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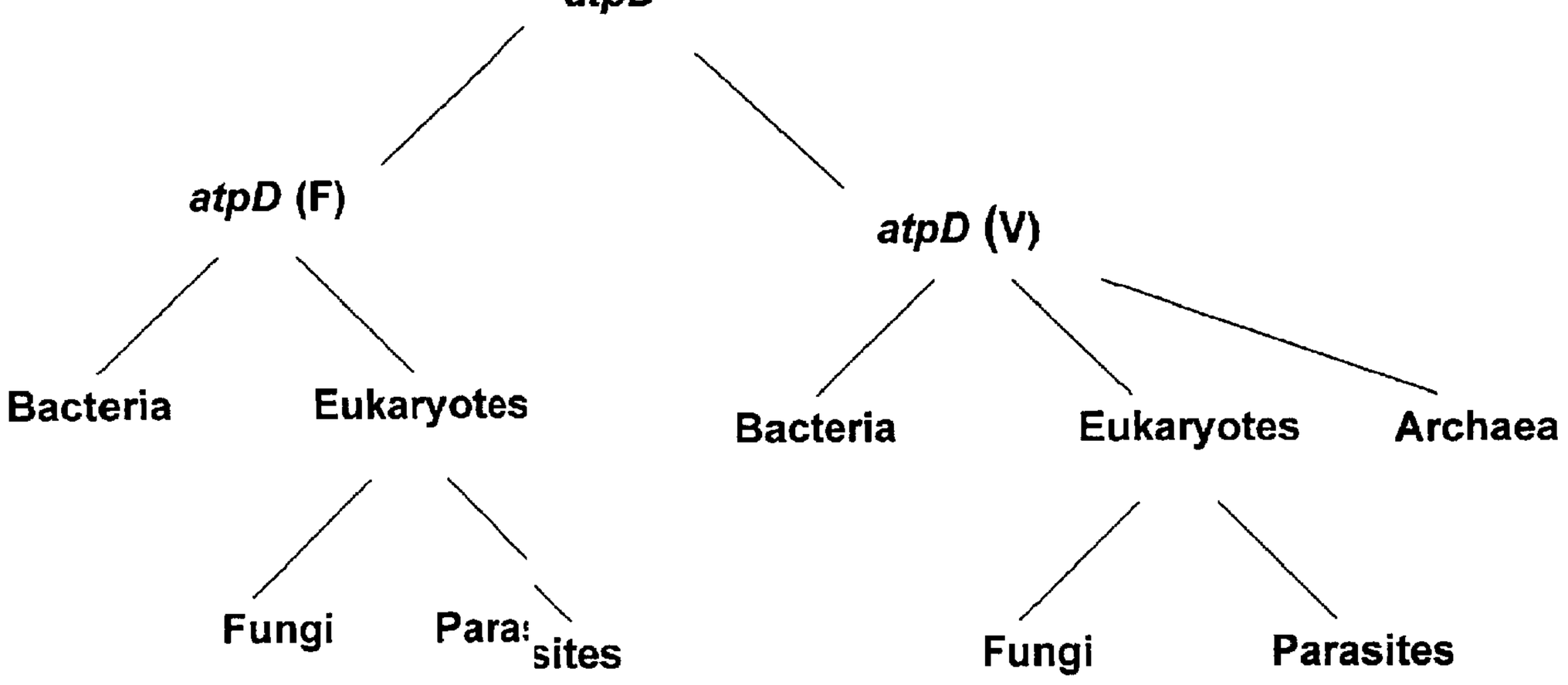
(54) Titre : ACIDES NUCLEIQUES ET METHODES DE DETECTION DE KLEBSIELLA
(54) Title: NUCLEIC ACIDS AND METHODS FOR THE DETECTION OF KLEBSIELLA



(57) Abrégé/Abstract:
Nucleic acids, methods and diagnostic kits for detecting in a sample at least one microorganism belonging to the Kiebsiella pneumoniae, Kiebsiella oxytoca, Kiebsiella planticola or Kiebsiella ornlthinolytica bacterial species and/or Kiebsiella genus are provided. The method comprises i) contacting the sample with at least one species-specific or genus-specific oligonucleotide selected to be sufficiently complementary to hybridize to at least one tuf nucleic acid specific to the species or genus; ii) Allowing the oligonucleotide and the tuf nucleic acid to hybridize under conditions such that the oligonucleotide hybridizes to the tuf nucleic acid, wherein the oligonucleotide hybridizes only to microorganisms belonging to the target Kiebsiella species or genus and does not detectably hybridize to tuf nucleic acids from nontarget microorganisms; and iii) Testing for hybridization of the oligonucleotide to the tuf nucleic acid.

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(13) C

(51) Cl.Int./Int.Cl. (suite/continued) *C12Q 1/68* (2006.01), *C40B 30/04* (2006.01), *C40B 40/06* (2006.01), *C40B 40/08* (2006.01), *C40B 50/06* (2006.01), *G01N 33/50* (2006.01), *G06F 19/20* (2011.01)

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ABSTRACT

Four highly conserved genes, encoding translation elongation factor Tu, translation elongation factor G, the catalytic subunit of proton-translocating ATPase and the RecA recombinase, are used to generate a sequence repertory or bank and species-specific, genus-specific, family-specific, group-specific and universal nucleic acid probes and amplification primers to rapidly detect and identify algal, archaeal, bacterial, fungal and parasitical microorganisms from specimens for diagnosis. The detection of associated antimicrobial agents resistance and toxin genes are also under the scope of the present invention.

CLAIMS

1. A method for detecting the presence or absence in a sample of at least one microorganism belonging to the *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella planticola* or *Klebsiella ornithinolytica* bacterial species and/or *Klebsiella* genus comprising:

i) Contacting said sample with at least one species-specific or genus-specific oligonucleotide selected to be sufficiently complementary to hybridize to at least one *tuf* nucleic acid specific to said species or genus;

ii) Allowing said oligonucleotide and said *tuf* nucleic acid to hybridize under conditions such that said oligonucleotide hybridizes to said *tuf* nucleic acid, wherein said oligonucleotide hybridizes only to microorganisms belonging to the target *Klebsiella* species or *Klebsiella* genus and does not detectably hybridize to *tuf* nucleic acids from non-target microorganisms; and

iii) Testing for hybridization of said at least one species-specific or genus-specific oligonucleotide to said *tuf* nucleic acid,

wherein said at least one species-specific oligonucleotide is capable of hybridizing to the following target nucleic acid(s): (a) a *tuf* nucleic acid consisting of SEQ ID NO: 99 or the complement thereof, for the detection of *K. ornithinolytica*; (b) *tuf* nucleic acids consisting of SEQ ID NOs: 100 and 1839 or complements thereof, for the detection of *K. oxytoca*; (c) *tuf* nucleic acids consisting of (i) SEQ ID NOs: 102, 103 and 104 or complements thereof; or (ii) SEQ ID NO: 2261 or a complement thereof, for the detection of *K. pneumoniae*; or (d) a *tuf* nucleic acid consisting of SEQ ID NO: 101 or a complement thereof for the detection of *K. Planticola;*

wherein said at least one genus-specific oligonucleotide is capable of hybridizing to each of the following target nucleic acids: (i) *tuf* nucleic acids consisting of SEQ ID NOs: 99-104 and 1839, or complements thereof; or (ii) *tuf* nucleic acids consisting of SEQ ID NOs: 1839 and 2261, or complements thereof; and

wherein said oligonucleotide is capable of:

(i) specifically amplifying its target nucleic acid(s) using PCR conditions comprising for each amplification cycle a denaturation step of 1 s at 95°C and an annealing-extension step of 30 s at 50-65°C ; or

(ii) specifically hybridizing to its target nucleic acid(s) under the following conditions:

- hybridization at 55°C for 30 min in a solution comprising 1.5M NaCl and 10mM EDTA followed by the following post-hybridization washings:

aa) twice in 2X SSC containing 0.1% SDS at 55°C for 15 min; and

bb) four times in 0.1X SSC containing 0.1% SDS at 55°C for 15 min.

2. The method of claim 1, wherein said method comprises hybridizing said sample with at least one species-specific oligonucleotide for the detection of *Klebsiella pneumoniae,* wherein said at least one species-specific oligonucleotide comprises a probe or primer which specifically hybridizes to a polynucleotide defined by SEQ ID NO: 1329 or 1330 or a complement thereof.

3. The method of claim 2, wherein said at least one oligonucleotide comprises a first primer which specifically hybridizes to a polynucleotide defined by SEQ ID NO: 1329 and a second primer which specifically hybridizes to a polynucleotide defined by SEQ ID NO: 1330, for the detection of *K. pneumoniae*.

4. The method of claim 2, wherein said at least one oligonucleotide comprises a primer pair consisting of a first primer consisting of the polynucleotide defined by SEQ ID NO: 1329 and a second primer consisting of the polynucleotide defined by SEQ ID NO: 1330, for the detection of *K. pneumoniae*.

5. The method of any one of claims 1-4, further comprising the detection of *K. pneumoniae* subs. *pneumoniae*, wherein said detection of *K. pneumoniae* subs. *pneumoniae* comprises contacting said sample with at least one oligonucleotide which specifically hybridizes to a polynucleotide defined by SEQ ID NO: 103 or a complement thereof.

6. The method of any one of claims 1-5, further comprising the detection of *K. pneumoniae* subs. *ozaenae*, wherein said detection of *K. pneumoniae* subs. *ozaenae* comprises contacting said sample with at least one oligonucleotide which specifically hybridizes to a polynucleotide defined by SEQ ID NO: 102 or a complement thereof.

7. The method of any one of claims 1-6, further comprising the detection of *K. pneumoniae* subs. *rhinoscleromatis*, wherein said detection of *K. pneumoniae* subs. *rhinoscleromatis* comprises contacting said sample with at least one oligonucleotide which specifically hybridizes to a polynucleotide defined by SEQ ID NO: 104 or a complement thereof.

8. The method of any one of claims 1-7, wherein said *Klebsiella* genus consists of *K. oxytoca*, *K. ornithinolytica*, *K. planticola* and *K. pneumoniae*.

9. The method of any one of claims 1-8, further comprising detecting the presence or absence in said sample of a shv-type beta-lactamase conferring antibiotic resistance in *Klebsiella pneumoniae* comprising detecting the presence of one or more of the following amino acid substitutions in said shv-type beta-lactamase:

   a. Ser 130 to Gly;
   b. Asp 179 to Ala or Asn;
   c. Gly 238 to Ser; and
   d. Glu 240 to Lys.

10. The method of claim 9, wherein said method comprises contacting said sample with at least one oligonucleotide which specifically hybridizes to a shv-type beta-lactamase polynucleotide defined by SEQ ID NO: 1884, 1885, 1896 or a complement thereof.

11. The method of claim 10, wherein said at least one oligonucleotide comprises a first primer consisting of the polynucleotide defined by SEQ ID NO: 1884 and a second primer consisting of the polynucleotide defined by SEQ ID NO: 1885.

12. The method of any one of claims 9-11, wherein said method comprises contacting said sample with at least one oligonucleotide which specifically

hybridizes to a shv-type beta-lactamase polynucleotide defined by SEQ ID NO: 1886, 1887, 1888, 1889, 1890, 1891, 1892, 1893, 1894, 1895, 1897 or 1898 or to a complement thereof.

13. The method of claim 12, wherein said at least one oligonucleotide comprises a probe consisting of the polynucleotide defined by SEQ ID NO: 1886, 1887, 1888, 1889, 1890, 1891, 1892, 1893, 1894, 1895, 1897 or 1898.

14. The method of claim 13, wherein said at least one oligonucleotide comprises multiple probes consisting of the polynucleotides defined by SEQ ID NOs: 1886, 1887, 1888, 1889, 1890, 1891, 1892, 1893, 1894, 1895, 1897 and 1898.

15. The method of any one of claims 9-14, wherein said antibiotic resistance comprises a resistance to third generation cephalosporins and to beta-lactamase inhibitors.

16. The method of any one of claims 1-15, further comprising detecting the presence or absence in said sample of one or more genes conferring antibiotic resistance to quinolone in *Klebsiella pneumoniae* comprising detecting the presence of one or more of the following amino acid substitutions:

    i) a) Ser 83 to Tyr or Phe; and b) Asp-87 to Gly, Ala or Asn in the *gyrA* subunit of DNA gyrase encoded by the *gyrA* gene; and
    ii) a) Ser 80 to Ile or Arg; and b) Glu 84 to Gly or Lys in the *parC* subunit of topoisomerase IV encoded by the *parC* gene.

17. The method of claim 16, wherein said method comprises contacting said sample with at least one oligonucleotide which specifically hybridizes to a *gyrA* polynucleotide defined by SEQ ID NO: 1936, 1937 or 1942 or to a complement thereof.

18. The method of claim 17, wherein said at least one oligonucleotide comprises a first primer consisting of the polynucleotide defined by SEQ ID NO: 1936 and a second primer consisting of the polynucleotide defined by SEQ ID NO: 1937 or 1942, for detecting the *gyrA* gene.

19. The method of any one of claims 16-18, wherein said method comprises contacting said sample with at least one oligonucleotide which specifically hybridizes to a polynucleotide defined by SEQ ID NO: 1945, 1946, 1947, 1948 or 1949 or to a complement thereof, for the detection of a *gyrA* gene conferring antibiotic resistance.

20. The method of claim 19, wherein said at least one oligonucleotide comprises a probe consisting of the polynucleotide defined by SEQ ID NO: 1945, 1946, 1947, 1948 or 1949 or to a complement thereof, for the detection of a *gyrA* gene conferring antibiotic resistance.

21. The method of claim 20, wherein said at least one oligonucleotide comprises multiple probes consisting of the polynucleotides defined by SEQ ID NOs: 1945, 1946, 1947, 1948 and 1949.

22. The method of any one of claims 16 -21, wherein said method comprises contacting said sample with at least one oligonucleotide which specifically hybridizes to a *parC* polynucleotide defined by SEQ ID NO: 1934, 1935 or 1936 or to a complement thereof.

23. The method of claim 22, wherein said at least one oligonucleotide comprises a first primer consisting of the polynucleotide defined by SEQ ID NO: 1935 and a second primer consisting of the polynucleotide defined by SEQ ID NO: 1934 or 1936, for detecting the *parC* gene.

24. The method of any one of claims 16-23, wherein said method comprises contacting said sample with at least one oligonucleotide which specifically hybridizes to a polynucleotide defined by SEQ ID NO: 1950, 1951, 1952 or 1953 or to a complement thereof, for the detection of a *parC* gene conferring antibiotic resistance.

25. The method of claim 24, wherein said at least one oligonucleotide comprises a probe consisting of the polynucleotide defined by SEQ ID NO: 1950, 1951, 1952 or 1953 or to a complement thereof, for the detection of a *parC* gene conferring antibiotic resistance.

26. The method of claim 25, wherein said at least one oligonucleotide comprises multiple probes consisting of the polynucleotides defined by SEQ ID NOs: 1950, 1951, 1952 and 1953.

27. The method of any one of claims 1-26, wherein step (iii) is performed by using a nucleic acid target amplification method.

28. The method of any one of claims 1-26, wherein step (iii) is based on a signal amplification method.

29. The method of any one of claims 1-28, wherein a plurality of primers and/or probes is used in a multiplex PCR assay.

30. An isolated *tuf* oligonucleotide primer or probe for detecting the presence or absence in a sample of at least one microorganism belonging to *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella planticola* or *Klebsiella ornithinolytica* bacterial species and/or *Klebsiella* genus, wherein said oligonucleotide hybridizes to the following target nucleic acid(s):

(a) a *tuf* nucleic acid consisting of SEQ ID NO: 99 or the complement thereof, for the detection of *K. ornithinolytica*;

(b) *tuf* nucleic acids consisting of SEQ ID NOs: 100 and 1839 or complements thereof, for the detection of *K. oxytoca*;

(c) *tuf* nucleic acids consisting of (i) SEQ ID NOs: 102, 103, and 104 or complements thereof; or (ii) SEQ ID NO: 2261 or a complement thereof, for the detection of *K. pneumoniae*;

(d) a *tuf* nucleic acid consisting of SEQ ID NO: 101 or a complement thereof, for the detection of *K. planticola*; or

(e) each of the *tuf* nucleic acids consisting of (i) SEQ ID NOs: 99-104 and 1839, or complements thereof; or (ii) SEQ ID NOs:1839 and 2261 or complements thereof, for the detection of *Klebsiella* genus,

for detecting the presence or absence in a sample of at least one microorganism belonging to *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella planticola* or *Klebsiella ornithinolytica* bacterial species and/or *Klebsiella* genus, wherein said oligonucleotide is capable of:

(i) specifically amplifying its target nucleic acid(s) using PCR conditions comprising for each amplification cycle a denaturation

step of 1 s at 95°C and an annealing-extension step of 30 s at 50-65°C ; or

(ii) specifically hybridizing to its target nucleic acid(s) under the following conditions:

- hybridization at 55°C for 30 min in a solution comprising 1.5M NaCl and 10mM EDTA followed by the following post-hybridization washings:

aa) twice in 2X SSC containing 0.1% SDS at 55°C for 15 min; and

bb) four times in 0.1X SSC containing 0.1% SDS at 55°C for 15 min.

