_length=0.19728663665716567, nam
e='Geobacillus_thermocatenulatus')
            Clade(branch_length=0.038457943927188765, na
me='Inner123')
                Clade(branch_length=0.00945822228625881,
name='Inner105')
                    Clade(branch_length=0.24412802456180
72, name='Geobacter_sulfurreducens')
                        Clade(branch_length=0.27684275771905
99, name='Inner2')
                            Clade(branch_length=0.0330466540
9990576, name='Inner1')
                                Clade(branch_length=0.011686
278634214398, name='Corynebacterium_variabile')
                                    Clade(branch_length=0.012818
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                                        Clade(branch_length=0.0442389255
4194159, name='Corynebacterium_glyciniphilum')
                                            Clade(branch_length=0.2651647748862199,
name='Corynebacterium_epidermidicanis')
                                                Clade(branch_length=0.15474884022416988, name='I
nner15')
                                                    Clade(branch_length=0.006547472909646107, na
me='Inner14')
                                                        Clade(branch_length=0.01823912669490618
3, name='Bacillus_atrophaeus')
                                                            Clade(branch_length=0.0110374991297875,
name='Inner13')
                                                                Clade(branch_length=0.00667886270636
0006, name='Bacillus_intestinalis')
                                                                    Clade(branch_length=0.00415996858487
469, name='Inner12')
                                                                        Clade(branch_length=0.0023886729
861339884, name='Bacillus_JS')
                                                                            Clade(branch_length=0.0013813552
890781233, name='Inner11')
                                                                                Clade(branch_length=0.000930
9502626761168, name='Bacillus_YP')
                                                                                    Clade(branch_length=1.155680
6126883368e-05, name='Inner10')
                                                                                        Clade(branch_length=0.00
09444793910730609, name='Bacillus_LM')
                                                                                            Clade(branch_length=0.00
09405347465329394, name='Inner5')
                                                                                                Clade(branch_length=
0.0, name='Bacillus_subtilis')
                                                                                                  Clade(branch_length=
0.0, name='Bacillus_BSA')
                                                                                                    Clade(branch_length=0.02263895204322852, nam
e='Inner9')
                                                                                                      Clade(branch_length=0.00163695151324758
1, name='Bacillus_amylolyticus')
                                                                                                        Clade(branch_length=0.000248062624358474
9, name='Inner8')
                                                                                                          Clade(branch_length=0.00140151009650
28005, name='Bacillus_SDLI')
                                                                                                            Clade(branch_length=0.00048350404110
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```
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02532e-05, name='Inner6')                                Clade(branch_length=0.001020
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9982638027909, name='Bacillus_velezensis')           Clade(branch_length=0.0008869738
879869749, name='Bacillus_Pc')                         Clade(branch_length=0.00016293628975172503, name='Inner232')
                                                       Clade(branch_length=0.09794938036317004, name='Inner69')
                                                       Clade(branch_length=0.04238604232713589, name='Inner
24')
                                                       Clade(branch_length=0.024329445491674567, name
='Caldicellulosiruptor_lactoaceticus')
                                                       Clade(branch_length=0.021853400879673163, name
='Caldicellulosiruptor_kronotskyensis')
                                                       Clade(branch_length=0.04055457972752957, name='Inner
25')
                                                       Clade(branch_length=0.02406084694857713, name='C
aldicellulosiruptor_kristjanssonii')
                                                       Clade(branch_length=0.0221219994227706, name='Ca
ldicellulosiruptor_hydrothermalis')
                                                       Clade(branch_length=0.0015934979800957955, name='Inner23
1')
                                                       Clade(branch_length=0.004562352880436479, name='Inne
r226')
                                                       Clade(branch_length=0.16889237083878136, name='C
ampylobacter_hominis')
                                                       Clade(branch_length=0.003075167029477466, name
='Inner224')
                                                       Clade(branch_length=0.15538600358769855, nam
e='Citrobacter_koseri')
                                                       Clade(branch_length=0.005077545264932537, na
me='Inner212')
                                                       Clade(branch_length=0.12364967830399184,
name='Bacillus_thuringiensis')
                                                       Clade(branch_length=0.00298721332621862
4, name='Inner206')
                                                       Clade(branch_length=0.01035793630019
538, name='Inner197')
                                                       Clade(branch_length=0.1144726743
0074673, name='Clostridium_perfringens')
                                                       Clade(branch_length=0.1089015010
0556803, name='Clostridium_acetobutylicum')
                                                       Clade(branch_length=0.00489335639680
1524, name='Inner201')
                                                       Clade(branch_length=0.1276252095
0351092, name='Bacillus_filamentosus')
                                                       Clade(branch_length=0.0052139484
351092225, name='Inner198')
                                                       Clade(branch_length=0.008982
522738692739, name='Inner191')
                                                       Clade(branch_length=0.10
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                                                       Clade(branch_length=0.00
1032652755732985, name='Inner189')
                                                       Clade(branch_length=
0.1032178866999326, name='Bacteroides_zoogloformans')
                                                       Clade(branch_length=
0.07538720283823895, name='Inner70')
```

```

Clade(branch_len
gth=0.030616573469575442, name='Bacillus_cereus') Clade(branch_len
gth=0.03158889307142357, name='Bacillus_anthracis') Clade(branch_length=0.003115
147864060794, name='Inner196') Clade(branch_length=0.10
023342217178036, name='Inner37') Clade(branch_length=
0.006896381335011081, name='Enterobacter_R') Clade(branch_length=
0.005356210559428143, name='Inner35') Clade(branch_len
gth=0.0020832691970692763, name='Citrobacter_rodentium') Clade(branch_len
gth=0.0007442520093398353, name='Inner34') Clade(branch_
_length=0.0005818508482564322, name='Citrobacter_farmeri') Clade(branch
_length=0.0013031632893495682, name='Citrobacter_amalonaticus') Clade(branch_length=0.00
3470341827763068, name='Inner192') Clade(branch_length=
0.1094088642126696, name='Inner27') Clade(branch_len
gth=0.0, name='Capnocytophaga_oral') Clade(branch_len
gth=0.0, name='Capnocytophaga_ochracea') Clade(branch_length=
0.002509995961252398, name='Inner190') Clade(branch_len
gth=0.004255762730228235, name='Inner187') Clade(branch
_length=0.09826949744915221, name='Capnocytophaga_gingivalis') Clade(branch
_length=0.0478190982153152, name='Inner121') Clade(br
anch_length=0.055000735030402065, name='Chryseobacterium_gallinaru
m') Clade(br
anch_length=0.05433008495074784, name='Bacteroides_salanitronis') Clade(branch_len
gth=0.00364705319372325, name='Inner188') Clade(branch
_length=0.09815672060136386, name='Bacillus_krulwichiae') Clade(branch
_length=0.005619842634969771, name='Inner186') Clade(br
anch_length=0.09069391110390561, name='Helicobacter_apodemus') Clade(br
anch_length=0.0034389823927956087, name='Inner183') Clad
e(branch_length=0.08402450149323785, name='Inner40')

Clade(branch_length=0.00884875676516031, name='Burkholderia_thailand
ensis')

Clade(branch_length=0.008116370473294027, name='Burkholderia_pseudom
allei') Clad
e(branch_length=0.006067761112794154, name='Inner177')

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Clade(branch_length=0.07825279907608049, name='Candidatus_Paracaedim  
onas')

Clade(branch_length=0.003436055777830918, name='Inner174')

Clade(branch_length=0.07430152941004796, name='Inner39')

Clade(branch_length=0.0036220063317142503, name='Enterobacter_hormae  
chei')

Clade(branch_length=0.0029755431499068064, name='Enterobacter_cloaca  
e')

Clade(branch_length=0.008208337460828594, name='Inner164')

Clade(branch_length=0.012354689567651178, name='Inner136')

Clade(branch_length=0.05697261379811111, name='Halomonas_GFAJ')

Clade(branch_length=0.0542432203206448, name='Eubacterium_eligens')

Clade(branch_length=0.004264360856477009, name='Inner159')

Clade(branch_length=0.062195205424815464, name='Candidatus_Doolittle  
a')

Clade(branch_length=0.004163182888096861, name='Inner139')

Clade(branch_length=0.059544550510938016, name='Dickeya_dadantii')

Clade(branch_length=0.0042513967086661025, name='Inner135')

Clade(branch_length=0.05213227348889464, name='Candidatus_Bathyarcha  
eota')

Clade(branch_length=0.006479884852292929, name='Inner124')

Clade(branch_length=0.03532423448076427, name='Inner55')

Clade(branch_length=0.012845690136321225, name='Bacillus_horikoshi  
i')

Clade(branch_length=0.010245733049352695, name='Inner38')

Clade(branch_length=0.0022913500932889297, name='Bacillus_paralichen  
iformis')

Clade(branch_length=0.0024211852507261267, name='Bacillus_glycinifer  
mentans')

Clade(branch_length=0.010033918205380896, name='Inner96')

Clade(branch_length=0.04023241677935782, name='Citrobacter_CFNIH')