31. An isolated *tuf* oligonucleotide consisting of the polynucleotide defined by SEQ ID NO: 1329 or 1330 or the complement thereof.

32. An isolated *tuf* nucleic acid comprising SEQ ID NOs: 99-104, 1839 or 2261 or a complement thereof.

33. The isolated oligonucleotide of claim 30, wherein said oligonucleotide consists of 12-30 nucleotides in length.

34. A kit for detecting the presence or absence in a sample of at least one microorganism belonging to the *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella planticola* or *Klebsiella ornithinolytica* bacterial species and/or *Klebsiella* genus, said kit comprising oligonucleotide probes and/or primers, wherein at least one of said oligonucleotide probes and/or primers is capable of specifically hybridizing to the following target nucleic acid(s):

(a) a *tuf* nucleic acid consisting of SEQ ID NO: 99 or the complement thereof, for the detection of *K. ornithinolytica*;

(b) *tuf* nucleic acids consisting of SEQ ID NOs: 100 and 1839 or complements thereof, for the detection of *K. oxytoca*;

(c) *tuf* nucleic acids consisting of (i) SEQ ID NOs: 102, 103 and 104 or complements thereof; or (ii) SEQ ID NO: 2261 or a complement thereof, for the detection of *K. pneumoniae*;

(d) a *tuf* nucleic acid consisting of SEQ ID NO: 101 or a complement thereof, for the detection of *K. planticola*; or

(e) each of the *tuf* nucleic acids consisting of (i) SEQ ID NOs: 99-104, and 1839, or complements thereof; or (ii) SEQ ID NOs: 1839 and 2261 or complements thereof, for the detection of *Klebsiella* genus,

wherein said at least one of said oligonucleotides probes and/or primers is capable of:

(i) specifically amplifying its target nucleic acid(s) using PCR conditions comprising for each amplification cycle a denaturation step of 1 s at 95ºC and an annealing-extension step of 30 s at 50-65ºC ; or

(ii) specifically hybridizing to its target nucleic acid(s) under the following conditions:

- hybridization at 55°C for 30 min in a solution comprising 1.5M NaCl and 10mM EDTA followed by the following post-hybridization washings:

aa) twice in 2X SSC containing 0.1% SDS at 55°C for 15 min; and

bb) four times in 0.1X SSC containing 0.1% SDS at 55°C for 15 min.

35. The kit of claim 34, wherein said kit comprises an oligonucleotide consisting of a polynucleotide defined by SEQ ID NO: 1329 or 1330 or a complement thereof.

36. A vector comprising the isolated oligonucleotide of any one of claims 30, 31 and 33 or the isolated nucleic acid of claim 32.

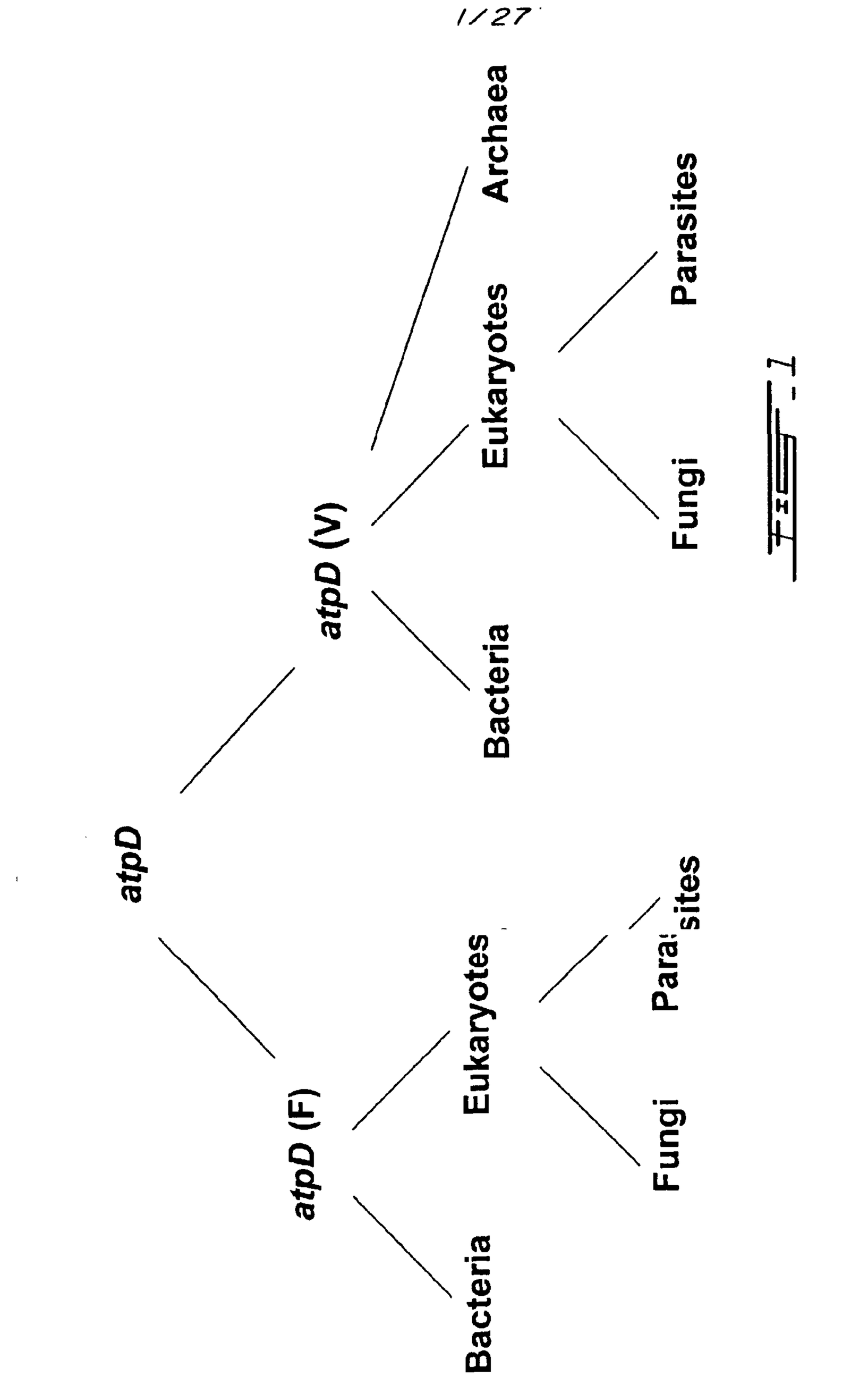

atpD
atpD (V)
Archaea
Eukaryotes
Parasites
Fungi
Bacteria
atpD (F)
Eukaryotes
Parasites
Fungi
Bacteria

Fig. 1

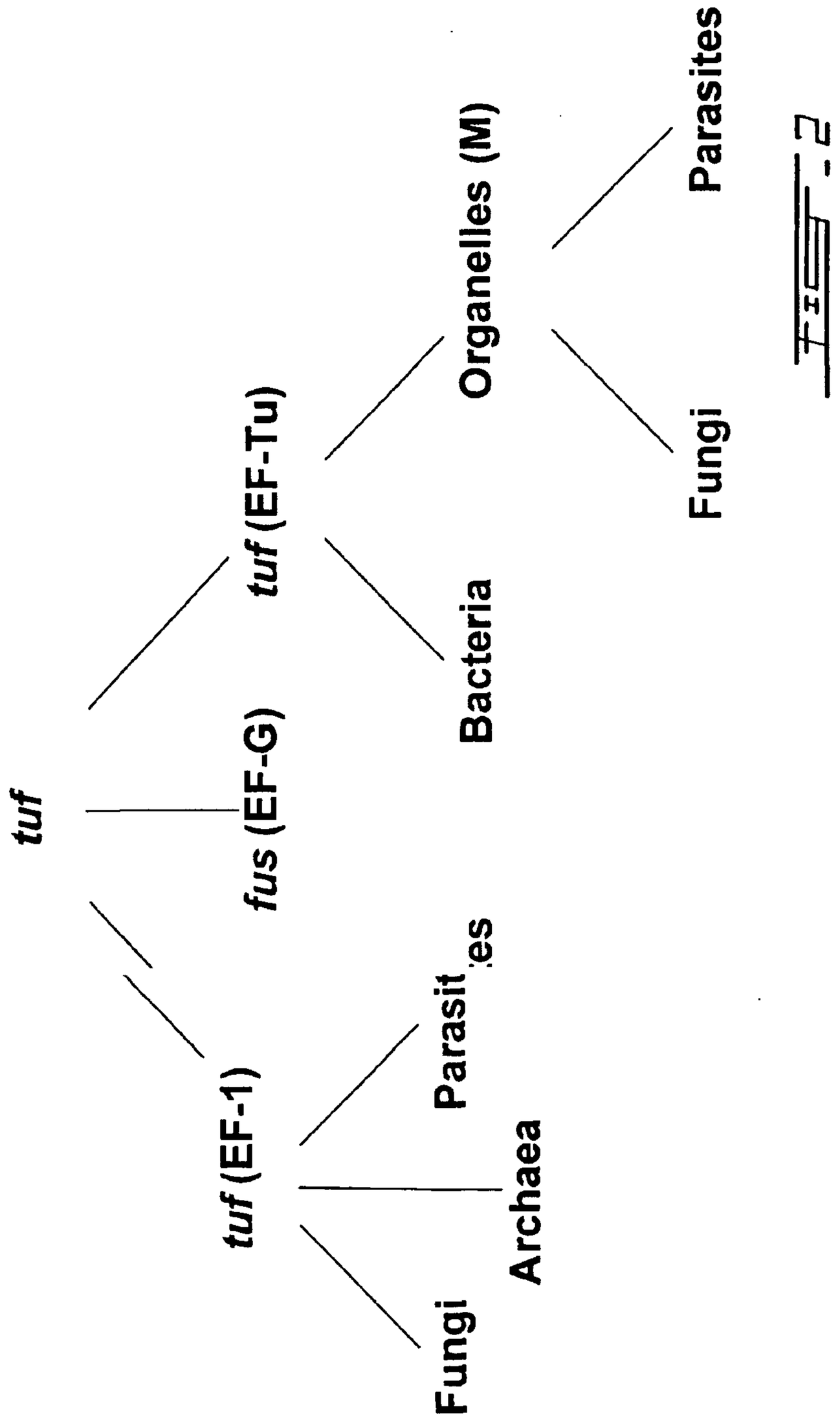
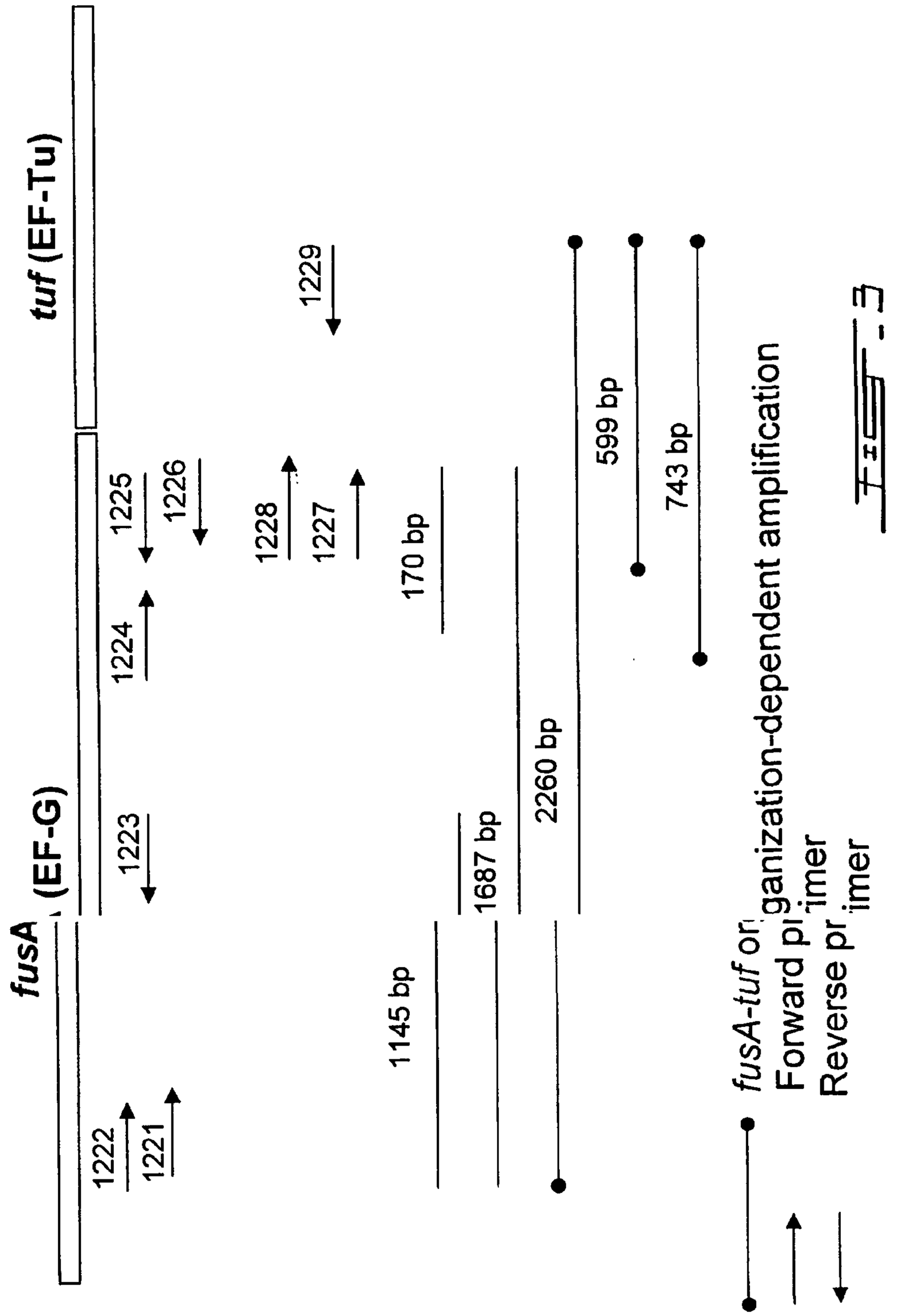

tuf
tuf (EF-Tu)
Parasites
Organelles (M)
Bacteria
Fungi
fus (EF-G)
tuf (EF-1)
Parasites
Archaea
Fungi

FIG 2


tuf (EF-Tu)
fusA (EF-G)
1223
1224
1225
1226
1228
1227
1229
1222
1221
1687 bp
170 bp
2260 bp
1145 bp
599 bp
743 bp
fusA-tuf organization-dependent amplification
Forward primer
Reverse primer

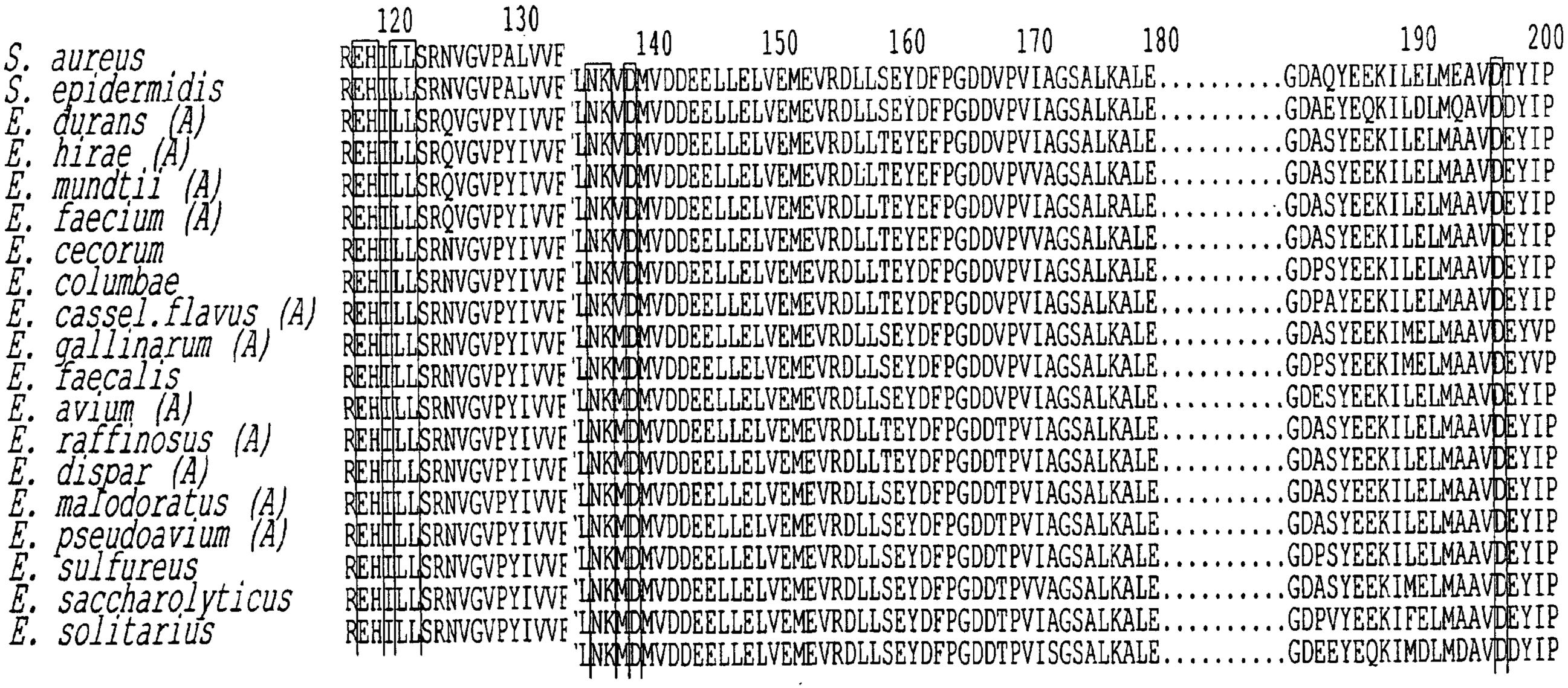


Fig. 4a

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                    210       220       230       240       250       260       270       280       290
S. aureus           TPERDSDKPFMMPVEDVI FSITGRGTVATGRVERGQIKVGEEVEIIGLH.DTSKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDVQRGQVLA
S. epidermidis      TPERDSDKPFMMPVEDVI FSITGRGTVATGRVERGQIKVGEEVEIIGMH.ETSKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDVQRGQVLA
E. durans (A)       TPERDNDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDVVDIVGIAEETAQTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. hirae (A)        TPERDNDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDVVDIVGIAEETAQTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. mundtii (A)      TPERDNDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDVVDIVGIAEETAQTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. faecium (A)      TPERDNDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDVIDIVGIAEETAQTTVTGVEMFRKLLDYAEAGDNIGALLRGVSREDIQRGQVLA
E. cecorum          TPERDNDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEVVGIAEETSKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. columbae         TPERDNDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEIVGIHDEISKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. casse1.flavus (A) TPERDTDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEIVGIADETSKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. gallinarum (A)   TPERDTDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEIVGIAEETAKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. faecalis         TPERDTDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEIVGIADETAKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. avium (A)        TPVRDTDKPFMMPVEDVI FSITGRGTVATGRVERGEVRVGDEVEIVGIKDETSKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIERGQVLA
E. raffinosus (A)   TPVRDTDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEIVGIADETAKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. dispar (A)       TPVRDTDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEIVGIAEETAKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. malodoratus (A)  TPVRDTDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEIVGIAEETAKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLS
E. pseudoavium (A)  TPVRDTDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEIVGIAEATAKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. sulfureus        TPTRDTDKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDEVEIVGIAEETAKTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. saccharolyticus  TPERDTEKPFMMPVEDVI FSITGRGTVATGRVERGQVRVGDVVDIVGIADETAQTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
E. solitarius       TPERDHDKPFMMPIEDVI FSITGRGTVATGRVERGQVRVGDVVEIVGIDEETAQTTVTGVEMFRKLLDYAEAGDNIGALLRGVAREDIQRGQVLA
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Fig. 4b

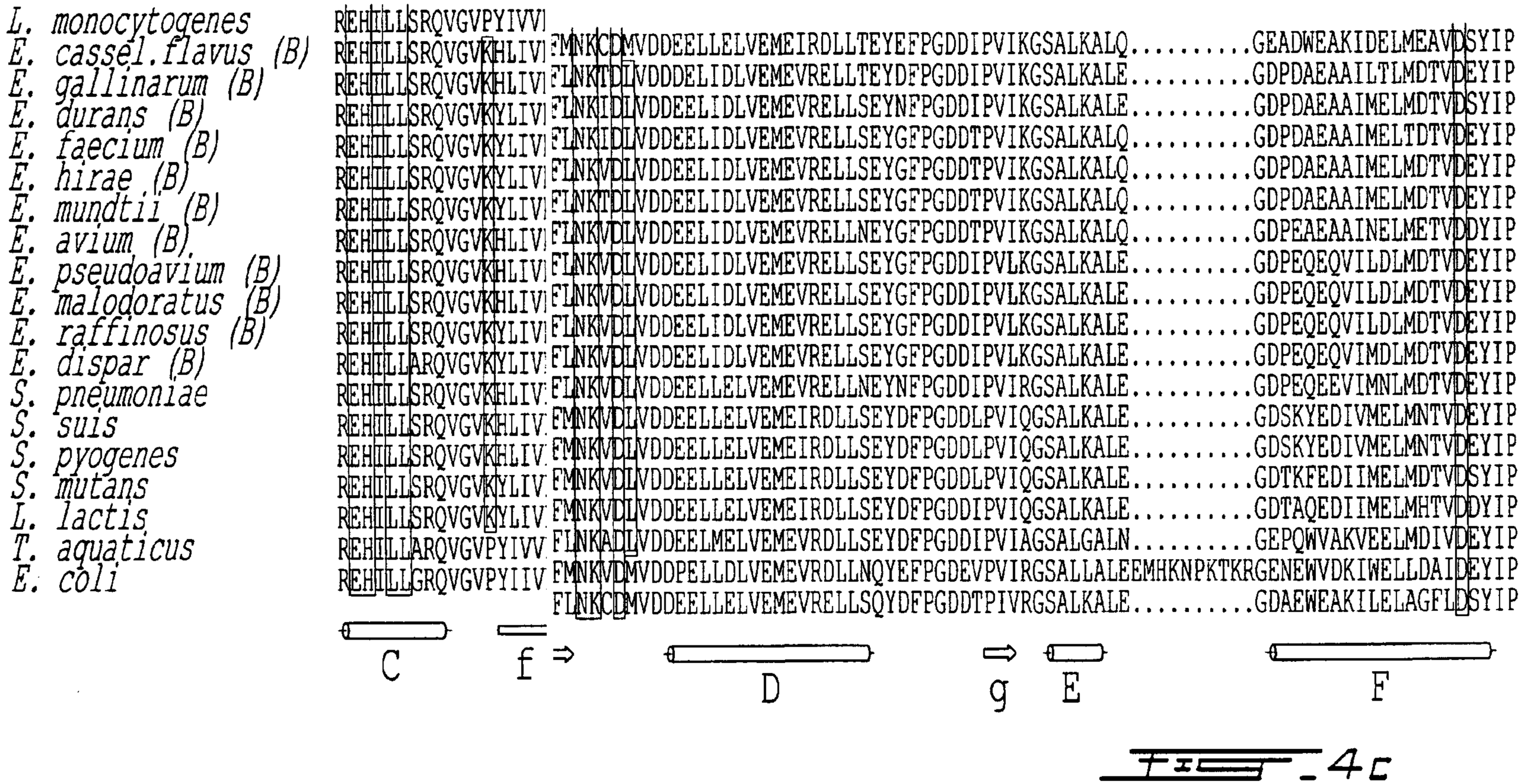

L. monocytogenes
E. casse1.flavus (B)
E. gallinarum (B)
E. durans (B)
E. faecium (B)
E. hirae (B)
E. mundtii (B)
E. avium (B)
E. pseudoavium (B)
E. malodoratus (B)
E. raffinosus (B)
E. dispar (B)
S. pneumoniae
S. suis
S. pyogenes
S. mutans
L. lactis
T. aquaticus
E. coli
C
f
D
g
E
F

Fig-4c

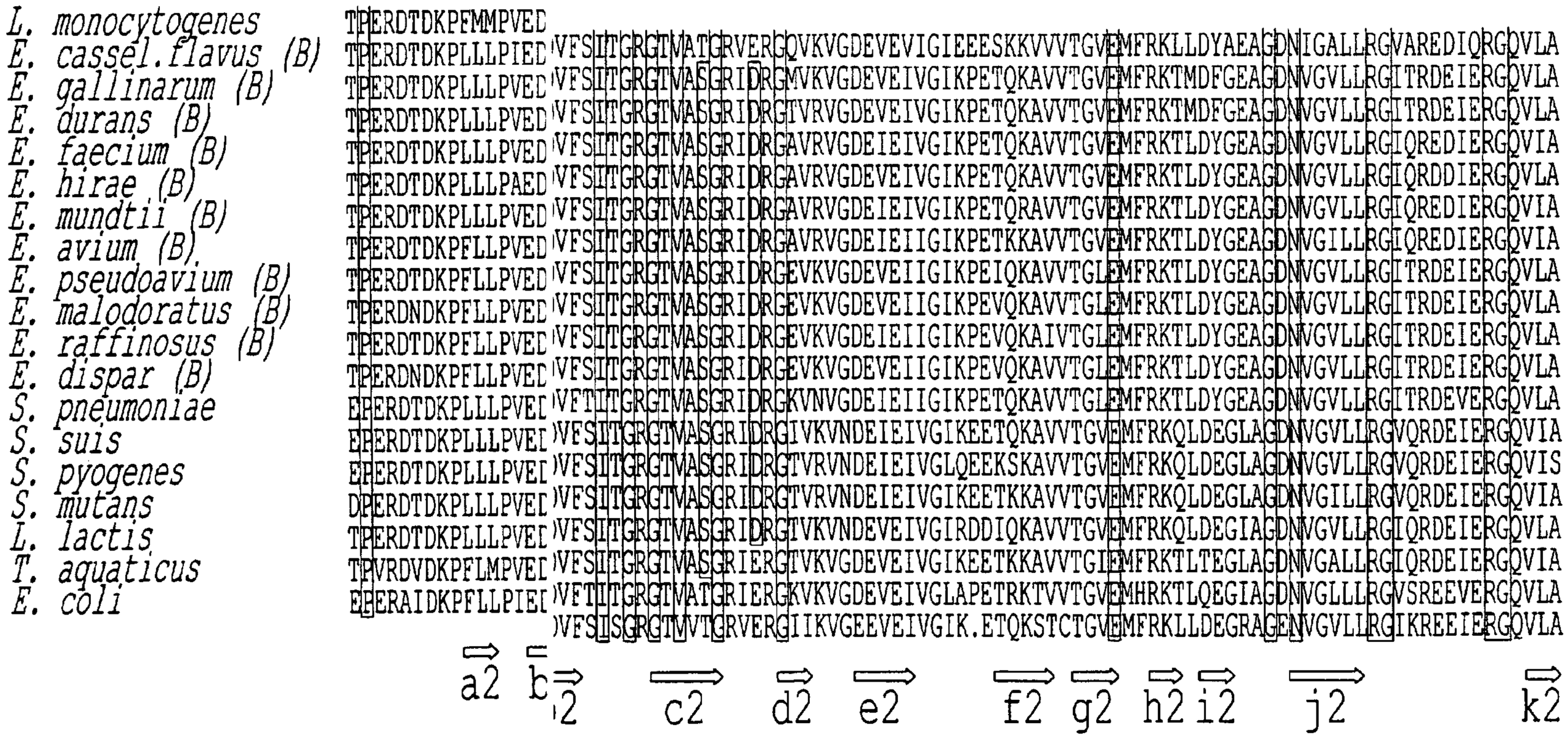
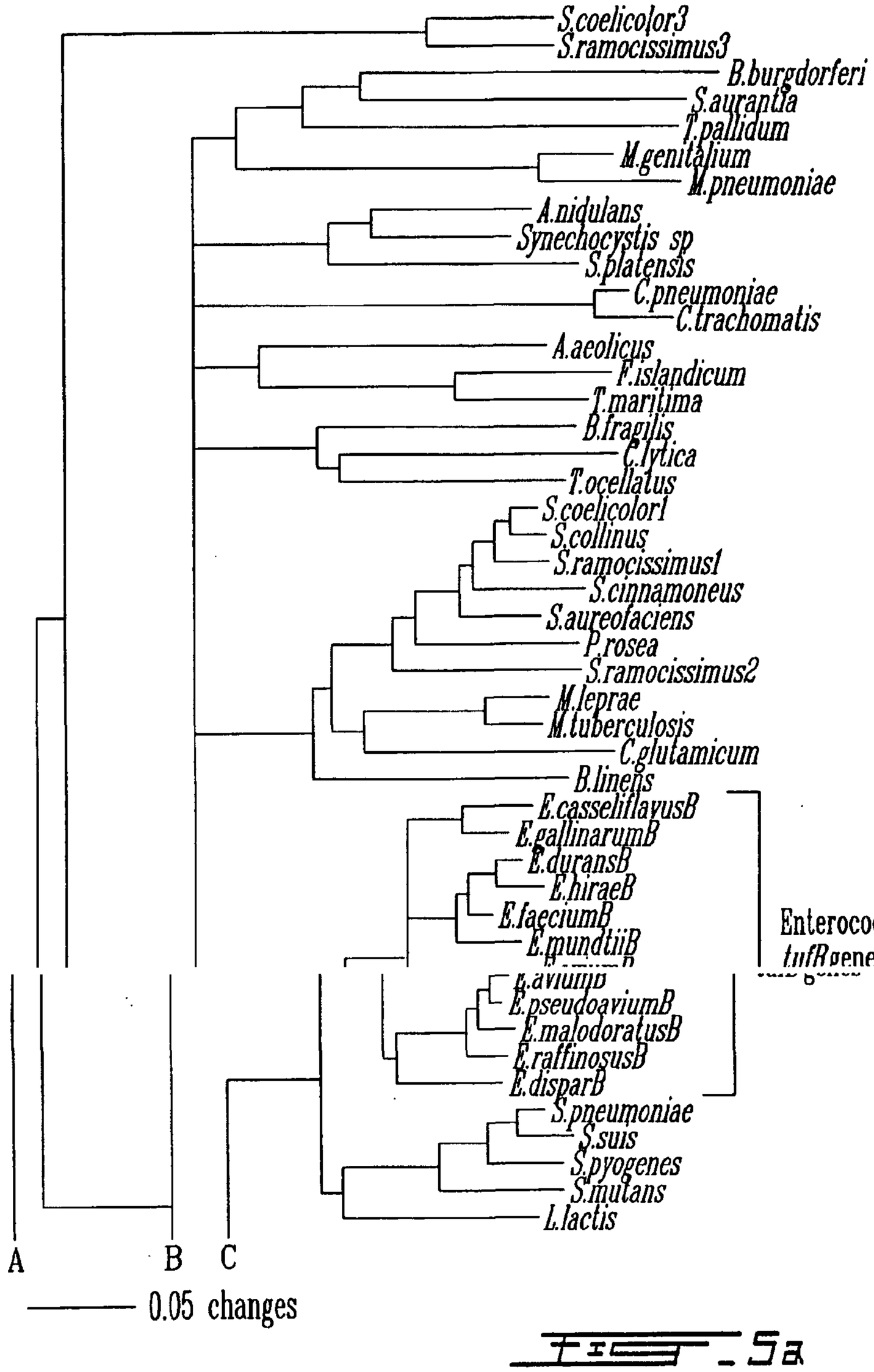