Clade(branch_length=0.03611065579368647, name='Bacillus_coagulans')
    Clade(branch_length=0.0036936723320418856, name='Inn  
er228')
        Clade(branch_length=0.006096729538827759, name  
='Inner222')
            Clade(branch_length=0.010733029364620908, na
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me='Inner211')
Clade(branch_length=0.02268563327655265
2, name='Inner181')
Clade(branch_length=0.09981221408025
54, name='Corynebacterium_camporealensis')
Clade(branch_length=0.10471181984999
903, name='Bifidobacterium_dentium')
Clade(branch_length=0.00331062614851805
6, name='Inner207')
Clade(branch_length=0.12465144566567
14, name='Candidatus_Hepatoplasma')
Clade(branch_length=0.00757566908456
4224, name='Inner199')
Clade(branch_length=0.0053936131
24738017, name='Inner194')
Clade(branch_length=0.011910
856230769054, name='Inner178')
Clade(branch_length=0.02
2703955082644875, name='Inner161')
Clade(branch_length=
0.08038327477631593, name='Helicobacter_typhlonius')
Clade(branch_length=
0.0751303915761817, name='HelicobacterMIT')
Clade(branch_length=0.01
1587102881539843, name='Inner171')
Clade(branch_length=
0.08579506774284167, name='Campylobacter_hyointestinalis')
Clade(branch_length=
0.018911576931993426, name='Inner137')
Clade(branch_len
gth=0.06561205343262044, name='Helicobacter_cinaedi')
Clade(branch_len
gth=0.012144779743628376, name='Inner111')
Clade(branch
_length=0.0033074550968177213, name='Inner107')
Clade(br
anch_length=0.04513771642411338, name='Inner44')
Clad
e(branch_length=0.0032553698100103168, name='Campylobacter_jejuni')
Clad
e(branch_length=0.00334217967161074, name='Campylobacter_coli')
Clad
e(br
anch_length=0.05005549752499125, name='Campylobacter_cunicolorum')
Clade(branch
_length=0.024437596741071058, name='Inner72')
Clade(br
anch_length=0.015123838592755429, name='Inner59')
Clad
e(branch_length=0.013803857546788784, name='Campylobacter_antarcticu
s')
Clad
e(branch_length=0.013528847448498721, name='Campylobacter_peloridi
s')
Clade(br
anch_length=0.015154200992541417, name='Inner60')
Clad
e(branch_length=0.007520759530696475, name='Inner48')
Clade(branch_length=0.007084321445582881, name='Campylobacter_lari')
Clade(branch_length=0.007053284586462399, name='Campylobacter_hepati

```

```

    cus')

                                         Clad
e(branch_length=0.01368564951737139, name='Campylobacter_RM')
                                         Clade(branch_length=0.018234
64329797741, name='Inner175')
                                         Clade(branch_length=0.08
96813163768902, name='Candidatus_Cyclonatronum')
                                         Clade(branch_length=0.00
3663700116983512, name='Inner173')
                                         Clade(branch_length=
0.03230267702999948, name='Inner120')
                                         Clade(branch_len
gth=0.020483348783421784, name='Inner82')
                                         Clade(branch
_length=0.036294563687434944, name='Chryseobacterium_indologenes')
                                         Clade(branch
_length=0.024968395784761066, name='Inner53')
                                         Clade(br
anch_length=0.007441770246978591, name='Chryseobacterium_STRB')
                                         Clade(br
anch_length=0.007638342853869634, name='Chryseobacterium_arthrosphae
rae')
                                         Clade(branch_len
gth=0.00873437034947172, name='Inner103')
                                         Clade(branch
_length=0.04491831980128205, name='Capnocytophaga_ChDC')
                                         Clade(branch
_length=0.02447376313934002, name='Inner68')
                                         Clade(br
anch_length=0.02065495724651678, name='Capnocytophaga_stomatis')
                                         Clade(br
anch_length=0.010919029558384252, name='Inner54')
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e(branch_length=0.008111451460885905, name='Inner43')

Clade(branch_length=0.0017838550019492194, name='Capnocytophaga_cyno
degmi')

Clade(branch_length=0.0019861732732628923, name='Capnocytophaga_cani
morsus')
                                         Clad
e(branch_length=0.008853675777568376, name='Capnocytophaga_H')
                                         Clade(branch_length=
0.06455465096246049, name='Inner65')
                                         Clade(branch_len
gth=0.03011648188322949, name='Bacteroides_heparinolyticus')
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472695354, name='Inner193')
                                         Clade(branch_length=0.007886
129506294581, name='Inner182')
                                         Clade(branch_length=0.07
80608363107645, name='Inner62')
                                         Clade(branch_length=
0.01837734667821056, name='Fusobacterium_periodonticum')
                                         Clade(branch_length=
0.018380429005107057, name='Fusobacterium_nucleatum')
                                         Clade(branch_length=0.05
241748602665301, name='Inner102')
                                         Clade(branch_length=

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0.005800942462442449, name='Inner95')                                Clade(branch_len
gth=0.03990101980620961, name='Enterococcus_thailandicus')                Clade(branch_len
gth=0.04021208104204679, name='Enterococcus_faecalis')                    Clade(branch_length=
0.00598039589759522, name='Inner94')                                         Clade(branch_len
gth=0.040315319105964725, name='Enterococcus_mundtii')                     Clade(branch_len
gth=0.0063387807997845325, name='Inner83')                                 Clade(branch_
_length=0.035540630793141514, name='Enterococcus_hirae')                   Clade(branch
_length=0.03420489229828172, name='Enterococcus_durans')                  Clade(branch_length=0.109367
64052198061, name='Bifidobacterium_bifidum')                           Clade(branch_length=0.0026173089970940346, n
ame='Inner220')                                                 Clade(branch_length=0.001658328805199740
8, name='Inner219')                                              Clade(branch_length=0.00292264735065
76145, name='Inner218')                                            Clade(branch_length=0.1376229060
4433622, name='Fusobacterium_mortiferum')                            Clade(branch_length=0.0049312881
12119986, name='Inner214')                                         Clade(branch_length=0.005244
472518942445, name='Inner209')                                         Clade(branch_length=0.12
491465698874973, name='Candidatus_Desulforudis')                      Clade(branch_length=0.12
484971624404947, name='Candidatus_Caldiarchaeum')                     Clade(branch_length=0.135660
33426710844, name='Candidatus_Symbiobacter')                         Clade(branch_length=0.01140640542973
824, name='Inner210')                                               Clade(branch_length=0.1236293910
248565, name='Helicobacter_cetorum')                                     Clade(branch_length=0.0659323431
881501, name='Inner108')                                             Clade(branch_length=0.434897
1628246413, name='Campylobacter_gracilis')                           Clade(branch_length=0.083717
35178421824, name='Inner20')                                           Clade(branch_length=0.06
673008196838642, name='Inner3')                                         Clade(branch_length=
0.08859209436495014, name='Corynebacterium_imitans')                  Clade(branch_length=
0.08765672750121384, name='Corynebacterium_flavescens')               Clade(branch_length=0.15
711534687232986, name='Bifidobacterium_animalis')                     Clade(branch_length=0.00567932115770458,
name='Inner217')                                                 Clade(branch_length=0.01431518179135
1439, name='Inner205')                                              Clade(branch_length=0.0602250376
49221116, name='Inner126')                                            Clade(branch_length=0.065677
91034510592, name='Campylobacter_sputorum')
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Clade(branch_length=0.007130
760719927076, name='Inner112')
Clade(branch_length=0.06
2065050128628854, name='Campylobacter_curvus')
Clade(branch_length=0.00
9094233565998852, name='Inner97')
Clade(branch_length=
0.0523914289473503, name='Campylobacter_concisis')
Clade(branch_length=
0.05222685568978444, name='Campylobacter_iguaniorum')
Clade(branch_length=0.0194436455
7452123, name='Inner172')
Clade(branch_length=0.034902
03906597382, name='Inner138')
Clade(branch_length=0.07
137751841447731, name='Campylobacter_fetus')
Clade(branch_length=0.00
700519836214851, name='Inner130')
Clade(branch_length=
0.009507262161399799, name='Inner115')
Clade(branch_
length=0.02918767795761093, name='Inner71')
Clade(branch
_length=0.023311329455369817, name='Eubacterium_maltosivorans')
Clade(branch
_length=0.024756531053584024, name='Eubacterium_limosum')
Clade(branch_
length=0.001973962004688773, name='Inner113')
Clade(branch
_length=0.05248939192715435, name='Clostridium_novyi')
Clade(branch
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Clade(br
anch_length=0.04015483268828418, name='Clostridium_saccharobylicu
m')
Clade(br
anch_length=0.0032004924766545484, name='Inner90')
Clad
e(branch_length=0.038458910946172375, name='Clostridium_formicacetic
um')
Clad
e(branch_length=0.037884161626871915, name='Clostridium_chauvoei')
Clade(branch_length=
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Clade(branch_
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Clade(branch
_length=0.036246375550046034, name='Clostridium_scatologenes')
Clade(branch
_length=0.0334991475413772, name='Clostridium_pasteurianum')
Clade(branch_
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Clade(branch
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Clade(branch
_length=0.04070553966654744, name='Clostridium_autoethanogenum')
Clade(branch_length=0.005082
891405721518, name='Inner170')
Clade(branch_length=0.02
049412084681937, name='Inner151')
Clade(branch_length=