L. monocytogenes
E. casseI.flavus (B)
E. gallinarum (B)
E. durans (B)
E. faecium (B)
E. hirae (B)
E. mundtii (B)
E. avium (B)
E. pseudoavium (B)
E. malodoratus (B)
E. raffinosus (B)
E. dispar (B)
S. pneumoniae
S. suis
S. pyogenes
S. mutans
L. lactis
T. aquaticus
E. coli
a2
b2
c2
d2
e2
f2
g2
h2
i2
j2
k2

Fig. 4d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S.platensis
C.pneumoniae
C.trachomatis
A.aeolicus
F.islandicum
T.maritima
B.fragilis
C.lytica
T.ocellatus
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M.leprae
M.tuberculosis
C.glutamicum
B.linens
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E.dransB
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E.faeciumB
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E.pseudoaviumB
E.malodoratusB
E.raffinosusB
E.disparB
S.pneumoniae
S.suis
S.pyogenes
S.mutans
L.lactis
Enterococcal
tufB genes
A
B
C
0.05 changes

Fig 5a

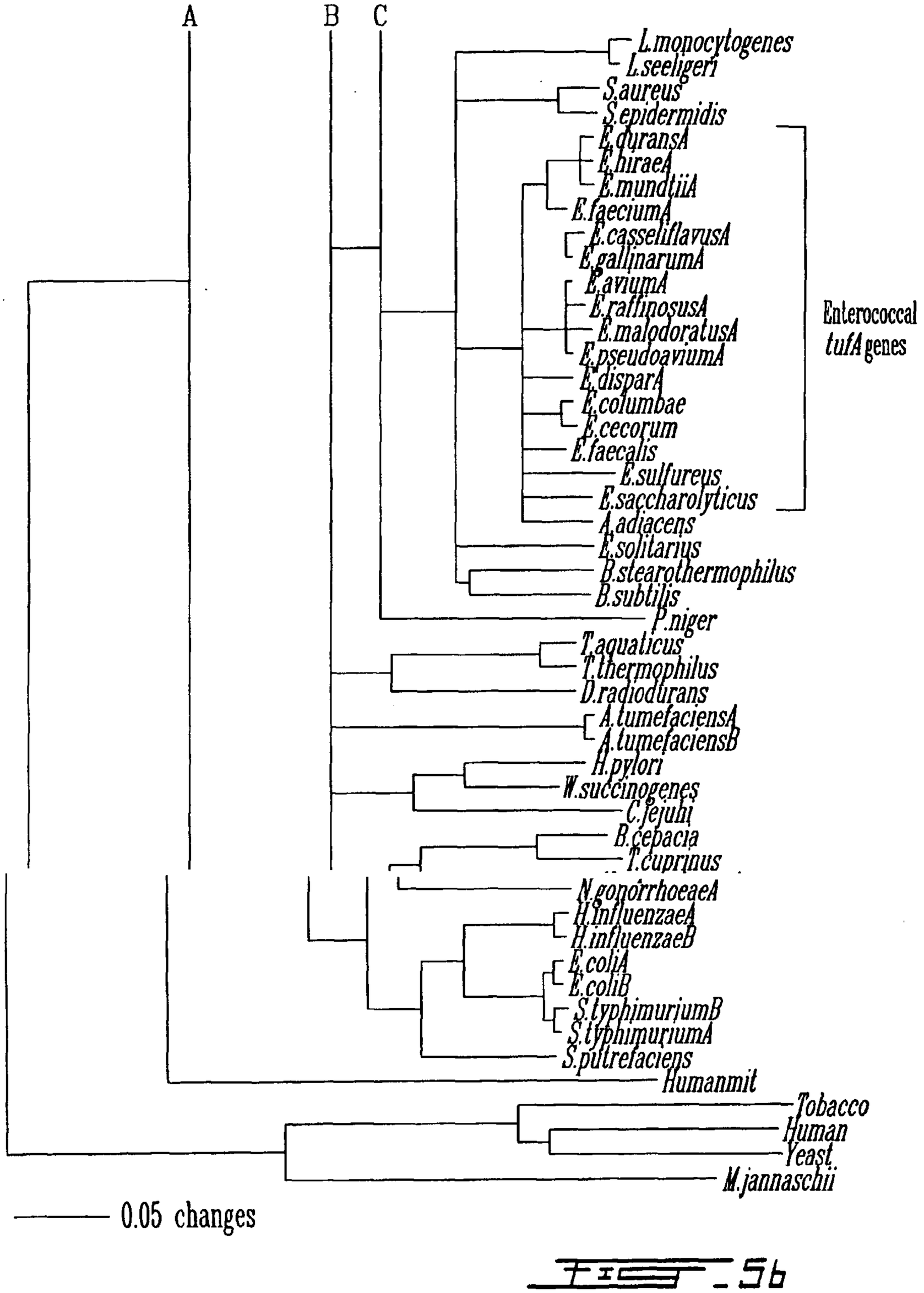

A
B
C
L.monocytogenes
L.seeligeri
S.aureus
S.epidermidis
E.duransA
E.hiraeA
E.mundtiiA
E.faeciumA
E.casseliflavusA
E.gallinarumA
E.aviumA
E.raffinosusA
E.malodoratusA
E.pseudoaviumA
E.disparA
E.columbae
E.cecorum
E.faecalis
E.sulfureus
E.saccharolyticus
A.adiacens
E.solitarius
B.stearothermophilus
B.subtilis
P.niger
T.aquaticus
T.thermophilus
D.radiodurans
A.tumefaciensA
A.tumefaciensB
H.pylori
W.succinogenes
C.jejuni
B.cepacia
T.cuprinus
N.gonorrhoeaeA
H.influenzaeA
H.influenzaeB
E.coliA
E.coliB
S.typhimuriumB
S.typhimuriumA
S.putrefaciens
Humanmit
Tobacco
Human
Yeast
M.jannaschii
Enterococcal
tufA genes
0.05 changes

Fig. 5b

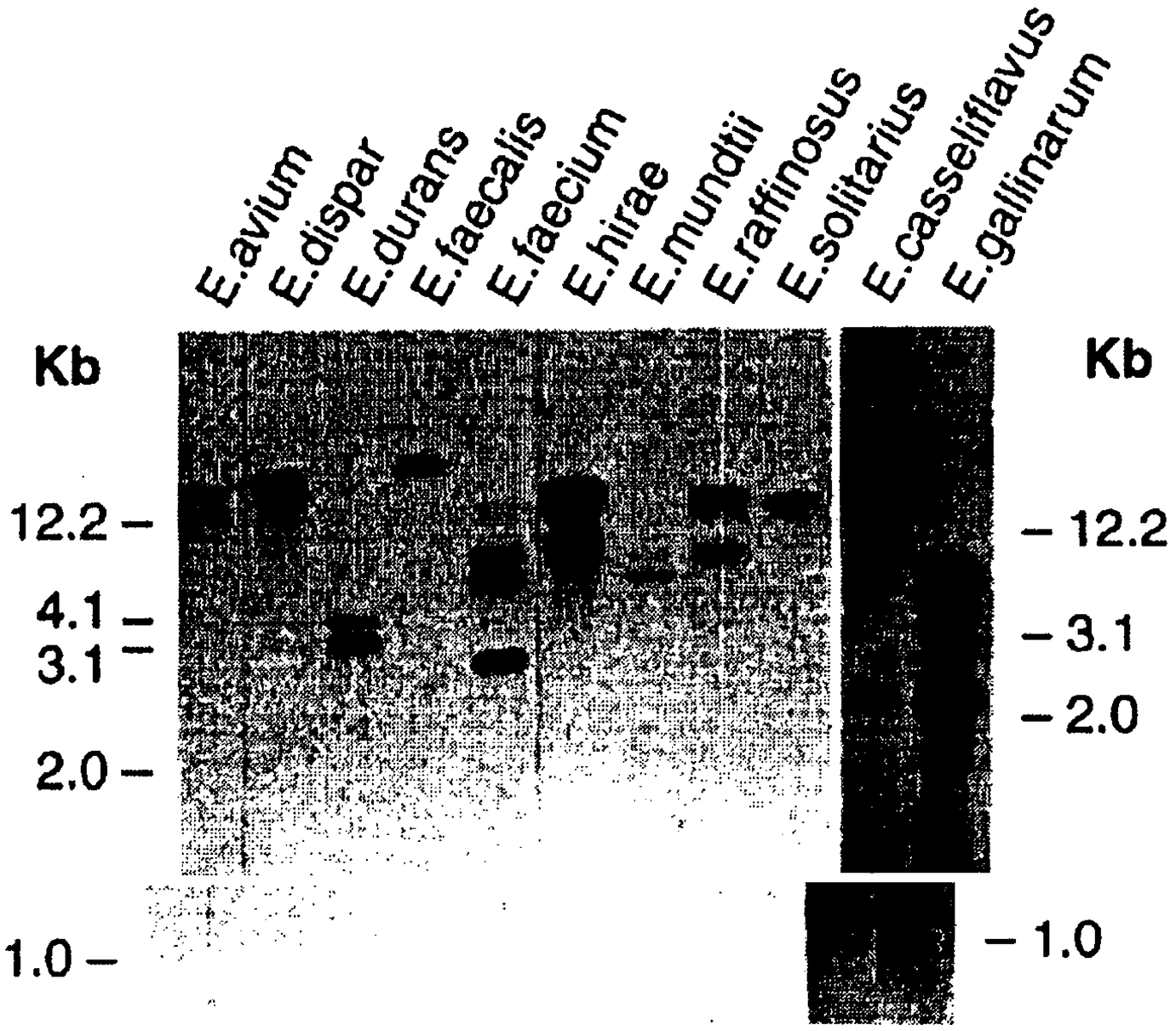
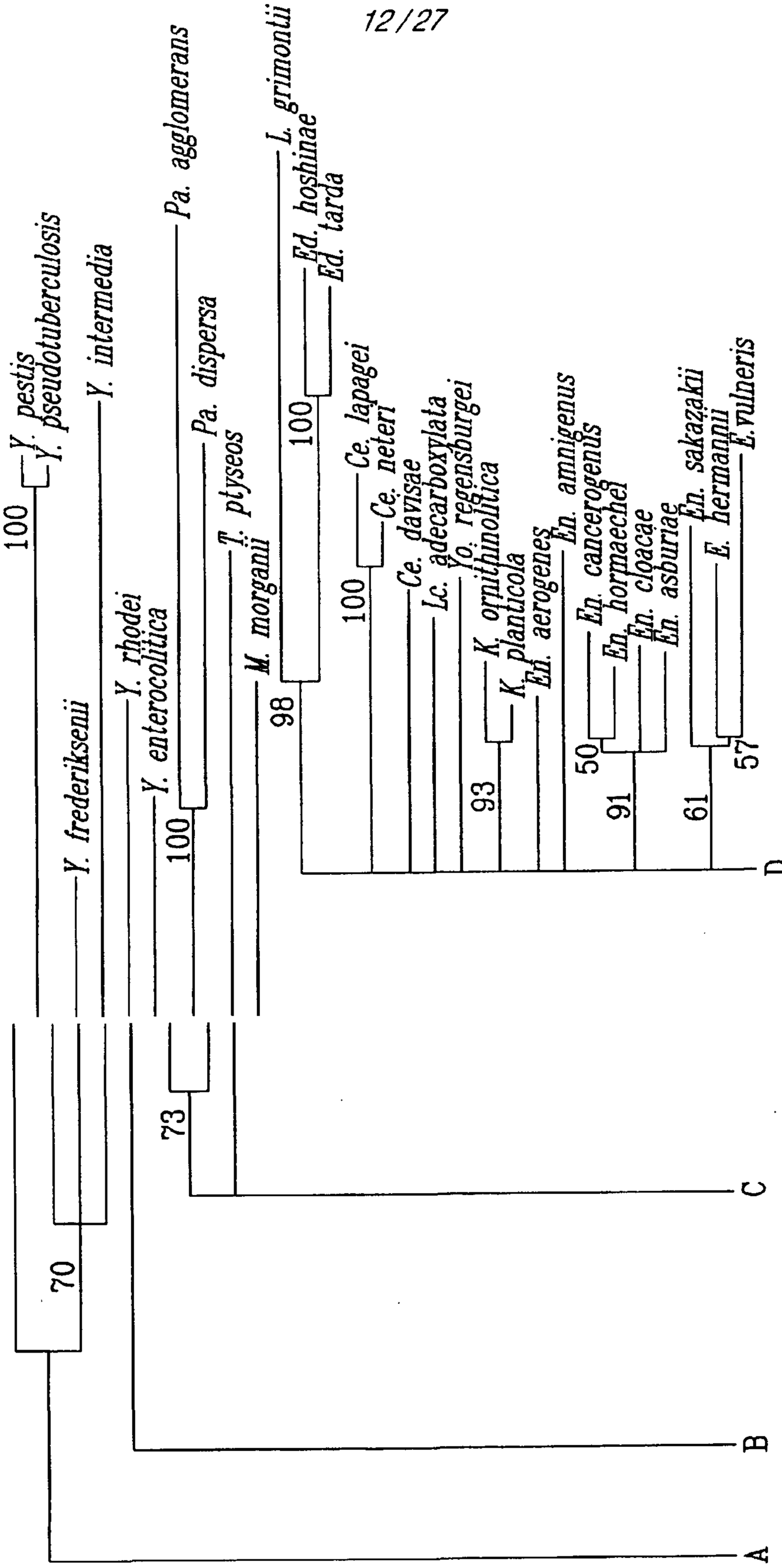

E.avium
E.dispar
E.durans
E.faecalis
E.faecium
E.hirae
E.mundtii
E.raffinosus
E.solitarius
E.casseliflavus
E.gallinarum
Kb
12.2 –
4.1 –
3.1 –
2.0 –
1.0 –
Kb
– 12.2
– 3.1
– 2.0
– 1.0

Fig 6

```
                 301         311         321                           331
E. coli          GAGATCGGTG AAGAAGAGCG TTGGG..... .......... CGATTCACCG
E. agglomerans   GACATCGGTG AAGAAGAGCG TTGGG..... .......... CGATCCACCG
P. agglomerans   GAGCTGAAAG AAGAAGATGG CAGCGCAGTA GAGATCGCCT CTATTCACCG
P. dispersa      GACCTGAAAG AAGAAGACGG CAGCGCTGTA GAGGTTTCCT CTATTCATCG
T. ptyseos       GACCTGAAGA ACGAAGATGG TAGCAATGTT GAGGTGAACT CTATTCACCG

E. coli          ~I~~G~~E~~ E~~E~~R~~W ~~A~~..... .......... I~~H~~R~~
E. agglomerans   ~I~~G~~E~~ E~~E~~R~~W ~~A~~..... .......... I~~H~~R~~
P. agglomerans   ~L~~K~~E~~ E~~D~~G~~S ~~A~~V~~E~ ~I~~A~~S~~ I~~H~~R~~
P. dispersa      ~L~~K~~E~~ E~~D~~G~~S ~~A~~V~~E~ ~V~~S~~S~~ I~~H~~R~~
T. ptyseos       ~L~~K~~N~~ E~~D~~G~~S ~~N~~V~~E~ ~V~~N~~S~~ I~~H~~R~~
```

FIG. 7


Y. pestis
Y. pseudotuberculosis
Y. frederiksenii
Y. intermedia
Y. rhodei
Y. enterocolitica
Pa. agglomerans
Pa. dispersa
T. ptyseos
M. morganii
L. grimontii
Ed. hoshinae
Ed. tarda
Ce. lapagei
Ce. neteri
Ce. davisae
Lc. adecarboxylata
Yo. regensburgei
K. ornithinolitica
K. planticola
En. aerogenes
En. amnigenus
En. cancerogenus
En. hormaechei
En. cloacae
En. asburiae
En. sakazakii
E. hermannii
E. vulneris
100
100
100
98
100
93
50
91
61
57
73
70
A
B
C
D