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0.030597457572987555, name='Inner106')                                Clade(branch_len
gth=0.04540670468602734, name='Geobacillus_WCH')                          Clade(branch_len
gth=0.04978650926307729, name='Inner29')                                    Clade(branch
_length=0.0, name='Geobacillus_YMC')                                         Clade(branch
_length=0.0, name='Geobacillus_stearothermophilus')                         Clade(branch_length=
0.0030971701367202564, name='Inner148')                                     Clade(branch_len
gth=0.07223291808682028, name='Caldicellulosiruptor_saccharolyticu
s')                                                               Clade(branch_len
gth=0.003638900951822538, name='Inner144')                                     Clade(branch
_length=0.07011516258246933, name='Caldicellulosiruptor_changbaiensi
s')                                                               Clade(branch
_length=0.06843337653157397, name='Clostridium_aceticum')                  Clade(branch_length=0.00
29581058261306734, name='Inner167')                                         Clade(branch_length=
0.003224321844266284, name='Inner166')                                     Clade(branch_len
gth=0.013687298753517096, name='Inner152')                                     Clade(branch
_length=0.07585112087940504, name='Enterococcus_avium')                      Clade(branch
_length=0.021227107207305584, name='Inner128')                               Clade(br
anch_length=0.058007392234097344, name='Fusobacterium_gonidiaforman
s')                                                               Clade(br
anch_length=0.055093456022264564, name='Clostridium_tetani')                 Clade(branch_len
gth=0.09258037325402294, name='Chryseobacterium')                           Clade(branch_length=
0.017435359470531098, name='Inner150')                                     Clade(branch_len
gth=0.05986814001112557, name='Inner66')                                     Clade(branch
_length=0.012037652596382509, name='Caldicellulosiruptor_owensensi
s')                                                               Clade(branch
_length=0.015295052398904996, name='Caldicellulosiruptor_obsidiansi
s')                                                               Clade(branch_len
gth=0.0010561059832193895, name='Inner149')                               Clade(branch
_length=0.005461199873004072, name='Inner143')                             Clade(br
anch_length=0.06903678678399247, name='Clostridium_baratii')                Clade(br
anch_length=0.004714391349843555, name='Inner140')                           Clad
e(branch_length=0.06253623897379527, name='Clostridium_septicum')            Clad
e(branch_length=0.005324269980021876, name='Inner133')
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Clade(branch_length=0.01974163364586934, name='Inner98')

Clade(branch_length=0.03686670974940398, name='Clostridium_saccharop
erbutyacetonicum')

Clade(branch_length=0.0357063345484282, name='Clostridium_bornimense')

Clade(branch_length=0.058015199530379535, name='Clostridium_argentinense')

Clade(branch_length=0.007498272323037375, name='Inner142')

Clade(branch_length=0.04346557512139898, name='Inner74')

Clade(branch_length=0.021745589361451434, name='Geobacillus_Y.MC')

Clade(branch_length=0.02160973580348735, name='Geobacillus_CT')

Clade(branch_length=0.0672790054629554, name='Bacillus_smithii')

Clade(branch_length=0.00373570904940057, name='Inner215')

Clade(branch_length=0.1278922325696153, name='Campylobacter_lanienae')

Clade(branch_length=0.0015790959787765624, name='Inner213')

Clade(branch_length=0.004710761189892457, name='Inner208')

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Clade(branch_length=0.006267741256616444, name='Inner204')

Clade(branch_length=0.11851201376523982, name='Fusobacterium_hwasookii')

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Clade(branch_length=0.12168018336077319, name='Geobacillus_lituanicus')

Clade(branch_length=0.12996920400963213, name='Candidatus_Cloacimonas')

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Clade(branch_length=0.10342933420286536, name='Eubacterium_rectale')

Clade(branch_length=0.015158120144448492, name='Inner169')

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Clade(branch_length=0.020259718265358523, name='Inner146')

Clade(branch_length=0.042071845412775255, name='Inner75')

Clade(branch_length=0.03972811783116684, name='Geobacter_metallicreducens')

Clade(branch_length=0.038499968879483454, name='Geobacter_daltonii')

Clade(branch_length=0.0743277758439726, name='Inner30')

Clade(branch_length=0.003401572693167737, name='Geobacillus_LC')
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anch_length=0.0031959767884533197, name='Geobacillus_AMOR')
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gth=0.0030369930589145694, name='Inner162')
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_length=0.09107135625294532, name='Candidatus_Endomicrobium')
                                         Clade(branch
_length=0.0785799161315976, name='Inner36')
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anch_length=0.007446341358923775, name='Bacteroides_helcogenes')
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anch_length=0.00763377174192445, name='Bacteroides_dorei')
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                                         Clade(branch
_length=0.006986402367830061, name='Inner147')
                                         Clade(br
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e(branch_length=0.06091072736878539, name='Clostridium_bolteae')
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e(branch_length=0.00338592673112037, name='Inner114')

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Clade(branch_length=0.0397384938230996, name='Clostridium_BNL')

Clade(branch_length=0.03848959288755069, name='Clostridium_cellulos
i')

Clade(branch_length=0.005610402185438304, name='Inner104')

Clade(branch_length=0.052739047886609144, name='Enterococcus_silesia
cus')

Clade(branch_length=0.009112978503588762, name='Inner91')

Clade(branch_length=0.004605947186649362, name='Inner86')

Clade(branch_length=0.03871960733117666, name='Bacillus_X()')

Clade(branch_length=0.03950847937947363, name='Bacillus_xiamenensi
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Clade(branch_length=0.02861742698865699, name='Inner50')

Clade(branch_length=0.015509747776912691, name='Bacillus_haloduran
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Clade(branch_length=0.015592985493586814, name='Bacillus_clausii')
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lum')
                                         Clad
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e(branch_length=0.005500024802817638, name='Inner92')

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Clade(branch_length=0.0032136224932578505, name='Inner31')

Clade(branch_length=0.0010713473208192326, name='Bifidobacterium_pseudocatenulatum')

Clade(branch_length=0.0008136668167867678, name='Bifidobacterium_kashwanohense')

Clade(branch_length=0.004326434057166262, name='Bifidobacterium_adolescentis')           Clade(branch_length=0.005986500911795246, name='Inner223')           Clade(branch_length=0.05708530573311614, name='Inner141')           Clade(branch_length=0.10524597938075764, name='Dickeya_chrysanthemi')           Clade(branch_length=0.03044558518097653, name='Inner87')           Clade(branch_length=0.03131374044669, name='Inner51')           Clade(branch_length=0.0408443469, name='Enterobacter_roggenkampii')           Clade(branch_length=0.0420962751, name='Enterobacter_cancerogenus')           Clade(branch_length=0.00862499659383, name='Inner76')           Clade(branch_length=0.0133528048, name='Inner61')           Clade(branch_length=0.049915, name='Dickeya_paradisiaca')           Clade(branch_length=0.050932, name='Cronobacter_muytjensii')           Clade(branch_length=0.0115058190, name='Inner63')           Clade(branch_length=0.010912, name='Inner52')           Clade(branch_length=0.03, name='Halomonas_elongata')           Clade(branch_length=0.03, name='Halomonas_campaniensis')           Clade(branch_length=0.018776, name='Inner41')           Clade(branch_length=0.03, name='Burkholderia_PAMC')           Clade(branch_length=0.03, name='Burkholderia_planitiae')           Clade(branch_length=0.013523512831626935, name='Inner200')           Clade(branch_length=0.00607189408971742, name='Inner195')           Clade(branch_length=0.06467089952284, name='Inner109')           Clade(branch_length=0.0221562205, name='Inner73')
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Clade(branch_length=0.045948
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Clade(branch_length=0.0103602733
2704989, name='Inner88')
Clade(branch_length=0.047368
14180858079, name='Inner28')
Clade(branch_length=0.00
23648463164445133, name='Corynebacterium_striatum')
Clade(branch_length=0.00
2347689027570543, name='Corynebacterium_simulans')
Clade(branch_length=0.050181
33981253137, name='Corynebacterium_glutamicum')
Clade(branch_length=0.00938999895147
3147, name='Inner184')
Clade(branch_length=0.0464556556
8670476, name='Inner131')
Clade(branch_length=0.062873
80102720697, name='Inner42')
Clade(branch_length=0.00
33701070629123253, name='Halomonas_alkaliphila')
Clade(branch_length=0.00
32274424187087314, name='Halomonas_K0')
Clade(branch_length=0.010759
563723028692, name='Inner116')
Clade(branch_length=0.03
4393692266450046, name='Inner64')
Clade(branch_length=
0.006548604950934123, name='Inner58')
Clade(branch_len
gth=0.0155324562483935, name='Geobacter_uraniireducens')
Clade(branch_len
gth=0.015570277022106005, name='Geobacter_lovleyi')
Clade(branch_length=
0.008767134917114922, name='Inner56')
Clade(branch_len
gth=0.010374196486248821, name='Geobacter_pickerlingii')
Clade(branch_len
gth=0.012245973165023516, name='Geobacter_anodireducens')
Clade(branch_length=0.00
8166392559186136, name='Inner99')
Clade(branch_length=
0.04669882535693094, name='Halomonas_beimenensis')
Clade(branch_length=
0.013268186895660956, name='Inner81')
Clade(branch_len
gth=0.033542885226663655, name='Citrobacter_youngae')
Clade(branch_len
gth=0.0007113560551459536, name='Inner80')
Clade(branch
_length=0.02635969267537369, name='Inner49')
Clade(br
anch_length=0.004246535649693017, name='Inner45')
Clad
e(branch_length=0.0013704376923037997, name='Dickeya_zeae')
Clad
e(branch_length=0.0014570835141052563, name='Dickeya_aquatica')
Clad
e(branch_length=0.006121042107140151, name='Dickeya_fangzhongdai')
Clade(branch

```

```

_length=0.011635123535747888, name='Inner67')                                Clade(br
anch_length=0.018009221895511685, name='Inner47')                                Clad
e(branch_length=0.0014402213631573568, name='Cronobacter_turicensi
s')                                         Clad
e(branch_length=0.000444792774448699, name='Inner46')                                         Clad

Clade(branch_length=0.000962560410692398, name='Cronobacter_sakazaki
i')                                         Clade(branch_length=0.0009224537269136024, name='Cronobacter_malonat
icus')                                         Clade(branch_length=0.020397941158211225, name='Cronobacter_dublinensis')                                         Clade(branch_length=0.0302565041
27150113, name='Inner156')                                         Clade(branch_length=0.091605
27484880615, name='Candidatus_Filomicrobium')                                         Clade(branch_length=0.043173
23599002515, name='Inner79')                                         Clade(branch_length=0.04
0461676446444356, name='Burkholderia_YI')                                         Clade(branch_length=0.04
342145267702405, name='Burkholderia_RPE')                                         Clade(branch_length=0.01852541409881897,
name='Inner185')                                         Clade(branch_length=0.02883854074512
454, name='Inner158')                                         Clade(branch_length=0.0685334791
5323185, name='Clostridium_SY')                                         Clade(branch_length=0.0634175104
7919039, name='Corynebacterium_casei')                                         Clade(branch_length=0.01014020130247
2063, name='Inner176')                                         Clade(branch_length=0.0986730193
1495598, name='Corynebacterium_kutscheri')                                         Clade(branch_length=0.0093802617
87777289, name='Inner165')                                         Clade(branch_length=0.033606
89087105813, name='Inner125')                                         Clade(branch_length=0.05
195850563991036, name='Corynebacterium_ureicelerivorans')                                         Clade(branch_length=0.04
794724365320932, name='Corynebacterium_resistens')                                         Clade(branch_length=0.010700
950898204366, name='Inner157')                                         Clade(branch_length=0.00
35094486429274874, name='Inner154')                                         Clade(branch_length=
0.013082501755695558, name='Inner132')                                         Clade(branch_len
gth=0.07023952762103017, name='Corynebacterium_jeikeium')                                         Clade(branch_len
gth=0.005161037883211109, name='Inner127')                                         Clade(branch
_length=0.06458725643183998, name='Corynebacterium_frankenforstens
e')                                         Clade(branch
_length=0.009281735085596418, name='Inner117')

```

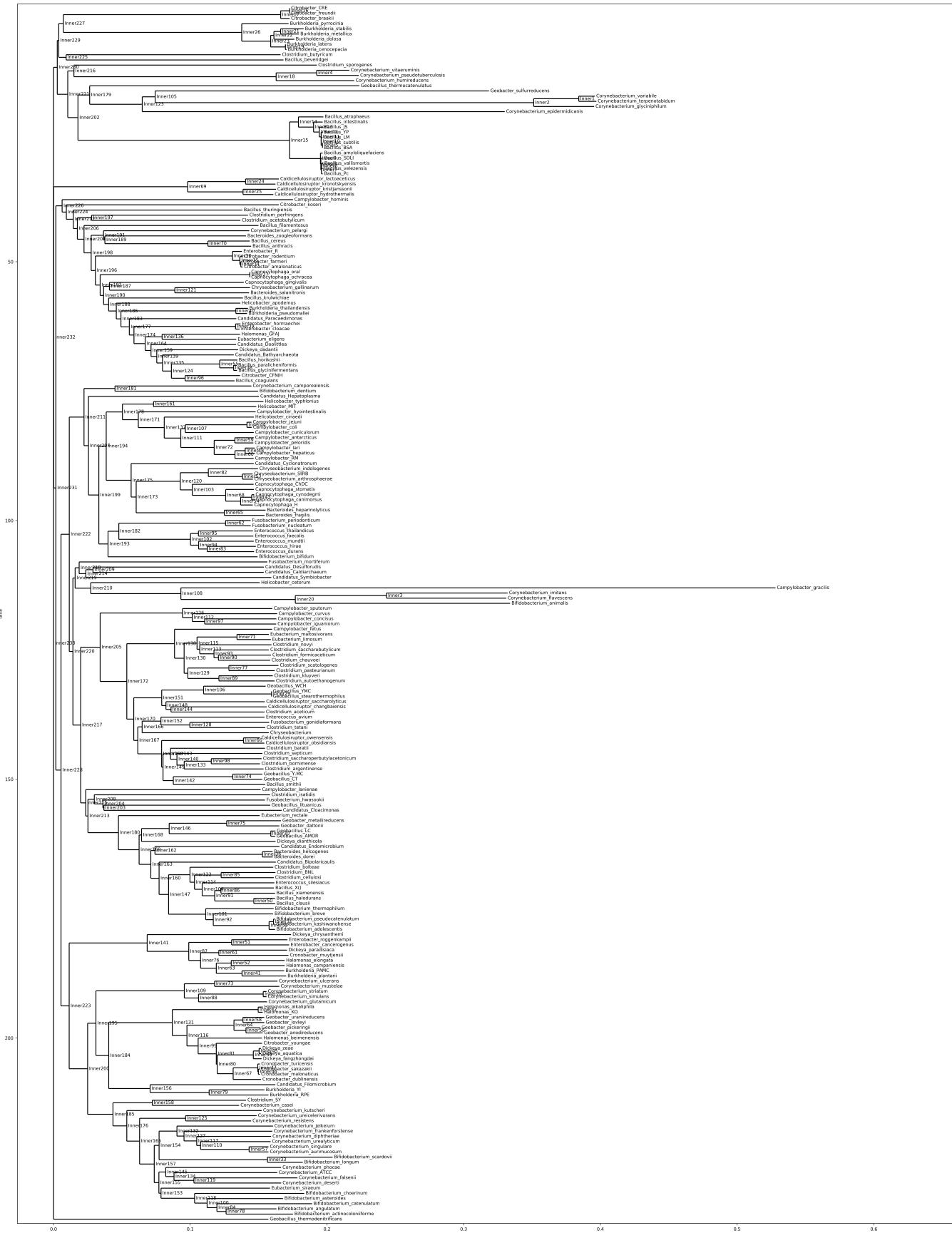
```
Clade(branch_length=0.0544016043010947, name='Corynebacterium_diphtheriae')
Clade(branch_length=0.002855700128688493, name='Inner110')
Clade(branch_length=0.05119864429099144, name='Corynebacterium_urealyticum')
Clade(branch_length=0.035512006038886074, name='Inner57')
Clade(branch_length=0.014119135289701101, name='Corynebacterium_singulare')
Clade(branch_length=0.014156076774389348, name='Corynebacterium_aurimucosum')
Clade(branch_length=0.07926846431404999, name='Inner33')
Clade(branch_length=0.04768408780789743, name='Bifidobacterium_scardovii')
Clade(branch_length=0.025831463558737805, name='Bifidobacterium_longum')
Clade(branch_length=0.0033442068854655366, name='Inner155')
Clade(branch_length=0.005031006928340326, name='Inner145')
Clade(branch_length=0.08437732069200143, name='Corynebacterium_phocae')
Clade(branch_length=0.006103357913088109, name='Inner134')
Clade(branch_length=0.07548049452167768, name='Corynebacterium_ATCC')
Clade(branch_length=0.01452893054901036, name='Inner119')
Clade(branch_length=0.0756869032496005, name='Corynebacterium_falsenii')
Clade(branch_length=0.06380414293324585, name='Corynebacterium_deserti')
Clade(branch_length=0.0015591792168057478, name='Inner153')
Clade(branch_length=0.07974019274033212, name='Eubacterium_siraeum')
Clade(branch_length=0.024642465129601887, name='Inner118')
Clade(branch_length=0.08009084421160496, name='Bifidobacterium_choerinum')
Clade(branch_length=0.00909388717388046, name='Inner100')
Clade(branch_length=0.055504280846005644, name='Bifidobacterium_asteroides')
Clade(branch_length=0.007172439229394895, name='Inner84')
Clade(branch_length=0.06961371936852032, name='Bifidobacterium_catenuatum')
Clade(branch_length=0.00672935320452403, name='Inner78')
Clade(branch_length=0.036817531990864905, name='Bifidobacterium_angulatum')
Clade(branch_length=0.0489506112702095, name='Bifidobacterium_actino')
```