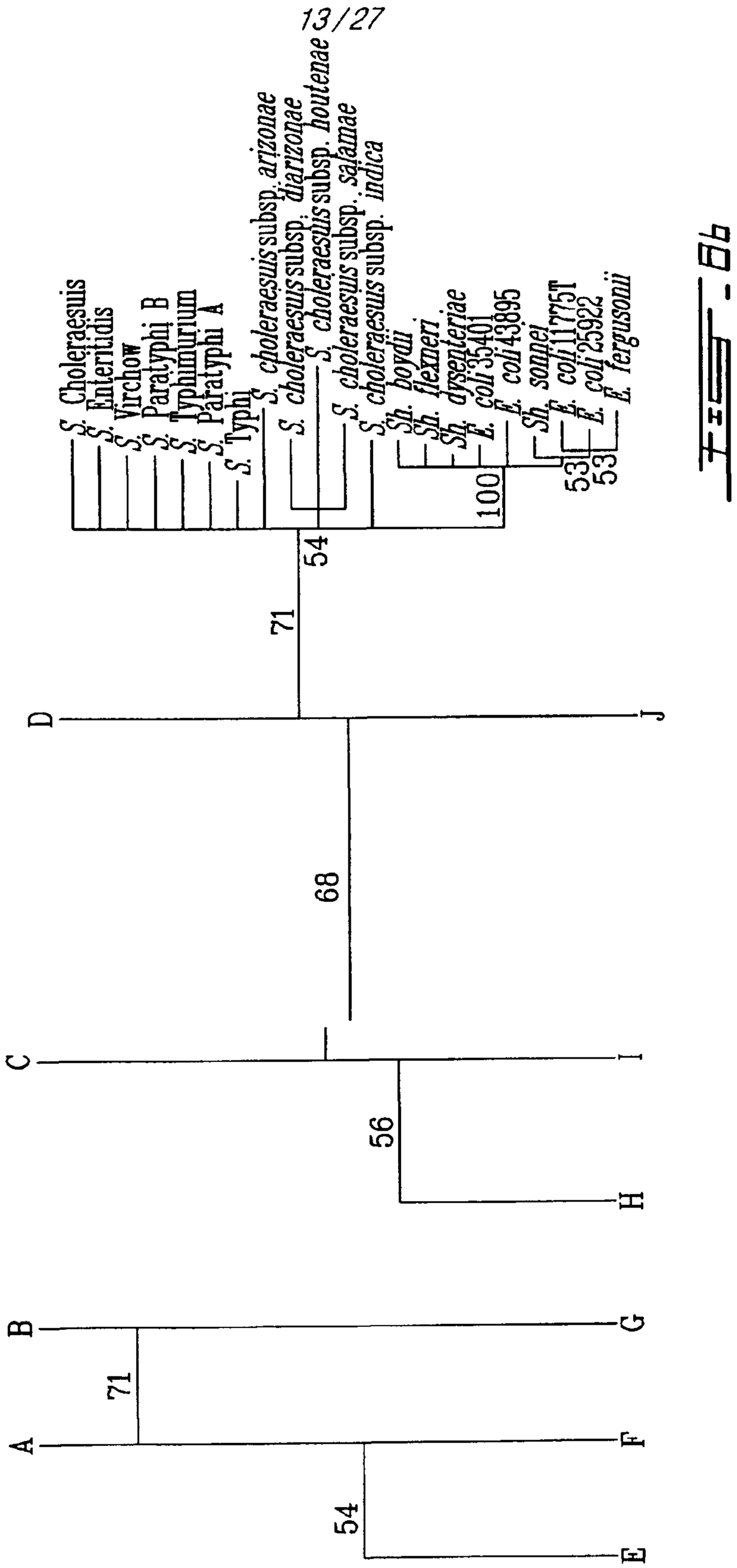

S. Choleraesuis
S. Enteritidis
S. Virchow
S. Paratyphi B
S. Typhimurium
S. Paratyphi A
S. Typhi
S. choleraesuis subsp. arizonae
S. choleraesuis subsp. diarizonae
S. choleraesuis subsp. houtenae
S. choleraesuis subsp. salamae
S. choleraesuis subsp. indica
Sh. boydii
Sh. flexneri
Sh. dysenteriae
E. coli 35401
E. coli 43895
Sh. sonnei
E. coli 11775T
E. coli 25922
E. fergusonii
100
53
53
54
71
68
56
71
54
D
J
C
I
H
B
G
A
F
E

Fig - 8b

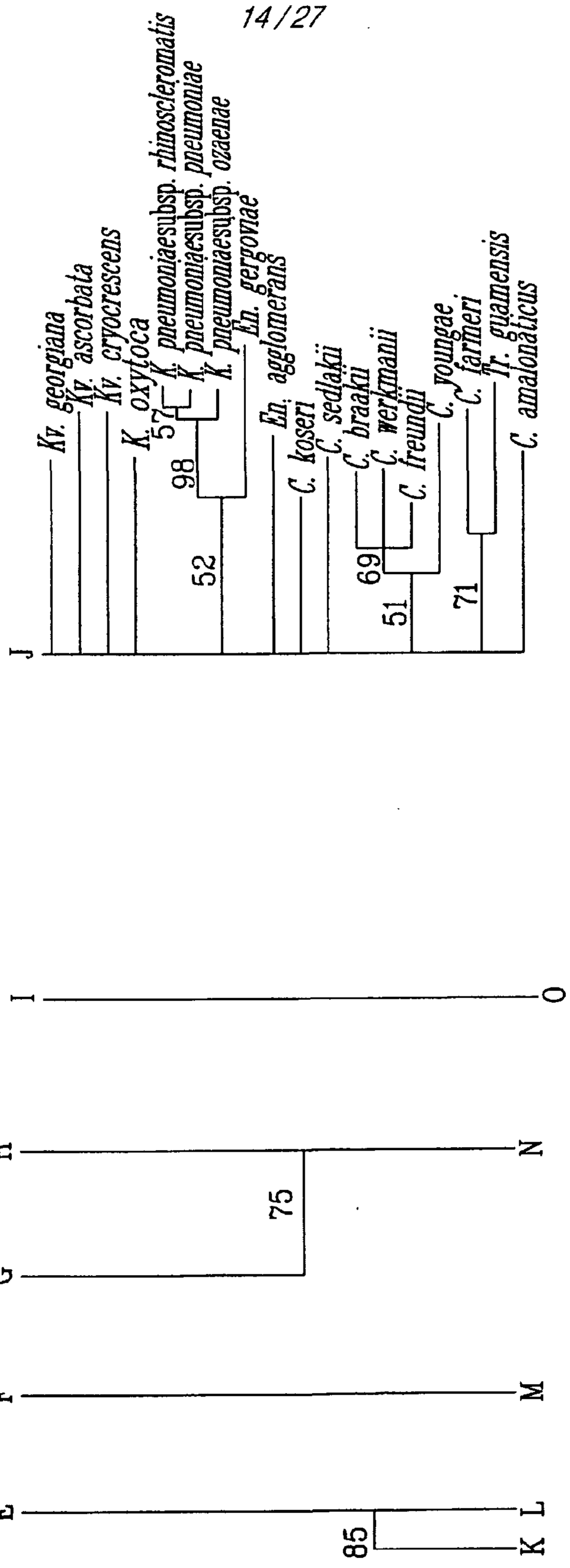
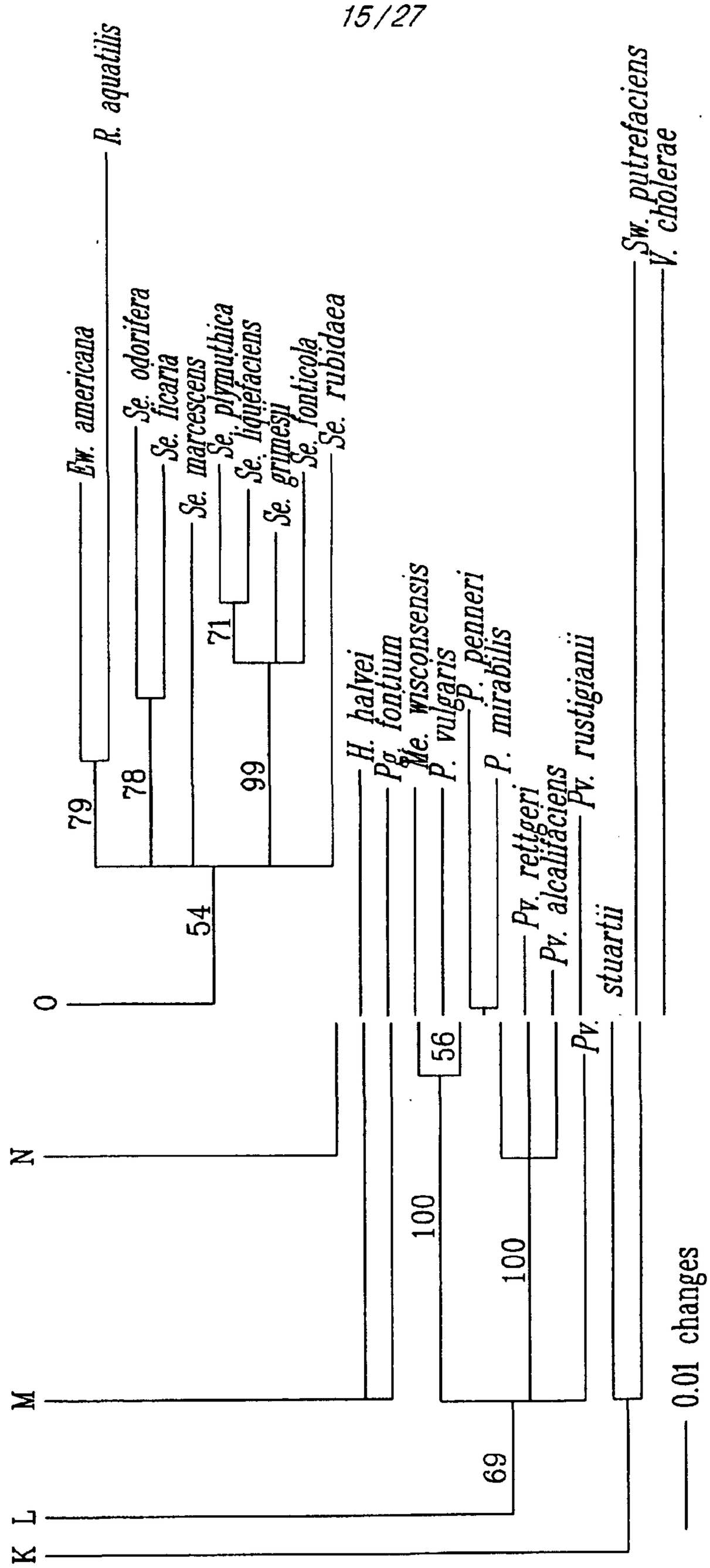
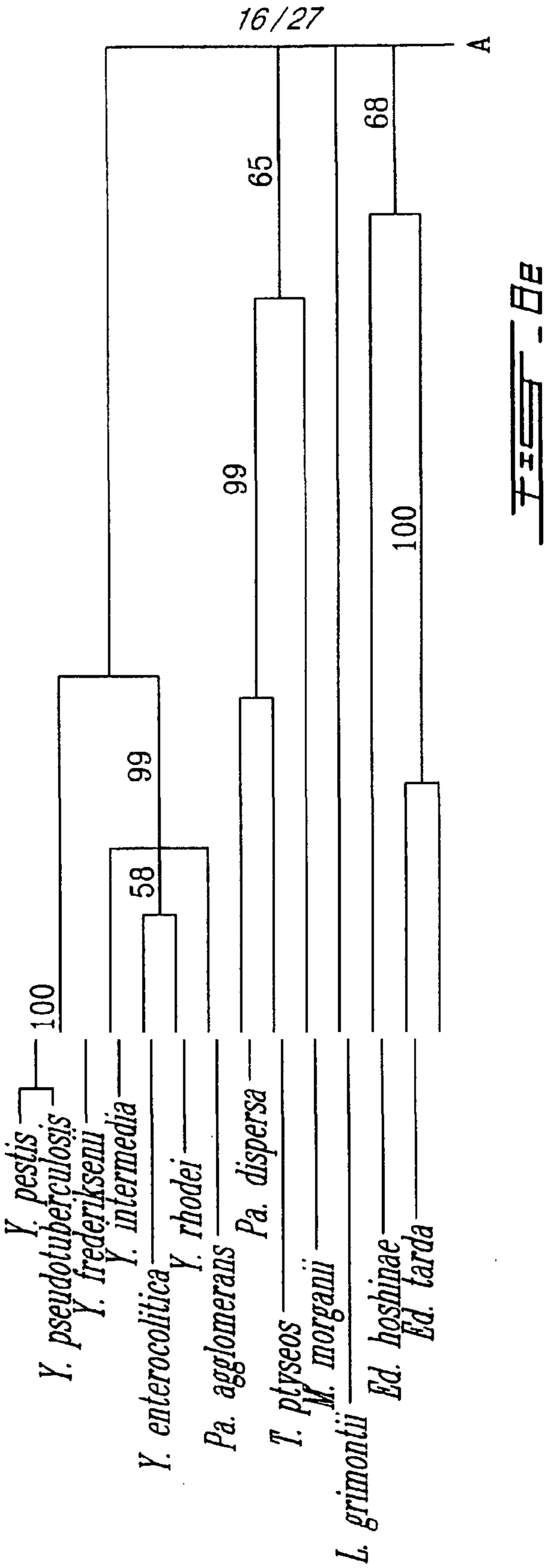

Kv. georgiana
Kv. ascorbata
Kv. cryocrescens
K. oxytoca
K. pneumoniaesubsp. rhinoscleromatis
K. pneumoniaesubsp. pneumoniae
K. pneumoniaesubsp. ozaenae
En. gergoviae
En. agglomerans
C. koseri
C. sedlakii
C. braakii
C. werkmanii
C. freundii
C. youngae
C. farmeri
Tr. guamensis
C. amalonaticus
57
98
52
69
51
71
J
I
O
H
N
75
G
F
M
E
L
85
K

Fig - 8c


R. aquatilis
Ew. americana
Se. odoriferia
Se. ficaria
Se. marcescens
Se. plymuthica
Se. liquefaciens
Se. grimesii
Se. fonticola
Se. rubidaea
H. halvei
Pg. fontium
Me. wisconsensis
P. vulgaris
P. penneri
P. mirabilis
Pv. rettgeri
Pv. alcalifaciens
Pv. rustigianii
stuartii
Sw. putrefaciens
V. cholerae
Bd
79
78
54
71
99
56
100
100
69
N
M
L
K
0.01 changes


A
65
68
99
100
99
58
100
Y. pestis
Y. pseudotuberculosis
Y. frederiksenii
Y. intermedia
Y. enterocolitica
Y. rhodei
Pa. agglomerans
Pa. dispersa
T. ptyseos
M. morganii
L. grimontii
Ed. hoshinae
Ed. tarda
TSP - Bz

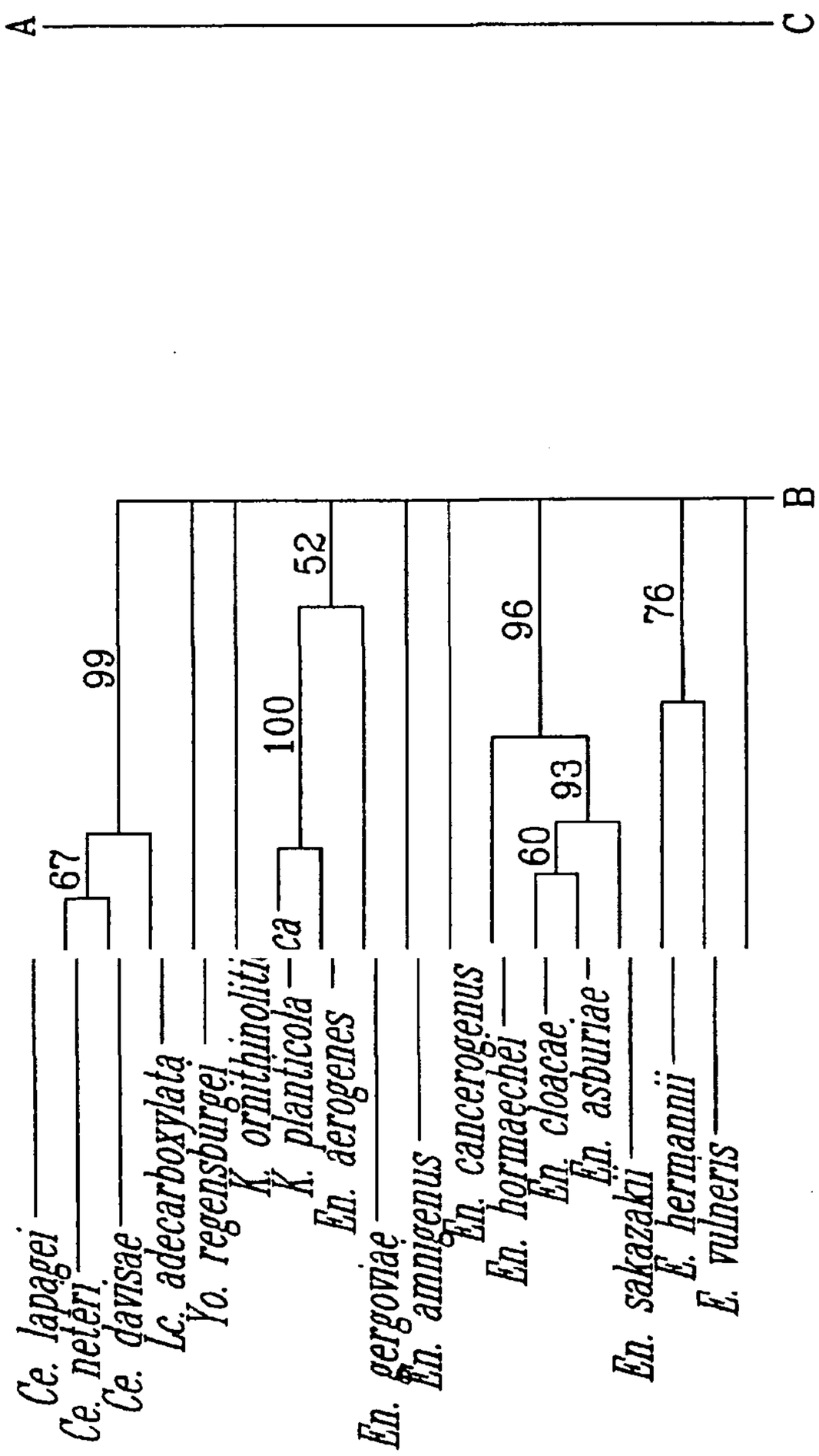

A
B
C
99
67
52
100
96
93
60
76
Ce. lapagei
Ce. netleri
Ce. davisae
Lc. adecarboxylata
Yo. regensburgei
K. ornithinolitica
K. planticola
En. aerogenes
En. gergoviae
En. amnigenus
En. cancerogenus
En. hormaechei
En. cloacae
En. asburiae
En. sakazakii
E. hermannii
E. vulneris

FIG - 8F

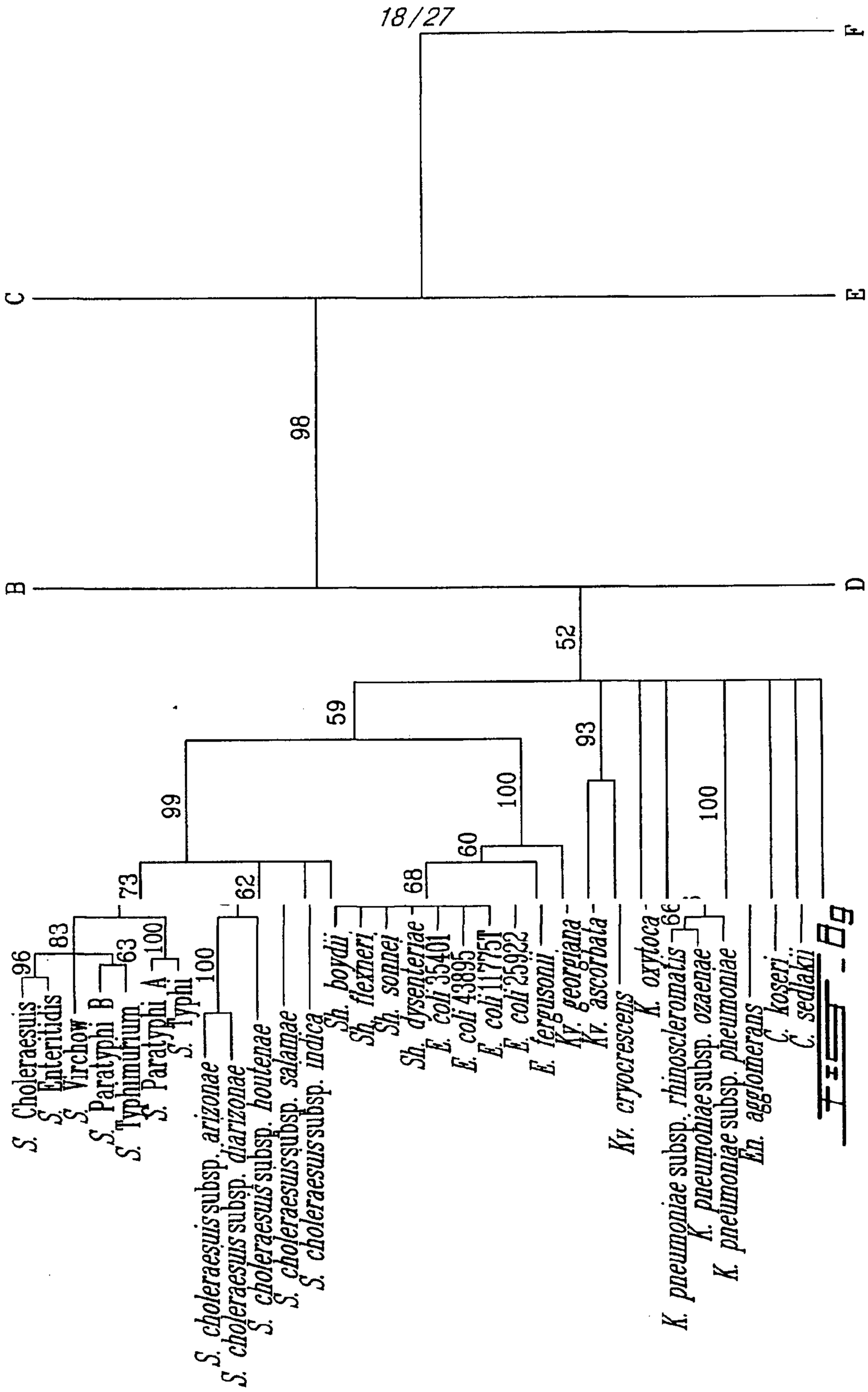
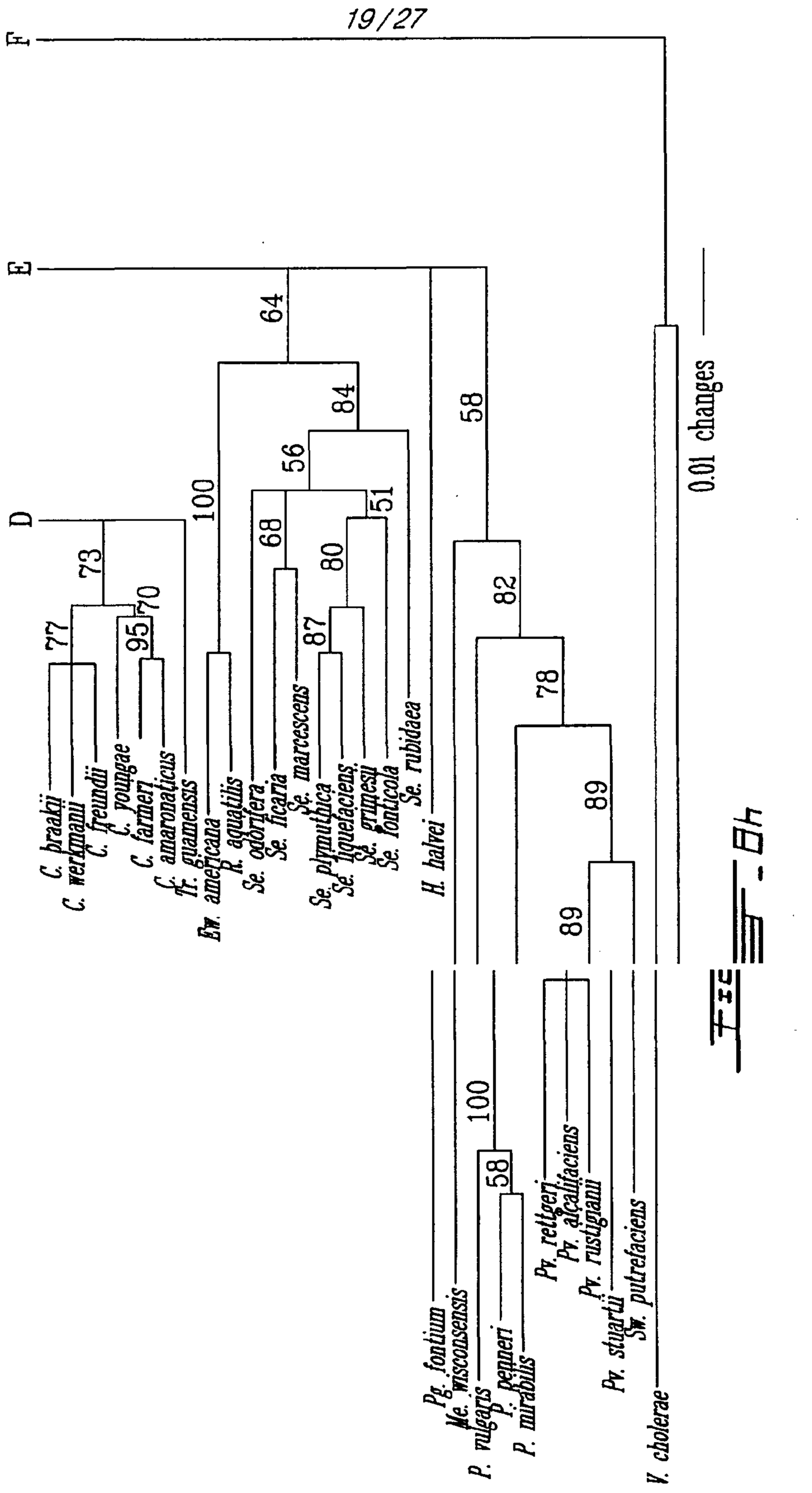

A
C
E
B
D
98
52
59
93
99
100
100
60
68
73
62
96
83
63
100
100
S. Choleraesuis
S. Enteritidis
S. Virchow
S. Paratyphi B
S. Typhimurium
S. Paratyphi A
S. Typhi
S. choleraesuis subsp. arizonae
S. choleraesuis subsp. diarizonae
S. choleraesuis subsp. houtenae
S. choleraesuis subsp. salamae
S. choleraesuis subsp. indica
Sh. boydii
Sh. flexneri
Sh. sonnei
Sh. dysenteriae
E. coli 35401
E. coli 43895
E. coli 11775T
E. coli 25922
E. fergusonii
Kv. georgiana
Kv. ascorbata
Kv. cryocrescens
K. oxytoca
K. pneumoniae subsp. rhinoscleromatis
K. pneumoniae subsp. ozaenae
K. pneumoniae subsp. pneumoniae
En. agglomerans
C. koseri
C. sedlakii

Fig - 8g


E1
E
64
D
100
73
77
95
70
56
68
84
80
87
51
58
82
78
89
89
100
0.01 changes
C. braakii
C. werkmanii
C. freundii
C. youngae
C. farmeri
C. amaronaticus
Tr. guamensis
Ew. americana
P. aquatilis
Se. odorifera
Se. ficaria
Se. marcescens
Se. plymuthica
Se. liquefaciens
Se. grimesii
Se. fonticola
Se. rubidaea
H. halvei
Pg. fontium
Me. wisconsensis
P. vulgaris
P. penneri
P. mirabilis
Pv. rettgeri
Pv. alcalifaciens
Pv. rustigianii
Pv. stuartii
Sw. putrefaciens
V. cholerae

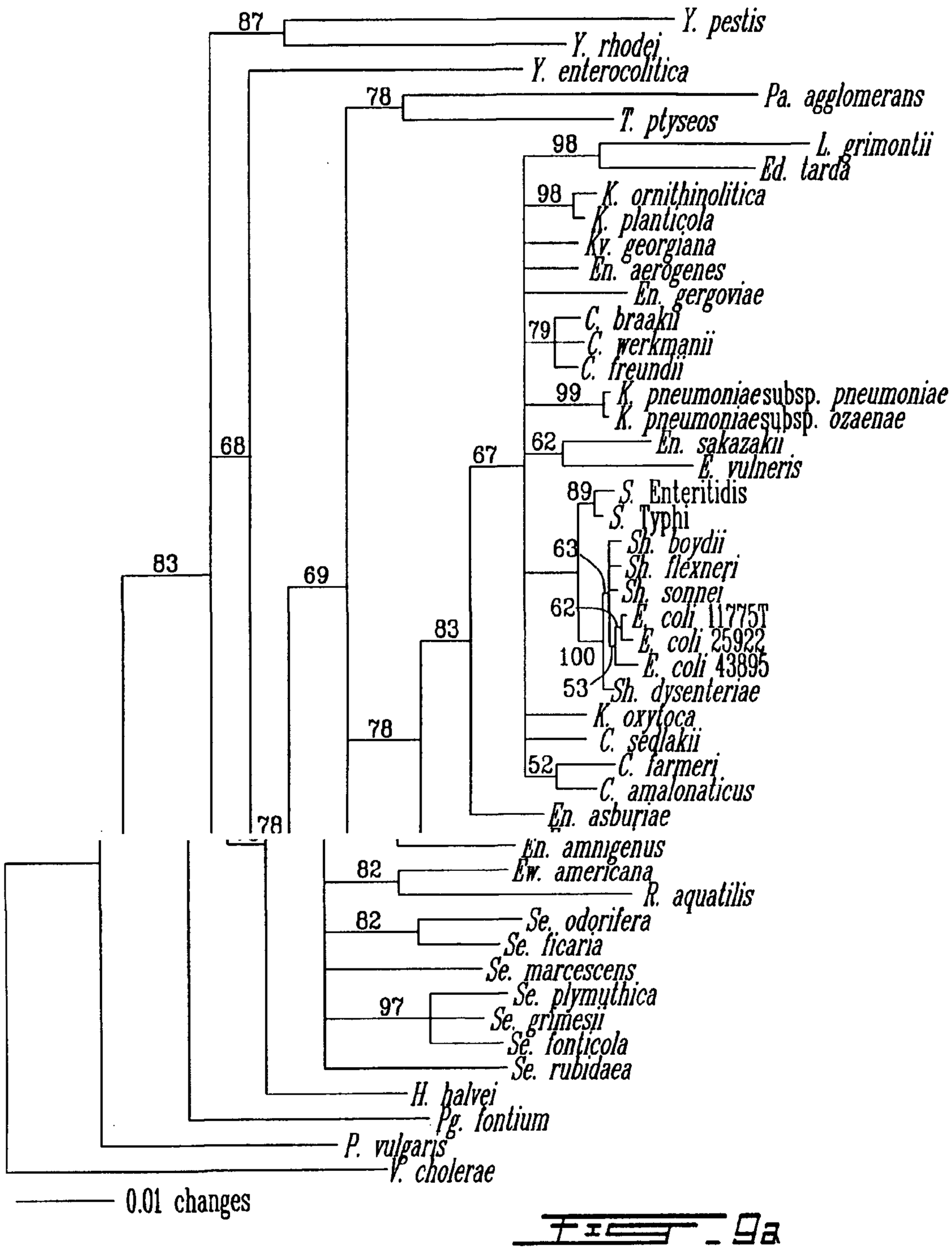

87
Y. pestis
Y. rhodei
Y. enterocolitica
78
Pa. agglomerans
T. ptyseos
98
L. grimontii
Ed. tarda
98
K. ornithinolitica
K. planticola
Kv. georgiana
En. aerogenes
En. gergoviae
79
C. braakii
C. werkmanii
C. freundii
99
K. pneumoniaesubsp. pneumoniae
K. pneumoniaesubsp. ozaenae
62
En. sakazakii
E. vulneris
89
S. Enteritidis
S. Typhi
63
Sh. boydii
Sh. flexneri
Sh. sonnei
62
E. coli 11775T
E. coli 25922
100
E. coli 43895
53
Sh. dysenteriae
K. oxytoca
C. sedlakii
52
C. farmeri
C. amalonaticus
En. asburiae
En. amnigenus
Ew. americana
R. aquatilis
Se. odorifera
Se. ficaria
Se. marcescens
Se. plymuthica
Se. grimesii
Se. fonticola
Se. rubidaea
H. halvei
Pg. fontium
P. vulgaris
V. cholerae
68
67
83
69
83
78
78
82
82
97
0.01 changes