```
coloniforme')
    Clade(branch_length=0.15719575566326555, name='Geobacillus_t
hermodenitrificans')
```

Out[11]:

1

visualization of tree in R was more beautiful, but more informative one was drawing using "ete3"



For project I used IQ_tree online version! the best model that it suggested was "VT+F+G4" I did bootstrap (1000 replicates). It shows "Bacillus_BSA" as an outgroup!

In [13]:

```
# Reading the resulting consensus tree from IQ_tree
tree = Phylo.read('/home/sedreh/Desktop/phyloproject_cas1/all_results/IQtree_res
ulta/bootstrap/cas1_clean_aln.contree', 'newick')
print (tree)
```

```

Tree(rooted=False, weight=1.0)
  Clade()
    Clade(branch_length=2.8398e-06, name='Bacillus_BSA')
    Clade(branch_length=0.002423471, confidence=99)
      Clade(branch_length=0.0024254074, confidence=92)
        Clade(branch_length=2.8398e-06, confidence=89)
          Clade(branch_length=0.0170170277, confidence=97)
            Clade(branch_length=0.0333432329, confidence
=99)
              Clade(branch_length=0.0921393181, confid
ence=100)
                Clade(branch_length=0.0024474798, co
nfidence=96)
                  Clade(branch_length=2.8398e-06,
confidence=50)
                    Clade(branch_length=2.8398e-
06, confidence=50)
                      Clade(branch_length=0.00
24492926, name='Bacillus_Pc')
                      Clade(branch_length=0.00
48990544, name='Bacillus_SDLI')
                      Clade(branch_length=0.002448
4914, name='Bacillus_vallismortis')
                      Clade(branch_length=0.004909201
9, name='Bacillus_amyloliquefaciens')
                      Clade(branch_length=2.4072e-06, name
='Bacillus_velezensis')
                      Clade(branch_length=0.0547222269, name
='Bacillus_atrophaeus')
                      Clade(branch_length=0.0129501021, name='Baci
llus_intestinalis')
                      Clade(branch_length=0.0097490584, name='Bacillus
_JS')
                      Clade(branch_length=2.8398e-06, name='Bacillus_YP')
                      Clade(branch_length=0.0011125045, confidence=76)
                        Clade(branch_length=3.5258988784, confidence=100)
                        Clade(branch_length=0.2496432916, confidence=35)
                          Clade(branch_length=0.3928248037, confidence
=38)
                            Clade(branch_length=0.1509259314, confid
ence=32)
                            Clade(branch_length=0.3211501356, co
nfidence=33)
                            Clade(branch_length=0.110044523
1, confidence=25)
                            Clade(branch_length=0.661203
2255, confidence=85)
                            Clade(branch_length=0.09
21759749, confidence=37)
                            Clade(branch_length=
0.3359938474, confidence=92)
                            Clade(branch_len
gth=0.3244109685, confidence=95)
                            Clade(branch_
length=0.239566793, confidence=99)
                            Clade(br
anch_length=0.1416158855, confidence=99)
                            Clad
e(branch_length=0.1099894433, confidence=97)
                            Clade(branch_
length=0.2398082087, confidence=100)

```

```
Clade(branch_length=0.1998093482, confidence=81)
Clade(branch_length=0.0987689011, confidence=81)
Clade(branch_length=0.0927497741, name='Bifidobacterium_actinocoloniiforme')
Clade(branch_length=0.3104080971, name='Bifidobacterium_asteroides')
Clade(branch_length=0.1022312207, confidence=100)
Clade(branch_length=0.0362313003, name='Bifidobacterium_angulatum')
Clade(branch_length=0.2350013872, name='Bifidobacterium_catenulatum')
Clade(branch_length=0.2204726331, name='Bifidobacterium_choerinum')
Clade(branch_length=0.1105490244, confidence=96)
Clade(branch_length=0.0607393899, confidence=47)
Clade(branch_length=0.1183842797, confidence=52)
Clade(branch_length=0.1456918736, confidence=73)
Clade(branch_length=0.4649560781, confidence=100)
Clade(branch_length=0.0514977393, name='Bifidobacterium_longum')
Clade(branch_length=0.1252107355, name='Bifidobacterium_scardovii')
Clade(branch_length=0.2314798439, confidence=100)
Clade(branch_length=0.1072417699, confidence=73)
Clade(branch_length=0.1318241457, confidence=96)
Clade(branch_length=0.0775880992, confidence=100)
Clade(branch_length=0.0614195311, confidence=100)
Clade(branch_length=0.0782960407, confidence=100)
Clade(branch_length=0.0152216544, name='Corynebacterium_aurimucosum')
Clade(branch_length=0.0274911987, confidence=99)
Clade(branch_length=0.4149698418, name='Corynebacterium_casei')
Clade(branch_length=0.0362062162, name='Corynebacterium_singulare')
Clade(branch_length=0.2000285103, name='Corynebacterium_resistens')
Clade(branch_length=0.0524380876, confidence=100)
Clade(branch_length=0.1793020999, name='Corynebacterium_urealyticum')
```

```
Clade(branch_length=0.1733426845, name='Corynebacterium_ureicelerivo  
rans')

Clade(branch_length=0.2215200877, name='Corynebacterium_diphtheria  
e')

Clade(branch_length=0.3672573393, name='Corynebacterium_frankenforst  
ense')

Clade(branch_length=0.3934217997, name='Corynebacterium_jeikeium')

Clade(branch_length=0.1486137145, confidence=100)

Clade(branch_length=0.3598671037, name='Corynebacterium_kutscheri')

Clade(branch_length=0.3129938305, name='Corynebacterium_phocae')

Clade(branch_length=0.2302358784, confidence=100)

Clade(branch_length=0.2250607721, name='Corynebacterium_deserti')

Clade(branch_length=0.2621970678, name='Corynebacterium_falsenii')

Clade(branch_length=0.3507765036, name='Corynebacterium_ATCC')  
Clad  
e(branch_length=0.3904766761, name='Eubacterium_siraeum')  
Clade(br  
anch_length=0.5318741605, name='Clostridium_SY')  
Clade(branch  
_length=0.7052293833, confidence=100)  
Clade(br  
anch_length=0.0676953515, confidence=72)  
Clad  
e(branch_length=0.1071952517, confidence=78)  
Clade(branch_length=0.1100945485, confidence=78)

Clade(branch_length=0.0425482619, name='Cronobacter_dublinensis')

Clade(branch_length=0.1030698637, confidence=75)

Clade(branch_length=0.1810042608, confidence=100)

Clade(branch_length=0.0033588753, confidence=54)

Clade(branch_length=0.038079533, name='Dickeya_fangzhongdai')

Clade(branch_length=0.006807365, name='Dickeya_zeae')

Clade(branch_length=2.8417e-06, name='Dickeya_aquatica')

Clade(branch_length=0.030736591, confidence=96)

Clade(branch_length=0.0033860676, confidence=94)

Clade(branch_length=0.0034026194, name='Cronobacter_malonaticus')

Clade(branch_length=0.0068216513, name='Cronobacter_turicensis')

Clade(branch_length=2.8398e-06, name='Cronobacter_sakazakii')
```

```
Clade(branch_length=0.1096020103, name='Citrobacter_youngae')  
e(branch_length=0.1065404579, confidence=94)  
  
Clade(branch_length=0.4471461799, confidence=100)  
  
Clade(branch_length=2.7575e-06, name='Halomonas_K0')  
  
Clade(branch_length=0.0238768307, name='Halomonas_alkaliphila')  
  
Clade(branch_length=0.0676401885, name='Halomonas_beimenensis')  
anch_length=0.2228294679, confidence=100  
  
Clade(branch_length=0.0108606208, confidence=94)  
  
Clade(branch_length=0.0441186528, name='Geobacter_anodireducens')  
  
Clade(branch_length=0.0313400317, name='Geobacter_pickeringii')  
e(branch_length=0.0581964501, confidence=100)  
  
Clade(branch_length=0.0552717366, name='Geobacter_lovleyi')  
  
Clade(branch_length=0.065938471, name='Geobacter_uraniireducens')  
gth=0.6534824776, confidence=100  
  
Clade(branch_length=0.3069838982, confidence=100)  
  
Clade(branch_length=0.1183458924, name='Burkholderia_RPE')  
  
Clade(branch_length=0.1064576044, name='Burkholderia_YI')  
  
Clade(branch_length=0.511296766, name='Candidatus_Filomicrobium')  
Clade(branch_length=0.944184903, confidence=98)  
gth=0.0913192743, confidence=99  
  