Fig - 9a

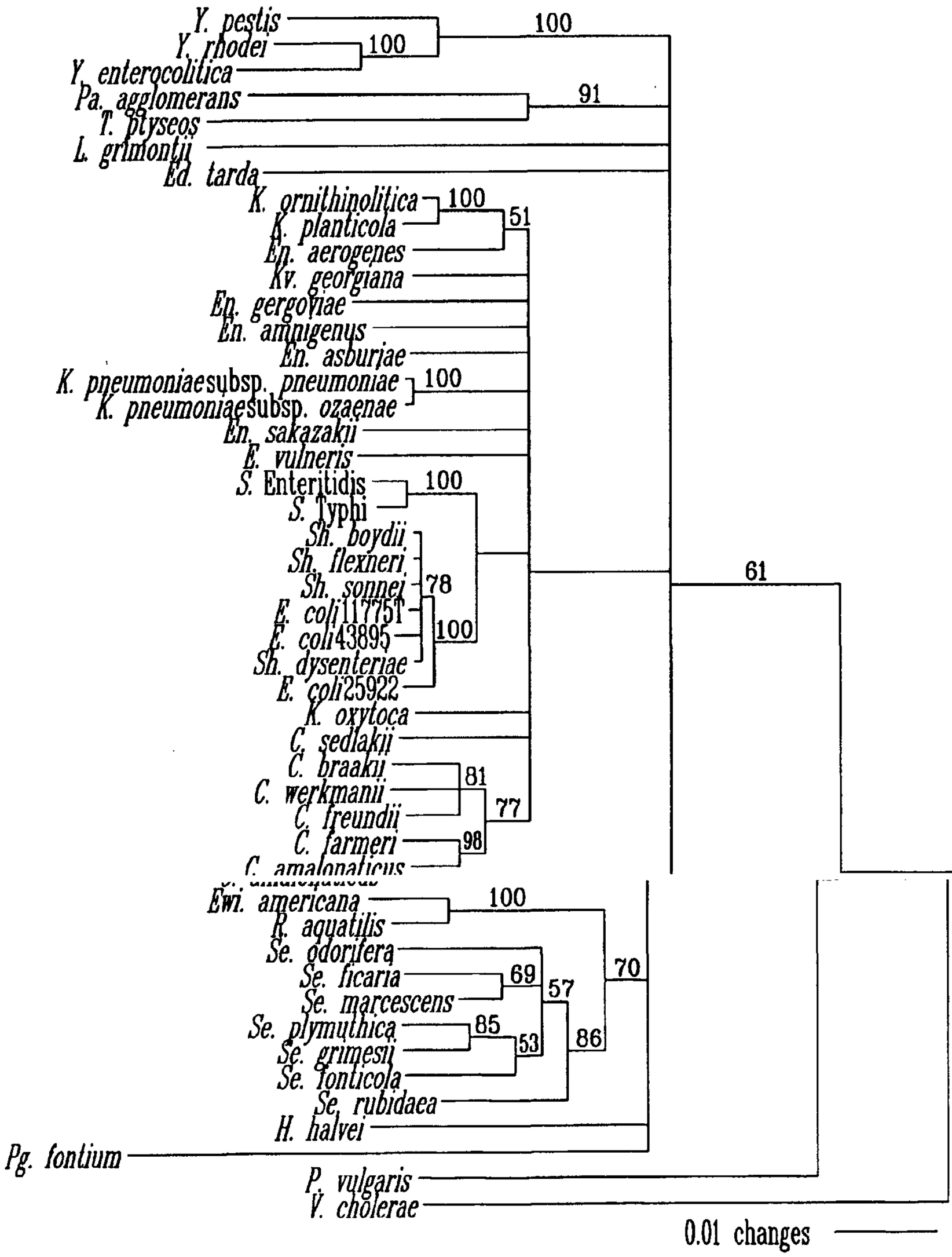

Y. pestis
Y. rhodei
Y. enterocolitica
Pa. agglomerans
T. ptyseos
L. grimontii
Ed. tarda
K. ornithinolitica
K. planticola
En. aerogenes
Kv. georgiana
En. gergoviae
En. amnigenus
En. asburiae
K. pneumoniaesubsp. pneumoniae
K. pneumoniaesubsp. ozaenae
En. sakazakii
E. vulneris
S. Enteritidis
S. Typhi
Sh. boydii
Sh. flexneri
Sh. sonnei
E. coli11775T
E. coli43895
Sh. dysenteriae
E. coli25922
K. oxytoca
C. sedlakii
C. braakii
C. werkmanii
C. freundii
C. farmeri
C. amalonaticus
Ewi. americana
R. aquatilis
Se. odorifera
Se. ficaria
Se. marcescens
Se. plymuthica
Se. grimesii
Se. fonticola
Se. rubidaea
H. halvei
Pg. fontium
P. vulgaris
V. cholerae
100
100
91
100
51
100
81
77
98
100
69
57
85
53
86
70
61
0.01 changes

Fig. 9b

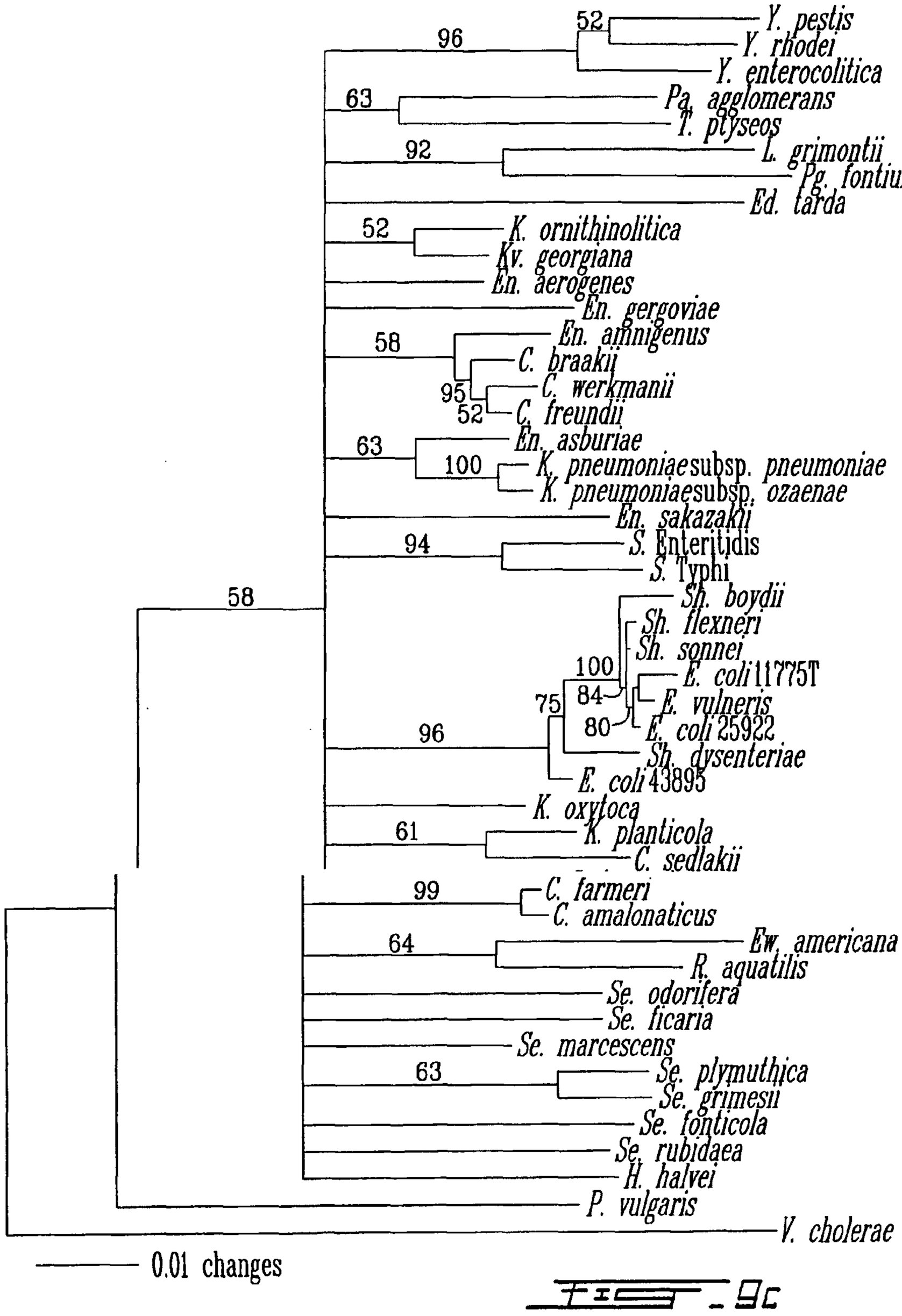
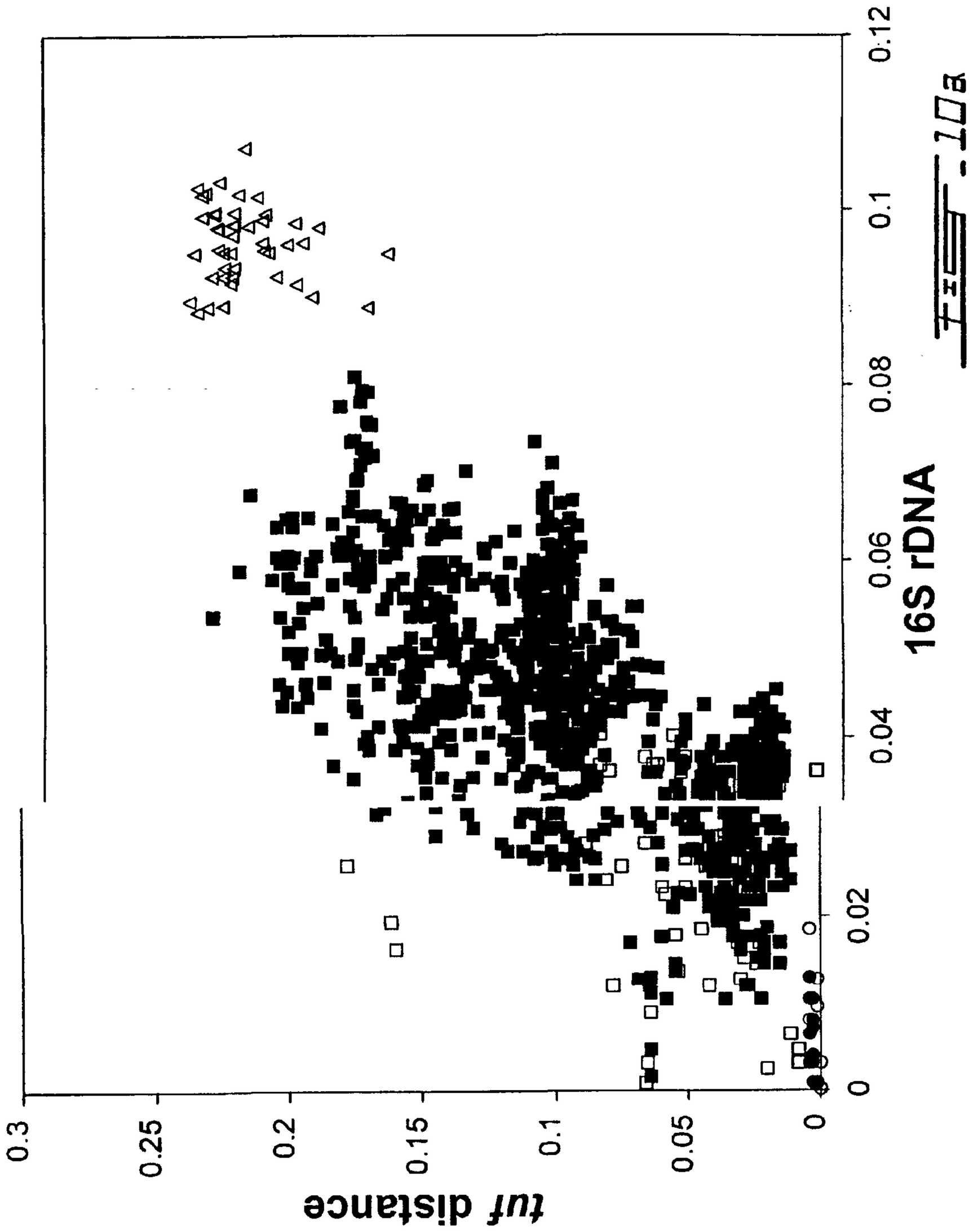
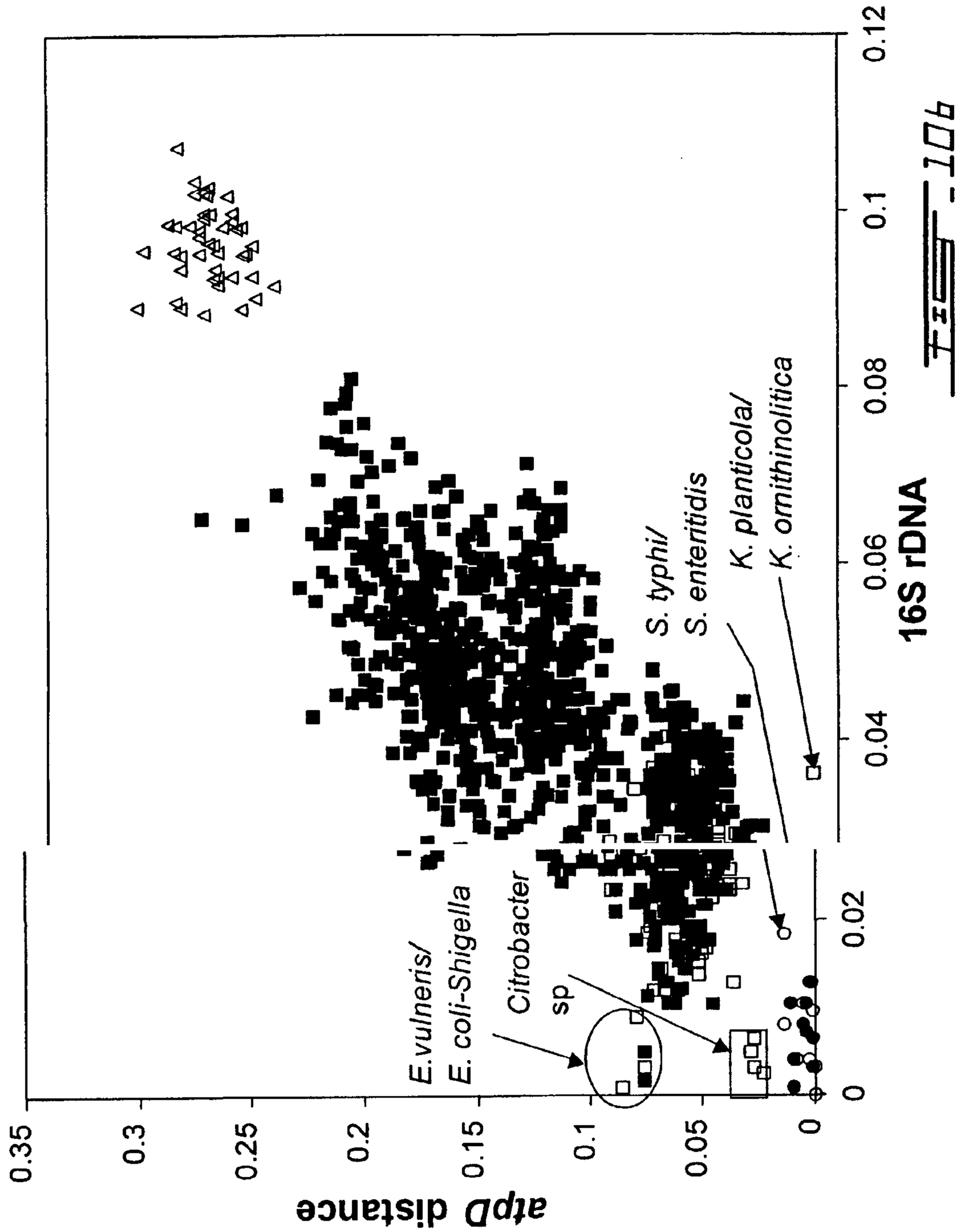
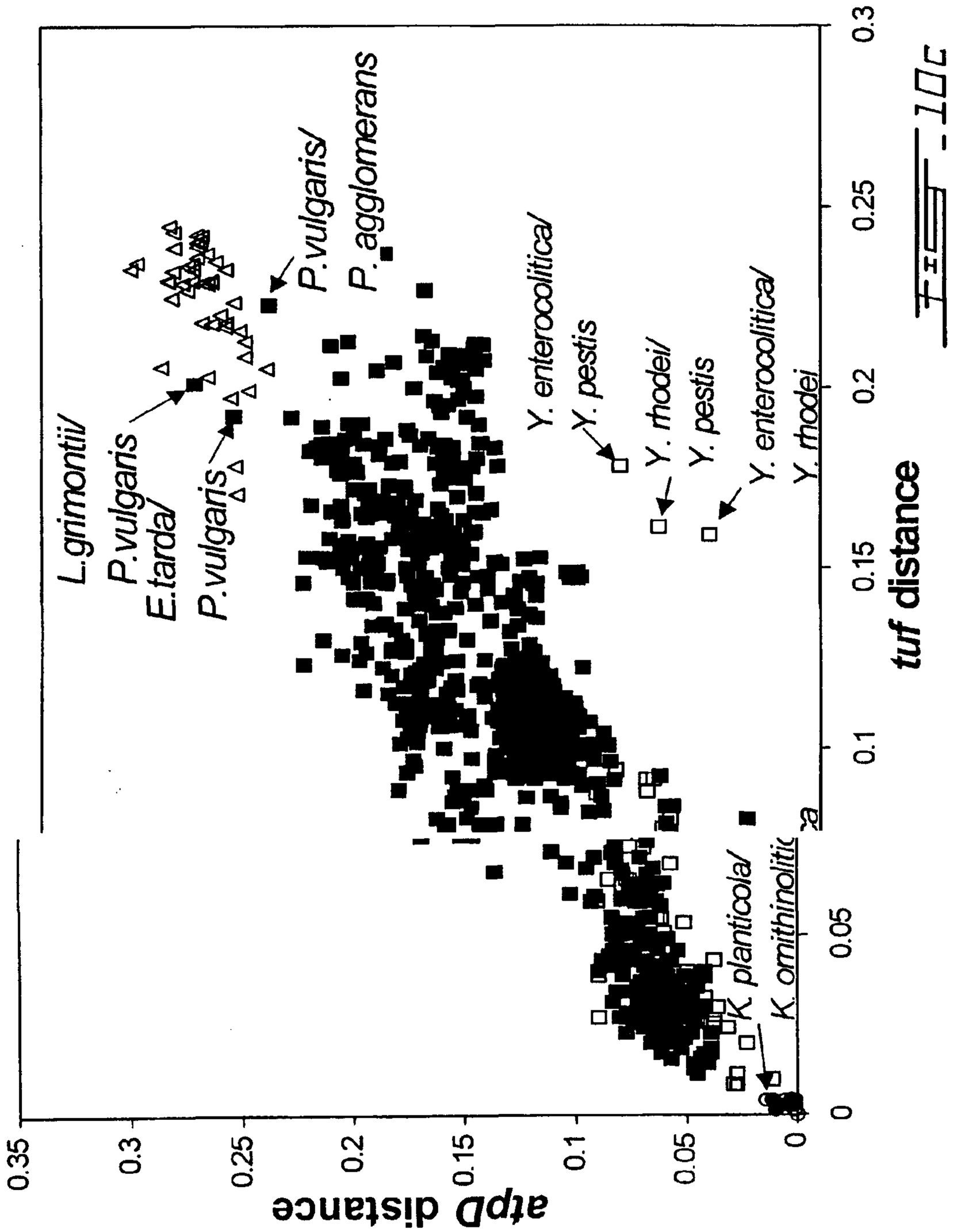
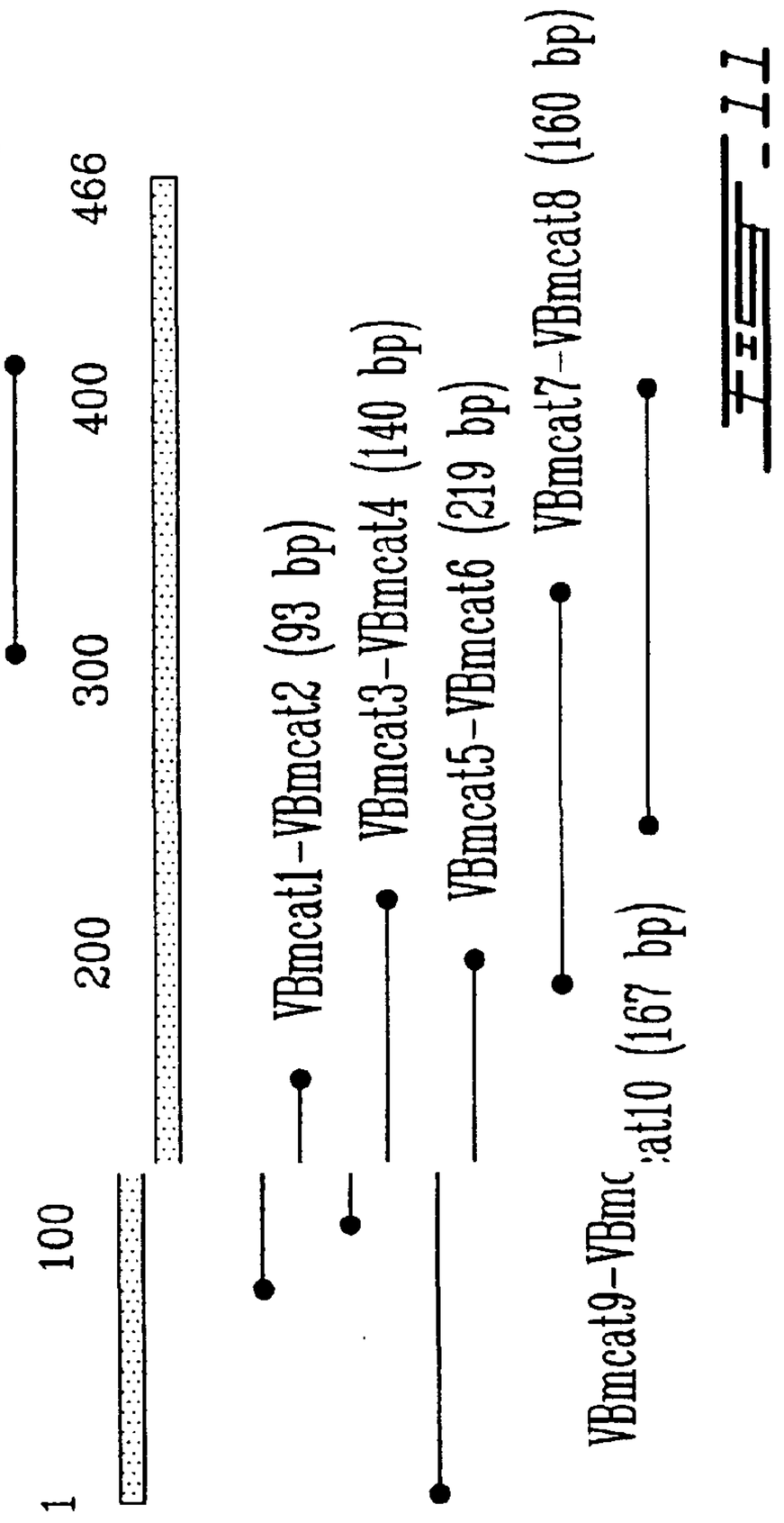