Clade(branch_length=0.1436291661, confidence=100)  
  
Clade(branch_length=0.2019489225, name='Corynebacterium_glutamicum')  
Clade(branch_length=0.2390299546, confidence=100)  
  
Clade(branch_length=2.1881e-06, name='Corynebacterium_simulans')  
Clade(branch_length=0.0158821725, name='Corynebacterium_striatum')  
Clade(branch_length=0.217928602, name='Corynebacterium_mustelae')  
gth=0.1159527488, name='Corynebacterium_ulcerans')  
Clade(branch_length=0.3559349215, confidence=69)  
Clade(branch_length=1.0031077304, confidence=99)  
gth=0.3780010995, confidence=88  
Clade(branch_length=0.1507476092, confidence=88)
```

```
Clade(br  
anch_length=0.2374426384, confidence=99)  
Clad  
e(branch_length=0.0702879078, confidence=99)  
Clade(branch_length=0.2167441173, confidence=100)  
Clade(branch_length=0.1126826258, name='Burkholderia_plantarii')  
Clade(branch_length=0.0968400512, name='Burkholderia_PAMC')  
Clade(branch_length=0.0851937054, name='Halomonas_campaniensis')  
Clad  
e(branch_length=0.1624378383, name='Halomonas_elongata')  
Clade(br  
anch_length=0.0836467411, confidence=100)  
Clad  
e(branch_length=0.1268691675, name='Cronobacter_muytjensii')  
Clad  
e(branch_length=0.2432431012, confidence=100)  
Clade(branch_length=0.281804859, name='Enterobacter_cancerogenus')  
Clade(branch_length=0.0336109143, name='Enterobacter_roggenkampii')  
Clade(branch_length=0.0382419321, name='Dickeya_paradisiaca')  
Clade(branch_length=0.1424412723, name='Dickeya_chrysanthemi')  
Clade(branch_length=3.9945224417, name='Geobacillus_thermodenitrificans')  
Clade(branch_length=0.214934  
707, confidence=28)  
Clade(branch_length=1.07  
56805384, confidence=74)  
Clade(branch_length=2.4630860776, confidence=100)  
Clade(branch_length=0.0029557896, confidence=89)  
Clade(branch_length=2.3822e-06, name='Burkholderia_cenocepacia')  
Clade(branch_length=0.0345724691, confidence=90)  
Clade(br  
anch_length=0.0152506911, confidence=92)  
Clad  
e(branch_length=0.0591192686, name='Burkholderia_dolosa')  
Clad  
e(branch_length=0.0297079839, confidence=98)  
Clade(branch_length=0.0331310736, name='Burkholderia_metallica')  
Clade(branch_length=0.1022565362, name='Burkholderia_pyrrocinia')  
Clade(br  
anch_length=0.0217707019, name='Burkholderia_stabilis')  
Clade(branch_length=2.3822e-06, name='Burkholderia_latens')  
Clade(branch_length=3.5936244002, name='Corynebacterium_epidermidicanis')  
Clade(branch_length=0.35  
728202, confidence=35)  
Clade(branch_length=
```

```
2.868617596, confidence=100)                                Clade(branch_len
gth=2.9473e-06, name='Burkholderia_pseudomallei')          Clade(branch_len
gth=0.056487695, name='Burkholderia_thailandensis')        Clade(branch_length=
3.5198409192, confidence=100)                                Clade(branch_len
gth=0.0404519658, name='Citrobacter_braakii')             Clade(branch_len
gth=2.7248e-06, confidence=92)                               Clade(branch
_length=2.8398e-06, name='Citrobacter_freundii')           Clade(branch
_length=2.8398e-06, name='Citrobacter_CRE')                 Clade(branch_length=3.323134579
5, name='Bacillus_filamentosus')                            Clade(branch_length=0.1904730188, co
nfidence=42)                                              Clade(branch_length=0.224453267
8, confidence=60)                                         Clade(branch_length=0.122745
161, confidence=37)                                       Clade(branch_length=0.25
70171946, confidence=56)                                     Clade(branch_length=
0.0950829135, confidence=36)                               Clade(branch_len
gth=0.2028244719, confidence=48)                           Clade(branch
_length=0.2130548032, confidence=63)                         Clade(br
anch_length=0.1722794121, confidence=93)                   Clad
e(branch_length=0.0814529633, confidence=98)
Clade(branch_length=0.0704901391, confidence=93)
Clade(branch_length=0.1570442941, confidence=100)
Clade(branch_length=0.1375686995, confidence=93)
Clade(branch_length=0.2929574255, confidence=100)
Clade(branch_length=0.0440165287, confidence=100)
Clade(branch_length=0.2157410228, confidence=100)
Clade(branch_length=0.0056767112, confidence=98)
Clade(branch_length=0.0230552418, name='Bifidobacterium_adolescenti
s')
Clade(branch_length=2.8398e-06, name='Bifidobacterium_pseudocatenula
tum')
Clade(branch_length=2.9123e-06, name='Bifidobacterium_kashiwanohens
e')
Clade(branch_length=0.1134978686, name='Bifidobacterium_breve')
```

```
Clade(branch_length=0.2185780882, name='Bifidobacterium_thermophilum')

Clade(branch_length=0.0673236199, confidence=100)

Clade(branch_length=0.0667204546, confidence=100)

Clade(branch_length=0.1228873945, confidence=100)

Clade(branch_length=0.0536702826, confidence=100)

Clade(branch_length=0.0903790083, confidence=100)

Clade(branch_length=0.0359424427, name='Bacillus_clausii')

Clade(branch_length=0.0627167861, name='Bacillus_halodurans')

Clade(branch_length=0.0742379051, confidence=100)

Clade(branch_length=0.1943872107, name='Bacillus_xiamenensis')

Clade(branch_length=0.0950228303, name='Bacillus_X__')

Clade(branch_length=0.2665048825, name='Enterococcus_silesiacus')

Clade(branch_length=0.0672378414, confidence=100)

Clade(branch_length=0.1444604845, name='Clostridium_cellulosi')

Clade(branch_length=0.1417523076, name='Clostridium_BNL')

Clade(branch_length=0.1518025321, confidence=100)

Clade(branch_length=0.1306351486, name='Clostridium_bolteae')

Clade(branch_length=0.3264404075, name='Eubacterium_rectale')

Clade(branch_length=0.2613776331, confidence=100)

Clade(branch_length=0.1250984376, confidence=100)

Clade(branch_length=0.0242635008, name='Bacteroides_dorei')

Clade(branch_length=0.0218306685, name='Bacteroides_helcogenes')

Clade(branch_length=0.8085938349, name='Bacteroides_zoogloformans')

Clade(branch_length=0.0904820408, confidence=100)

Clade(branch_length=0.4743152332, name='Candidatus_Bipolaricaulis')

Clade(branch_length=0.4509723514, name='Candidatus_Endomicrobium')

Clade(branch_length=0.5643777033, name='Dickeya_dianthicola')      Clad
e(branch_length=0.1274291133, confidence=96)

Clade(branch_length=0.3238864519, confidence=100)

Clade(branch_length=2.5306e-06, name='Geobacillus_AMOR')
```

```
Clade(branch_length=0.0196783769, name='Geobacillus_LC')

Clade(branch_length=0.3101596297, confidence=100)

Clade(branch_length=0.1244656698, name='Geobacter_daltonii')

Clade(branch_length=0.1535009985, name='Geobacter_metallicreducens')
    Clade(branch_length=0.4662285604, confidence=61)
        Clade(branch_length=0.0885662892, confidence=53)

Clade(branch_length=1.1908759673, confidence=86)

Clade(branch_length=0.9732023608, name='Bacillus_coagulans')

Clade(branch_length=3.3011160447, name='Campylobacter_hominis')

Clade(branch_length=2.9606845305, name='Citrobacter_koseri')
    Clade(branch_length=0.3559316345, confidence=68)

Clade(branch_length=1.5199888797, confidence=100)

Clade(branch_length=0.4291468233, name='Bacteroides_salanitronis')

Clade(branch_length=0.2011119511, name='Chryseobacterium_gallinarum')

Clade(branch_length=0.7883894061, confidence=98)

Clade(branch_length=1.7956741886, name='Capnocytophaga_gingivalis')

Clade(branch_length=2.4764058982, name='Helicobacter_apodemus')
    Clade(branch_length=0.986091747, confidence=100)
        Clade(branch_length=0.5255947416, name='Bifidobacterium_animalis')
            Clade(branch_length=0.3947211277, confidence=100)
                Clade(branch_length=0.1970484217, name='Corynebacterium_flavescens')
                    Clade(branch_length=0.2482406723, name='Corynebacterium_imitans')
                        Clade(branch_length=0.1144044546, confidence=56)
                            Clade(branch_length=0.9146961976, name='Candidatus_Cloacimonas')
                                Clade(branch_length=1.0906604609, name='Geobacillus_lituicanicus')
                                    Clade(branch_length=0.1228749576, confidence=53)
                                        Clade(branch_length=0.4727032225, confidence=88)
                                            Clade(branch_length=0.2329229576, confidence=98)
                                                Clade(branch_length=0.0413884878, confidence=88)
                                                    Clade(branch_length=0.1765750286, confidence=100)
```

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Clade(branch_length=0.1103033992, confidence=100)

Clade(branch_length=0.0762788418, confidence=100)

Clade(branch_length=0.2789203368, name='Bacillus_smithii')

Clade(branch_length=0.2056138629, confidence=100)

Clade(branch_length=0.0586956721, name='Geobacillus_CT')

Clade(branch_length=0.0888136614, name='Geobacillus_Y.MC')

Clade(branch_length=0.0673637762, confidence=100)

Clade(branch_length=0.2701241305, name='Clostridium_argentinense')

Clade(branch_length=0.0403777624, confidence=100)

Clade(branch_length=0.0931393088, confidence=100)

Clade(branch_length=0.3177345858, name='Clostridium_baratii')

Clade(branch_length=0.0352560366, confidence=45)

Clade(branch_length=0.0542051174, confidence=76)

Clade(branch_length=0.1025304213, name='Clostridium_bornimense')

Clade(branch_length=0.300781273, name='Clostridium_perfringens')

Clade(branch_length=0.1536578353, name='Clostridium_saccharoperbutylacetonicum')

Clade(branch_length=0.2879064841, name='Clostridium_septicum')

Clade(branch_length=0.2977859519, confidence=100)

Clade(branch_length=0.0134224786, name='Caldicellulosiruptor_obsidiansis')

Clade(branch_length=0.0330341273, name='Caldicellulosiruptor_owensis')          Clad

e(branch_length=0.1350092735, confidence=100)

Clade(branch_length=0.191821179, confidence=100)

Clade(branch_length=0.2176377351, name='Clostridium_tetani')

Clade(branch_length=0.1535733476, name='Fusobacterium_gonidiaformans')          Clad

Clade(branch_length=0.3438568457, name='Enterococcus_avium')                      Clade(br

anch_length=0.0501694787, confidence=48)                                         anch

e(branch_length=0.2174067564, confidence=100)                                     e

Clade(branch_length=0.0542470267, confidence=82)

Clade(branch_length=0.2926059296, name='Clostridium_aceticum')
```

```
Clade(branch_length=0.3645177604, name='Caldicellulosiruptor_saccharolyticus')

Clade(branch_length=0.0591193396, confidence=99)

Clade(branch_length=0.3591373927, name='Caldicellulosiruptor_changbaiensis')

Clade(branch_length=0.2355729151, confidence=100)

Clade(branch_length=0.1924479095, confidence=100)

Clade(branch_length=2.8398e-06, name='Geobacillus_stearothermophilus')

Clade(branch_length=2.8398e-06, name='Geobacillus_YMC')

Clade(branch_length=0.0571331079, name='Geobacillus_WCH')                                Clad

e(branch_length=0.1062349653, confidence=92)

Clade(branch_length=0.4908563008, confidence=100)

Clade(branch_length=0.1161224097, confidence=92)

Clade(branch_length=0.0883687772, confidence=99)

Clade(branch_length=0.168547876, confidence=100)

Clade(branch_length=0.0576581231, name='Clostridium_autoethanogenum')

Clade(branch_length=0.1630077905, name='Clostridium_kluyveri')

Clade(branch_length=0.1345687349, confidence=100)

Clade(branch_length=0.0935909067, name='Clostridium_pasteurianum')

Clade(branch_length=0.0954640844, name='Clostridium_scatologenes')

Clade(branch_length=0.0530208988, confidence=47)

Clade(branch_length=0.0844843319, confidence=99)

Clade(branch_length=0.050654228, confidence=100)

Clade(branch_length=0.1304791921, name='Clostridium_chauvoei')

Clade(branch_length=0.0898156221, name='Clostridium_saccharobutylicum')

Clade(branch_length=0.1271066878, name='Clostridium_formicaceticum')

Clade(branch_length=0.0273479403, confidence=46)

Clade(branch_length=0.1824481088, name='Clostridium_novyi')

Clade(branch_length=0.1833638242, confidence=100)

Clade(branch_length=0.0139208523, name='Eubacterium_limosum')
```

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Clade(branch_length=0.0880436492, name='Eubacterium_maltosivorans')

Clade(branch_length=0.283306176, name='Campylobacter_fetus')

Clade(branch_length=0.484512478, name='Chryseobacterium')
                                         Clade(branch_length=0.1187058558, confidence=64)
                                         Clade(branch_length=3.7531996844, confidence=100)
                                         Clade(branch_length=0.0420261342, confidence=75)

Clade(branch_length=0.0087057696, confidence=74)

Clade(branch_length=0.0043845056, name='Citrobacter_amalonaticus')

Clade(branch_length=0.0042586253, name='Citrobacter_farmeri')

Clade(branch_length=0.0053992104, name='Citrobacter_rodentium')
                                         Clade(branch_length=0.0189694093, name='Enterobacter_R')
                                         Clade(branch_length=0.7626787296, confidence=92)
                                         Clade(branch_length=0.1626484311, name='Campylobacter_iguaniorum')
                                         Clade(branch_length=0.0631396308, confidence=90)

Clade(branch_length=0.1150863925, confidence=100)

Clade(branch_length=0.1102162289, name='Campylobacter_concisus')

Clade(branch_length=0.1454433423, name='Campylobacter_curvus')

Clade(branch_length=0.3160379547, name='Campylobacter_sputorum')
                                         Clade(branch_length=0.3125966741, confidence=71)
                                         Clade(branch_length=3.3441736945, confidence=100)
                                         Clade(branch_length=0.1155006688, name='Corynebacterium_humireducens')
                                         Clade(branch_length=0.1374800605, confidence=94)
                                         Clade(branch_length=0.0774834195, name='Corynebacterium_pseudotuberculosis')
                                         Clade(branch_length=0.042730966, name='Corynebacterium_vitaeruminis')
                                         Clade(branch_length=0.4840137393, name='Fusobacterium_hwasookii')
                                         Clade(branch_length=0.223351575, confidence=51)
                                         Clade(branch_length=2.1694e-06, confidence=38)
                                         Clade(branch_length=0.3066715348, confidence=77)
                                         Clade(branch_length=0.3621714608, confidence=77)
                                         Clade(branch_length=0.1485550469, confidence=51)
```

Clad

e(branch_length=0.6282325688, confidence=97)

Clade(branch_length=1.9663791803, name='Candidatus_Bathyarchaeota')

Clade(branch_length=2.2720843676, name='Bacillus_thuringiensis')

Clad

e(branch_length=3.912760253, confidence=100)

Clade(branch_length=0.0647157327, confidence=53)

Clade(branch_length=0.2923874817, confidence=66)

Clade(branch_length=0.1434712108, name='Caldicellulosiruptor_hydrothermialis')

Clade(branch_length=2.9301e-06, name='Caldicellulosiruptor_kristjanssonii')

Clade(branch_length=2.7634e-06, name='Caldicellulosiruptor_lactoaceticus')

Clade(branch_length=0.0784950545, name='Caldicellulosiruptor_kronotskyensis')

Clade(branch_length=0.8158792108, name='Campylobacter_lanienae')

Clade(branch_length=0.3861621466, confidence=97)

Clade(branch_length=0.6334883524, name='Campylobacter_gracilis')

Clade(branch_length=0.9368325491, name='Helicobacter_cetorum')

Clade(branch_length=0.2731565795, confidence=89)

Clade(branch_length=1.3395582191, name='Candidatus_Caldiarchaeum')

Clade(branch_length=0.6768252017, name='Candidatus_Desulforudis')

Clade(branch_length=3.5328775612, name='Corynebacterium_pelargi')

Clade(branch_length=0.0539117041, confidence=30)

Clade(branch_length=0.1198817545, confidence=56)

Clade(branch_length=0.2874492447, confidence=93)

Clade(branch_length=0.3337603785, confidence=63)

Clade(branch_length=0.2355921537, confidence=68)

Clade(branch_length=0.2078477153, confidence=100)

Clade(branch_length=1.1168685961, name='Bifidobacterium_bifidum')

Clade(branch_length=0.1397627817, confidence=100)

Clade(branch_length=0.5763988951, confidence=100)

Clade(branch_length=0.0909725705, confidence=86)

```
Clade(branch_length=0.1101330042, confidence=86)

Clade(branch_length=0.1292688758, name='Enterococcus_durans')

Clade(branch_length=0.1917377133, name='Enterococcus_hirae')

Clade(branch_length=0.0990450186, name='Enterococcus_mundtii')

Clade(branch_length=0.0466011606, confidence=97)

Clade(branch_length=0.1869342922, name='Enterococcus_faecalis')

Clade(branch_length=0.1879810583, name='Enterococcus_thailandicus')

Clade(branch_length=0.6953107616, confidence=100)

Clade(branch_length=0.0332879551, name='Fusobacterium_nucleatum')