Y. pestis
Y. rhodei
Y. enterocolitica
Pa. agglomerans
T. ptyseos
L. grimontii
Pg. fontium
Ed. tarda
K. ornithinolitica
Kv. georgiana
En. aerogenes
En. gergoviae
En. amnigenus
C. braakii
C. werkmanii
C. freundii
En. asburiae
K. pneumoniae subsp. pneumoniae
K. pneumoniae subsp. ozaenae
En. sakazakii
S. Enteritidis
S. Typhi
Sh. boydii
Sh. flexneri
Sh. sonnei
E. coli 11775T
E. vulneris
E. coli 25922
Sh. dysenteriae
E. coli 43895
K. oxytoca
K. planticola
C. sedlakii
C. farmeri
C. amalonaticus
Ew. americana
R. aquatilis
Se. odorifera
Se. ficaria
Se. marcescens
Se. plymuthica
Se. grimesii
Se. fonticola
Se. rubidaea
H. halvei
P. vulgaris
V. cholerae
96
52
63
92
52
58
95
52
63
100
94
58
100
84
75
80
96
61
99
64
63
0.01 changes

Fig. 9c


0.12
0.1
0.08
0.06
0.04
16S rDNA
0.02
0
0.3
0.25
0.2
0.15
0.1
0.05
0
tuf distance


0.12
0.1
0.08
0.06
0.04
0.02
0
16S rDNA
S. typhi/
S. enteritidis
K. planticola/
K. ornithinolitica
E.vulneris/
E. coli-Shigella
Citrobacter
sp
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
atpD distance


L.grimontii/
P.vulgaris
E.tarda/
P.vulgaris
P.vulgaris
P.vulgaris
P. agglomerans
Y. enterocolitica/
Y. pestis
Y. rhodei/
Y. pestis
Y. enterocolitica/
Y. rhodei
K. planticola/
K. omithinolitic
atpD distance
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
tuf distance
0.1
0.15
0.2
0.25
0.3
0.05
0


SEQ ID NO: 118+SEQ ID NO: 119 (118 bp
1
100
200
300
400
466
VBmcat1-VBmcat2 (93 bp)
VBmcat3-VBmcat4 (140 bp)
VBmcat5-VBmcat6 (219 bp)
VBmcat7-VBmcat8 (1
at10 (167 bp)
VBmcat9-VBmc

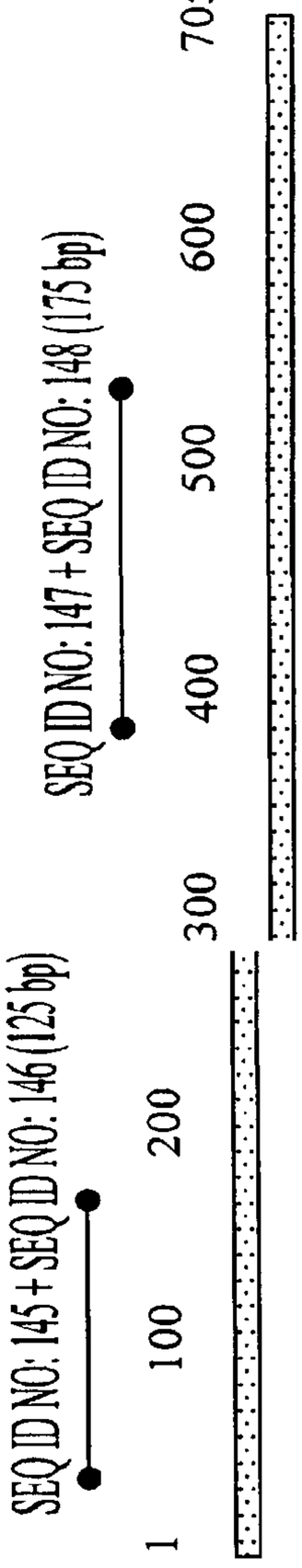
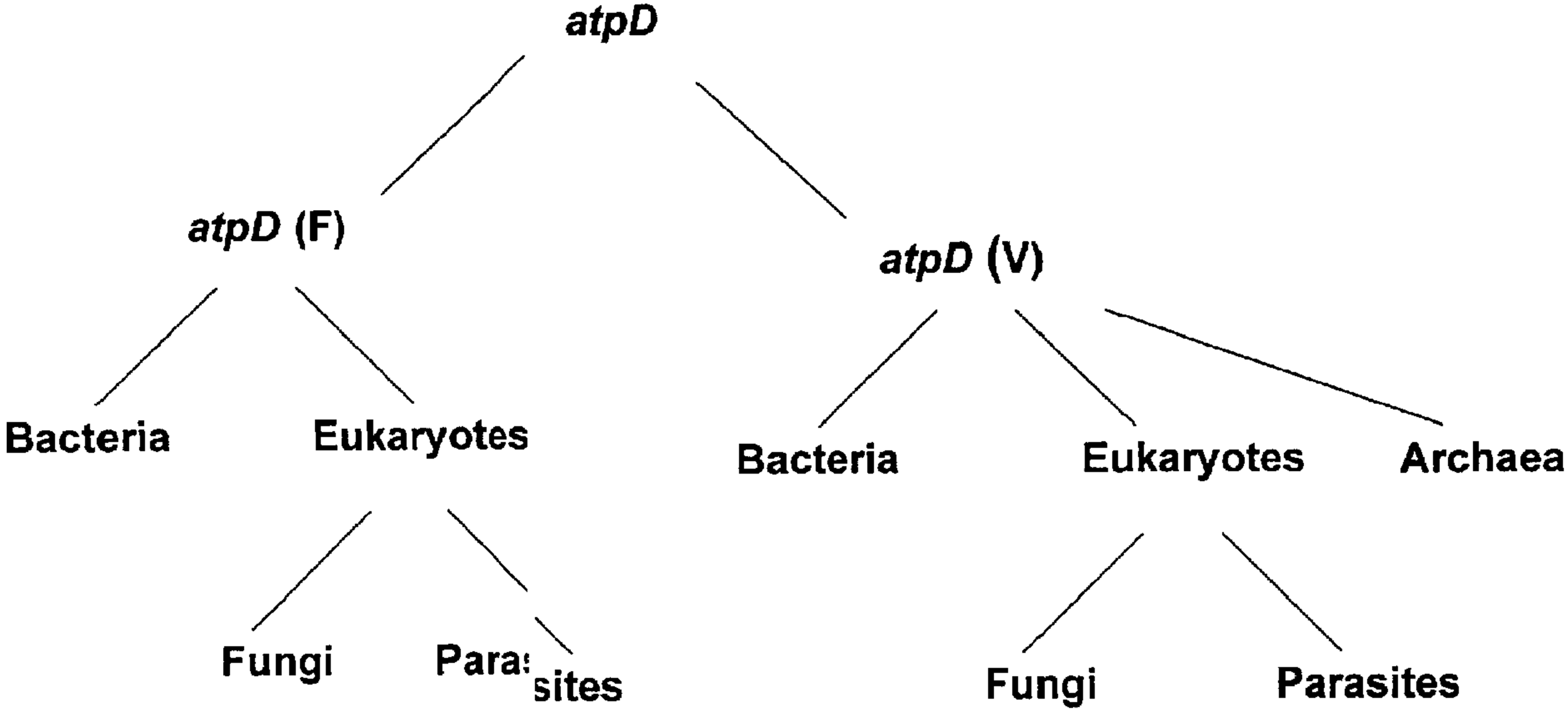

SEQ ID NO: 147 + SEQ ID NO: 148 (175 bp)
705
600
500
400
300
VBSep3-VBSep4 (208 bp)
VBSep5-VBSep6 (208 bp)
VBSep7-VBSep8 (177 bp)
(153 bp)
VBSep11-VBSep12 (135 bp)
VBSep9-VBSep10
SEQ ID NO: 145 + SEQ ID NO: 146 (125 bp)
1
100
200

Fig. 27


atpD
atpD (F)
atpD (V)
Bacteria
Eukaryotes
Bacteria
Eukaryotes
Archaea
Fungi
Para
sites
Fungi
Parasites