Clade(branch_length=0.0649666323, name='Fusobacterium_periodonticum')

Clade(branch_length=0.1959248862, confidence=98)

Clade(branch_length=0.1524403428, confidence=100)

Clade(branch_length=0.7363640211, confidence=100)

Clade(branch_length=0.9081228755, name='Bifidobacterium_dentium')

Clade(branch_length=0.6855685251, name='Corynebacterium_camporealensis')

Clade(branch_length=0.3343839216, confidence=100)

Clade(branch_length=0.1312235703, confidence=99)

Clade(branch_length=0.4098695755, confidence=100)

Clade(branch_length=0.1390216367, name='Bacteroides_fragilis')

Clade(branch_length=0.0858734702, name='Bacteroides_heparinolyticus')

Clade(branch_length=0.5664221952, name='Candidatus_Cyclonatronum')

Clade(branch_length=0.373505457, confidence=100)

Clade(branch_length=0.0675923215, confidence=99)

Clade(branch_length=0.1210956405, confidence=100)

Clade(branch_length=0.0009160469, confidence=99)

Clade(branch_length=2.844e-06, name='Capnocytophaga_H')

Clade(branch_length=0.1129567597, name='Capnocytophaga_stomatis')

Clade(branch_length=0.0269902137, confidence=100)

Clade(branch_length=0.0065587133, name='Capnocytophaga_canimorsus')
```

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Clade(branch_length=0.006652314, name='Capnocytophaga_cynodegmi')

Clade(branch_length=0.1690115611, confidence=100)

Clade(branch_length=0.0421761537, confidence=100)

Clade(branch_length=0.0438930197, name='Chryseobacterium_arthrosphaerae')

Clade(branch_length=0.0127154371, name='Chryseobacterium_StRB')

Clade(branch_length=0.0785277869, name='Chryseobacterium_indologenes')

Clade(branch_length=0.1740067293, name='Capnocytophaga_ChDC')      Clad
e(branch_length=0.3705618775, confidence=100)

Clade(branch_length=0.1819771459, confidence=100)

Clade(branch_length=0.2408138884, confidence=100)

Clade(branch_length=0.0683231374, confidence=66)

Clade(branch_length=0.050577318, confidence=67)

Clade(branch_length=0.1776663879, confidence=100)

Clade(branch_length=0.0708125435, confidence=100)

Clade(branch_length=0.0693800092, name='Campylobacter_RM')

Clade(branch_length=0.007055235, confidence=100)

Clade(branch_length=0.0395468433, name='Campylobacter_hepaticus')

Clade(branch_length=0.0098129232, name='Campylobacter_lari')

Clade(branch_length=0.0421612412, confidence=100)

Clade(branch_length=0.0700650796, name='Campylobacter_peloridis')

Clade(branch_length=0.0252939394, name='Campylobacter_antarcticus')

Clade(branch_length=0.2346873765, name='Campylobacter_cuniculorum')

Clade(branch_length=0.2201478979, confidence=100)

Clade(branch_length=0.0195350482, name='Campylobacter_coli')

Clade(branch_length=0.0037515131, name='Campylobacter_jejuni')

Clade(branch_length=0.3280029173, name='Helicobacter_cinaedi')

Clade(branch_length=0.6406302311, name='Campylobacter_hyointestinalis')

Clade(branch_length=0.2860197503, confidence=100)

Clade(branch_length=0.4436066651, name='Helicobacter/MIT')
```

```
Clade(branch_length=0.3659203459, name='Helicobacter_typhlonius')
    Clade(branch_length=1.1967410754, name='Candidatus_Hepatoplasma')
        Clade(branch_length=4.2667141115, name='Geobacter_sulfurreducens')
            Clade(branch_length=0.3462872136, confidence=100)
                Clade(branch_length=0.6454502405, name='Clostridium_isatidis')
                    Clade(branch_length=1.3211238121, name='Fusobacterium_mortiferum')
                        Clade(branch_length=0.7431680938, confidence=94)
                            Clade(branch_length=0.5269696345, confidence=100)
                                Clade(branch_length=0.364413921, name='Candidatus_Symbiobacter')
                                    Clade(branch_length=1.036664138, name='Halomonas_GFAJ')
                                        Clade(branch_length=1.7263068154, name='Eubacterium_eligens')
                                            Clade(branch_length=0.4809377696, confidence=59)
                                                Clade(branch_length=0.1027560461, confidence=69)
                                                    Clade(branch_length=0.3961527604, confidence=76)
                                                        Clade(branch_length=0.9670187476, confidence=92)
                                                            Clade(branch_length=1.9021658926, confidence=97)
                                                                Clade(branch_length=0.0300909762, confidence=50)
                                                                    Clade(branch_length=0.0175846836, name='Bacillus_glycinifementans')
                                                                        Clade(branch_length=0.3658600164, name='Bacillus_horikoshii')
                                                                            Clade(branch_length=2.5758e-06, name='Bacillus_paralicheniformis')
                                                                                Clade(branch_length=1.8099402071, confidence=100)
                                                                                    Clade(branch_length=2.8398e-06, name='Capnocytophaga_ochracea')
                                                                                        Clade(branch_length=2.8398e-06, name='Capnocytophaga_oral')
                                                                                            Clade(branch_length=3.3366228108, name='Dickeya_dadantii')
                                                                                                Clade(branch_length=0.4954042118, confidence=73)
                                                                                                    Clade(branch_length=0.3714877245, confidence=86)
                                                                                                        Clade(branch_length=0.1786667261, confidence=83)
                                                                                                            Clade(branch_length=3.1674479251, name='Clostridium_acetobutylicum')
                                                                                                                Clade(branch_length=2.378107607, name='Citrobacter_CFNIH')
                                                                                                                    Clade(branch_length=3.1277452562, name='Geobacillus_thermocatenulatus')
                                                                                                                        Clade(branch_length=2.8315432579, confidence=99)
```

```

Clade(branch_len
gth=0.0047789634, confidence=99)
    Clade(branch
_length=0.131702578, name='Corynebacterium_glyciniphilum')
        Clade(branch
_length=0.0338650653, name='Corynebacterium_terpenotabidum')
            Clade(branch_len
gth=2.2156e-06, name='Corynebacterium_variabile')
                Clade(branch_length=3.695540
1839, name='Bacillus_krulwichiae')
                    Clade(branch_length=2.6955157952, name
='Candidatus_Doolittlea')
                        Clade(branch_length=2.2890377879, confidence
=100)
                            Clade(branch_length=2.4955e-06, name='En
terobacter_cloacae')
                                Clade(branch_length=0.0208250654, name
='Enterobacter_hormaechei')
                                    Clade(branch_length=0.0921023417, confidence=35)
                                        Clade(branch_length=0.1224463808, confidence
=30)
                                            Clade(branch_length=2.8112068476, confid
ence=100)
                                                Clade(branch_length=0.121609736, nam
e='Bacillus_anthracis')
                                                    Clade(branch_length=0.0471874386, na
me='Bacillus_cereus')
                                                        Clade(branch_length=0.4383711062, confid
ence=94)
                                                            Clade(branch_length=1.5800238079, na
me='Candidatus_Paracaedimona')
                                                                Clade(branch_length=2.6733979337, na
me='Clostridium_sporogenes')
                                                                    Clade(branch_length=0.9096411712, confidence
=61)
                                                                        Clade(branch_length=2.0553890107, name
='Bacillus_beveridgei')
                                                                            Clade(branch_length=2.8886666441, name
='Clostridium_butyricum')
                                                                                Clade(branch_length=0.0013174194, name='Bacillus_L
M')
                                                                                    Clade(branch_length=2.8398e-06, name='Bacillus_subtilis')

```

In [14]:

```

#Root the tree at Bacillus_BSA as an outgroup and draw rooted tree using ete3
#in the file called "rooted_cas1_clean_aln.treefile.pdf"

tree.root_with_outgroup({'name': 'Bacillus_BSA'})

Phylo.write(tree, 'rooted_iqtree.newick', 'newick')

```

Out[14]:

1

In [15]:

```
#Look at the branch length of origin tree
iqtree_rooted = Phylo.read('/home/sedreh/Desktop/phyloproject_cas1/all_results/I
Qtree_resulta/bootstrap/rooted_iqtree.newick', 'newick')
print("Total branch length %0.2f" % iqtree_rooted.total_branch_length())
```

Total branch length 187.38

In [20]:

```
# “collapse” clades with bootstrap support < 95%. Because the tree is huge and I
need the most confident branches.
iqtree_rooted.collapse_all(lambda c: c.confidence is not None and c.confidence <
95)
print("Total branch length %0.2f" % iqtree_rooted.total_branch_length())
```

Total branch length 291.33

In [21]:

```
#I wrote collapsesed rooted tree in new file and draw it using ete3. the pdf file
attached called
#"collapsed_rooted_cas1.tree.pdf" has attached to the results.
Phylo.write(iqtree_rooted, 'collapsed_iqtree.nwk', 'newick')
```

Out[21]:

1

In []: