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A Common Origin of Rickettsiae and Certain Plant Pathogens

Abstract. *On the basis of ribosomal RNA sequence comparisons, the rickettsia Rochalimaea quintana has been found to be a member of subgroup 2 of the α subdivision of the so-called purple bacteria, which is one of about ten major eubacterial divisions. Within subgroup α -2, R. quintana is specifically related to the agrobacteria and rhizobacteria, organisms that also have close associations with eukaryotic cells. This genealogical grouping of the rickettsiae with certain plant pathogens and intracellular symbionts suggests a possible evolution of the rickettsiae from plant-associated bacteria.*

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The *Rickettsiaceae* (1) are small Gram-negative bacteria that, with few exceptions, are capable of growth only inside eukaryotic cells. They are associated with arthropods or other invertebrate hosts and often infect vertebrates. The group as taxonomically defined contains well-known human pathogens, such as the agent of epidemic typhus (*Rickettsia prowazekii*), Rocky Mountain spotted fever (*Rickettsia rickettsii*), and Q fever (*Coxiella burnetii*), as well as animal pathogens, such as agents of tropical canine pancytopenia (*Ehrlichia canis*) and tick-borne fever of sheep and

cattle (*Ehrlichia phagocytophila*). A few species are epicellular in their natural environment and can be grown axenically (for example, *Wolbachia melophagi* and the genus *Rochalimaea*); others invade and grow in the cytoplasm of their host cells (genus *Rickettsia*); still others remain in the phagosome but do not stimulate a lysosomal response (genus *Ehrlichia*); and others are adapted to grow in the phagolysosome (*Coxiella burnetii*). The designation "rickettsia" or "rickettsia-like" is sometimes applied more broadly to include certain of the endosymbionts of protozoa, insects, and other invertebrates (2), as well as certain plant pathogens, such as the agent of Pierce's disease of grapevines (3). It is not known whether rickettsiae in this broadened definition thereof constitute a phylogenetically coherent (monophyletic) grouping. Nucleic acid hybridization studies (4) leave no doubt, however, that the genera *Rickettsia* and *Rochalimaea* are related to one another.

Because of the difficulty associated with the culture of pathogens such as the rickettsiae, relatively little is known about their biochemistry and molecular biology. If free-living, readily culturable, close relatives of the rickettsiae can be found, many of their properties might be easily studied in these more readily manipulated systems.

Comparisons of nucleic acid sequences provide the best method for ascertaining microbial phylogenies. Partial sequencing of ribosomal RNA's (rRNA's) has, over the last decade, begun to reveal the evolutionary relationships among the bacteria (5). If one uses full sequencing methods (6), these relationships can be further refined and the deeper ones, such as the branching order among the various eubacterial divisions, can now be determined. A precise phylogenetic placement of *Rochalimaea quintana*, the subject of this report, demonstrates the power of sequencing approaches to (microbial) taxonomy.

Figure 1 shows the sequence of the *R. quintana* 16S rRNA gene aligned with four other eubacterial sequences, from *Agrobacterium tumefaciens* (7), *Escherichia coli* (8), *Bacillus subtilis* (9), and *Anacystis nidulans* (10). The percent difference between the various pairs of sequences is shown in the lower left triangular portion of Table 1. The numbers in the upper right triangular portion are tallies of the specific homology between them, that is, the number of positions in which composition is both common and unique to a given pair of sequences. (These numbers are basically a measure of derived characters, characters specific for particular lines of descent.)

The *R. quintana* and *A. tumefaciens* sequences (7 percent difference between the two) are much closer to one another than any of the other pairings in Table 1 (19 percent difference or more). This nearly threefold difference makes it obvious, without any formal analysis, that *R. quintana* and *A. tumefaciens* are specific relatives of one another. The count of "derived" characters (upper right portion of Table 1) shows this specific relationship as well.

Agrobacterium tumefaciens and *E. coli* are members of the purple bacteria, which is one of about ten major divisions of eubacteria (5). *Agrobacterium tumefaciens* represents the so-called α subdivision of the purple bacteria (11) and *E. coli* its γ subdivision (12). Thus, not only are the rickettsiae typical eubacteria but also they can be placed in a particular subdivision, α , of a particular eubacterial division, the purple bacteria.

Table 1. Homology measurements for the sequences in Fig. 1. The lower left triangular section shows the percentage of total positions wherein any two sequences differ. Only those positions in the Fig. 1 alignment represented in all of the sequences are used in this analysis, a total of 1477. The upper right triangular section represents the number of positions (from the 1477) in which composition is both common and unique to a given pair of sequences.

Species	<i>R. quin-</i> <i>tana</i>	<i>A. tume-</i> <i>faciens</i>	<i>E. coli</i>	<i>B. sub-</i> <i>tilis</i>	<i>A. nidu-</i> <i>lans</i>
<i>R. quintana</i>	----	94	6	10	17
<i>A. tumefaciens</i>	7.1	----	8	4	11
<i>E. coli</i>	20.5	20.2	----	27	46
<i>B. subtilis</i>	20.0	20.4	21.1	----	63
<i>A. nidulans</i>	21.3	21.7	21.9	19.0	----

R quintana	UUCAAUUGAGA	GUUUGAUCCU	GGCUCAGAAC	GAACGCGUGC	GGCAGGCUUA	ACACAUGCAA	GUCCGAGCGA	CUCU.....	..UUU....	AGAGUG
A tumefaciens	CUCAACUUGAGA	GUUUGAUCCU	GGCUCAGAAC	GAACGCGUGC	GGCAGGCUUA	ACACAUGCAA	GUCCGAGCGC	CC.....	..GCAA....	GGGG
E coli	..AAAUUGAAGA	GUUUGAUCAU	GGCUCAGAUU	GAACGCGUGC	GGCAGGCUUA	ACACAUGCAA	GUCCGAGCGU	AACAGGAAGA	AGCUUGCUUC	UUUGUCUGAG
B subtilis	..UUUAUCCGAGA	GUUUGAUCCU	GGCUCAGAGC	GAACGCGUGC	GGCAGGCUUA	ACACAUGCAA	GUCCGAGCGA	CAGGUGGAGU	..CUUG...C	UCCCGAUGUU
A nidulans	..CAAAUUGGAGA	GUUUGAUCCU	GGCUCAGAUU	GAACGCGUGC	GGCAGGCUUA	ACACAUGCAA	GUCCGAGCGG	CUC.....	..UUCG....	GAGCU
R quintana	AGCGGCAAAAC	GGGUGAGUAA	CGCGUGGGA.	UCUACCCAU	UCUACGGAU	AACACAGAGA	AAUUGUGCU	AAUACCGU	ACGUCCUC.....	UG GGAGAAAG.
A tumefaciens	AGUGGCGAC	GGGUGAGUAA	CGCGUGGGA.	UCUACCGUG	CCUGCGGAU	AGCUCCGGA	AACUGGAU	AAUACCGCAU	ACGCCUA.....	CG GGGAAAGA.
E coli	AGUGGCGGAC	GGGUGAGUAA	UGUCUGGGA.	ACUGCGGUA	GGAGGUGUA	AACUGGUA	AACUGGUA	AAUACCGCAU	AACGUGC.....	AA GACCAAGAG
B subtilis	AGCGGCGGAC	GGGUGAGUAA	CAGUGGUGUA	CCUGCGGUA	AGACUGGUA	AACUGGUA	AACCGGUA	AAUACCGCAU	GGUUGUUGAACCGCAUGGUUCAA	CAUAAAGGU
A nidulans	AGUGGCGGAC	GGGUGAGUAA	CGCGUGGGA.	UCUGCGUA	GGACGGGAC	AACUGGUA	AACUGGUA	AAUACCGCAU	G.UCCGCA.....	GA GGUGAAACA.
R quintana	UUU	A.....UGG	GAGUGGAUG	AGCGCGGUA	GGAUUGCUA	GUUGUGAGG	UAAAGGCUA	CCAAGGCGAC	GAUCCAUAGC	UGGUCUGAGA
A tumefaciens	UUU	A.....UGG	GGGUGAUG	AGCGCGGUA	GGAUUGCUA	GUUGUGAGG	UAAAGGCUA	CCAAGGCGAC	GAUCCAUAGC	UGGUCUGAGA
E coli	GGGCGGCUU	GGGCGGCUU	CCAUCCGAG	UGCGCGGUA	GGAUUGCUA	GUUGUGAGG	UAAAGGCUA	CCAAGGCGAC	GAUCCAUAGC	UGGUCUGAGA
B subtilis	GGG.....UUC	GG.....UUC	CCUACAGAG	AGCGCGGUA	GGAUUGCUA	GUUGUGAGG	UAAAGGCUA	CCAAGGCGAC	GAUCCAUAGC	UGGUCUGAGA
A nidulans	UUU	A.....UGG	CCUUGAUG	AGCGCGGUA	GGAUUGCUA	GUUGUGAGG	UAAAGGCUA	CCAAGGCGAC	GAUCCAUAGC	UGGUCUGAGA
R quintana	GGAUGAUCAG	CCACACUGGG	ACUGAGACAC	GGCCACACU	CCUACGGGAG	GCAGCAGUGG	GGAUUAUUGG	ACAAUGGGGG	CAACCCUGAU	CCAGCCAUCC
A tumefaciens	GGAUGAUCAG	CCACACUGGG	ACUGAGACAC	GGCCACACU	CCUACGGGAG	GCAGCAGUGG	GGAUUAUUGG	ACAAUGGGGG	CAACCCUGAU	CCAGCCAUCC
E coli	GGAUGAUCAG	CCACACUGGG	ACUGAGACAC	GGCCACACU	CCUACGGGAG	GCAGCAGUGG	GGAUUAUUGG	ACAAUGGGGG	CAACCCUGAU	CCAGCCAUCC
B subtilis	GGGUGAUCAG	CCACACUGGG	ACUGAGACAC	GGCCACACU	CCUACGGGAG	GCAGCAGUGG	GGAUUAUUGG	ACAAUGGGGG	CAACCCUGAU	CCAGCCAUCC
A nidulans	GGAUGAUCAG	CCACACUGGG	ACUGAGACAC	GGCCACACU	CCUACGGGAG	GCAGCAGUGG	GGAUUAUUGG	ACAAUGGGGG	CAACCCUGAU	CCAGCCAUCC
R quintana	CGCGUGAGUG	AUCAAGGCC	UAGGUGUUA	AAGCUCUUC	ACCGGUAAG	AUA.....		U	CAGCUUAACC	GGAGAAGAAG
A tumefaciens	CGCGUGAGUG	AUCAAGGCC	UAGGUGUUA	AAGCUCUUC	ACCGGUAAG	AUA.....		U	CAGCUUAACC	GGAGAAGAAG
E coli	CGCGUGAGUG	AUCAAGGCC	UAGGUGUUA	AAGCUCUUC	ACCGGUAAG	AAGG.GAGUAA	AGUUAUACC	UUUGCUUACU	CAGCUUAACC	GGAGAAGAAG
B subtilis	CGCGUGAGUG	AUCAAGGCC	UAGGUGUUA	AAGCUCUUC	ACCGGUAAG	AACAAGUACCG	UUCGAAUAGG	CGCGUACCUU	CAGCUUAACC	GGAGAAGAAG
A nidulans	CGCGUGAGUG	AUCAAGGCC	UAGGUGUUA	AAGCUCUUC	ACCGGUAAG	AAGA.....		AAGU	CAGCUUAACC	GAGGAUAGG
R quintana	CCCCGGCUAA	CUUCGUGCCA	CGACCGCGG	UAAUACGAAG	GGGCGUAGG	UUUUUUGGAA	UUUACUGGGG	UAAAGCGCAU	GUAGGCGGAU	AUUUAAGUCA
A tumefaciens	CCCCGGCUAA	CUUCGUGCCA	CGACCGCGG	UAAUACGAAG	GGGCGUAGG	UUUUUUGGAA	UUUACUGGGG	UAAAGCGCAU	GUAGGCGGAU	AUUUAAGUCA
E coli	CACCGGCUAA	CUUCGUGCCA	CGACCGCGG	UAAUACGAAG	GGGCGUAGG	UUUUUUGGAA	UUUACUGGGG	UAAAGCGCAU	GUAGGCGGAU	UUGUUAAGUCA
B subtilis	CCACGGCUAA	CUUCGUGCCA	CGACCGCGG	UAAUACGAAG	GGGCGUAGG	UUUUUUGGAA	UUUACUGGGG	UAAAGCGCAU	GUAGGCGGAU	UUGUUAAGUCA
A nidulans	CCCCGGCUAA	CUUCGUGCCA	CGACCGCGG	UAAUACGAAG	GGGCGUAGG	UUUUUUGGAA	UUUACUGGGG	UAAAGCGCAU	GUAGGCGGAU	UUGUUAAGUCA
R quintana	GAGGUGAAU	CCGAGGCGC	AACCCUGGA	CUGCCUUGA	UACUGGAUG	CUCGAGUGG	GAAGAGGUGA	GUGGAAUUC	GAGUGUAGG	GUAAAAUCC
A tumefaciens	GAGGUGAAU	CCGAGGCGC	AACCCUGGA	CUGCCUUGA	UACUGGAUG	CUCGAGUGG	GAAGAGGUGA	GUGGAAUUC	GAGUGUAGG	GUAAAAUCC
E coli	GAGGUGAAU	CCGAGGCGC	AACCCUGGA	CUGCCUUGA	UACUGGAUG	CUCGAGUGG	GAAGAGGUGA	GUGGAAUUC	GAGUGUAGG	GUAAAAUCC
B subtilis	GAGGUGAAU	CCGAGGCGC	AACCCUGGA	CUGCCUUGA	UACUGGAUG	CUCGAGUGG	GAAGAGGUGA	GUGGAAUUC	GAGUGUAGG	GUAAAAUCC
A nidulans	GAGGUGAAU	CCGAGGCGC	AACCCUGGA	CUGCCUUGA	UACUGGAUG	CUCGAGUGG	GAAGAGGUGA	GUGGAAUUC	GAGUGUAGG	GUAAAAUCC
R quintana	UAGAUUUCG	GAGGAACACC	AGUGGCGAAG	GCGGCUACU	GGUCCAUUAC	UGACGCGUAG	GUGGGAAGC	GUGGGGAGCA	AACAGGAUUA	GAUACCCUGG
A tumefaciens	UAGAUUUCG	GAGGAACACC	AGUGGCGAAG	GCGGCUACU	GGUCCAUUAC	UGACGCGUAG	GUGGGAAGC	GUGGGGAGCA	AACAGGAUUA	GAUACCCUGG
E coli	UAGAUUUCG	GAGGAACACC	AGUGGCGAAG	GCGGCUACU	GGUCCAUUAC	UGACGCGUAG	GUGGGAAGC	GUGGGGAGCA	AACAGGAUUA	GAUACCCUGG
B subtilis	UAGAUUUCG	GAGGAACACC	AGUGGCGAAG	GCGGCUACU	GGUCCAUUAC	UGACGCGUAG	GUGGGAAGC	GUGGGGAGCA	AACAGGAUUA	GAUACCCUGG
A nidulans	UAGAUUUCG	GAGGAACACC	AGUGGCGAAG	GCGGCUACU	GGUCCAUUAC	UGACGCGUAG	GUGGGAAGC	GUGGGGAGCA	AACAGGAUUA	GAUACCCUGG
R quintana	UAGUCCACGC	CGUAAACGAU	GAAUGUAGC	CGUGGCGUG	..UUA..CUACU	CGUGGCGCA	CGUAAACGCU	UAAACAUUCC	CGUGGGGAG	UACCGUGCA
A tumefaciens	UAGUCCACGC	CGUAAACGAU	GAAUGUAGC	CGUGGCGUG	..UUA..CUACU	CGUGGCGCA	CGUAAACGCU	UAAACAUUCC	CGUGGGGAG	UACCGUGCA
E coli	UAGUCCACGC	CGUAAACGAU	GAAUGUAGC	CGUGGCGUG	..UUA..CUACU	CGUGGCGCA	CGUAAACGCU	UAAACAUUCC	CGUGGGGAG	UACCGUGCA
B subtilis	UAGUCCACGC	CGUAAACGAU	GAAUGUAGC	CGUGGCGUG	..UUA..CUACU	CGUGGCGCA	CGUAAACGCU	UAAACAUUCC	CGUGGGGAG	UACCGUGCA
A nidulans	UAGUCCACGC	CGUAAACGAU	GAAUGUAGC	CGUGGCGUG	..UUA..CUACU	CGUGGCGCA	CGUAAACGCU	UAAACAUUCC	CGUGGGGAG	UACCGUGCA
R quintana	AGAUUAAAC	UCAAAGGAU	UGACGGGGC	CGGCACAAGC	GGUGGAGCAU	GUGGUUUAU	UGCAAGCAAC	GCGCAGAAC	UUACAGCCCC	UUGACAUCC
A tumefaciens	AGAUUAAAC	UCAAAGGAU	UGACGGGGC	CGGCACAAGC	GGUGGAGCAU	GUGGUUUAU	UGCAAGCAAC	GCGCAGAAC	UUACAGCCCC	UUGACAUCC
E coli	AGAUUAAAC	UCAAAGGAU	UGACGGGGC	CGGCACAAGC	GGUGGAGCAU	GUGGUUUAU	UGCAAGCAAC	GCGCAGAAC	UUACAGCCCC	UUGACAUCC
B subtilis	AGAUUAAAC	UCAAAGGAU	UGACGGGGC	CGGCACAAGC	GGUGGAGCAU	GUGGUUUAU	UGCAAGCAAC	GCGCAGAAC	UUACAGCCCC	UUGACAUCC
A nidulans	AGAUUAAAC	UCAAAGGAU	UGACGGGGC	CGGCACAAGC	GGUGGAGCAU	GUGGUUUAU	UGCAAGCAAC	GCGCAGAAC	UUACAGCCCC	UUGACAUCC
R quintana	GAUGCGGAAAG	UGGAGACACC	UCCCUUAGU	AGGUGGAUCC	GUGCAGGUG	CUGCAUGGCU	GUGGUGAGCU	CGUGUGGUA	GAUGUUGGU	UAGUCCCGC
A tumefaciens	GGGUUUGGCGAG	UGGAGACAUU	GUCCUUAGU	AGGUGGCCCC	AGAAGAGGUG	CUGCAUGGCU	GUGGUGAGCU	CGUGUGGUA	GAUGUUGGU	UAGUCCCGC
E coli	GGGUUUGGCGAG	UGGAGACAUU	GUCCUUAGU	AGGUGGCCCC	AGAAGAGGUG	CUGCAUGGCU	GUGGUGAGCU	CGUGUGGUA	GAUGUUGGU	UAGUCCCGC
B subtilis	GGGUUUGGCGAG	UGGAGACAUU	GUCCUUAGU	AGGUGGCCCC	AGAAGAGGUG	CUGCAUGGCU	GUGGUGAGCU	CGUGUGGUA	GAUGUUGGU	UAGUCCCGC
A nidulans	GGGUUUGGCGAG	UGGAGACAUU	GUCCUUAGU	AGGUGGCCCC	AGAAGAGGUG	CUGCAUGGCU	GUGGUGAGCU	CGUGUGGUA	GAUGUUGGU	UAGUCCCGC
R quintana	AACGAGCGCA	ACCCUGCGCC	UUAGUUGCA	UCAUUAGU.	UGGGCACUCU	AGGGGACUC	CCGGUGAUAA	GCCGAGAGGA	GGUGGGGAG	ACGUCAAGUC
A tumefaciens	AACGAGCGCA	ACCCUGCGCC	UUAGUUGCA	UCAUUAGU.	UGGGCACUCU	AGGGGACUC	CCGGUGAUAA	GCCGAGAGGA	GGUGGGGAG	ACGUCAAGUC
E coli	AACGAGCGCA	ACCCUGCGCC	UUAGUUGCA	UCAUUAGU.	UGGGCACUCU	AGGGGACUC	CCGGUGAUAA	GCCGAGAGGA	GGUGGGGAG	ACGUCAAGUC
B subtilis	AACGAGCGCA	ACCCUGCGCC	UUAGUUGCA	UCAUUAGU.	UGGGCACUCU	AGGGGACUC	CCGGUGAUAA	GCCGAGAGGA	GGUGGGGAG	ACGUCAAGUC
A nidulans	AACGAGCGCA	ACCCUGCGCC	UUAGUUGCA	UCAUUAGU.	UGGGCACUCU	AGGGGACUC	CCGGUGAUAA	GCCGAGAGGA	GGUGGGGAG	ACGUCAAGUC
R quintana	CUCAUGGCCC	UUACGGGCG	GGCUACACAC	GUGCUACAAU	GGUGGUGACA	GUGGGCAGCG	AGACCGCGAG	GUCGAGCUAA	UUCUCAA...	GCCAUUCAG
A tumefaciens	CUCAUGGCCC	UUACGGGCG	GGCUACACAC	GUGCUACAAU	GGUGGUGACA	GUGGGCAGCG	AGACCGCGAG	GUCGAGCUAA	UUCUCAA...	GCCAUUCAG
E coli	CUCAUGGCCC	UUACGGGCG	GGCUACACAC	GUGCUACAAU	GGUGGUGACA	GUGGGCAGCG	AGACCGCGAG	GUCGAGCUAA	UUCUCAA...	GCCAUUCAG
B subtilis	CUCAUGGCCC	UUACGGGCG	GGCUACACAC	GUGCUACAAU	GGUGGUGACA	GUGGGCAGCG	AGACCGCGAG	GUCGAGCUAA	UUCUCAA...	GCCAUUCAG
A nidulans	CUCAUGGCCC	UUACGGGCG	GGCUACACAC	GUGCUACAAU	GGUGGUGACA	GUGGGCAGCG	AGACCGCGAG	GUCGAGCUAA	UUCUCAA...	GCCAUUCAG
R quintana	UUCGGAUUGC	ACUCUGCAAC	UCGAGUGCAU	GAAGUUGAA	UCGCUAGUAA	UCGUGGAUAA	GCAUGCCAGC	GUGAAUACCU	UCCCGGGCCU	UGUACACACC
A tumefaciens	UUCGGAUUGC	ACUCUGCAAC	UCGAGUGCAU	GAAGUUGAA	UCGCUAGUAA	UCGUGGAUAA	GCAUGCCAGC	GUGAAUACCU	UCCCGGGCCU	UGUACACACC
E coli	UUCGGAUUGC	ACUCUGCAAC	UCGAGUGCAU	GAAGUUGAA	UCGCUAGUAA	UCGUGGAUAA	GCAUGCCAGC	GUGAAUACCU	UCCCGGGCCU	UGUACACACC
B subtilis	UUCGGAUUGC	ACUCUGCAAC	UCGAGUGCAU	GAAGUUGAA	UCGCUAGUAA	UCGUGGAUAA	GCAUGCCAGC	GUGAAUACCU	UCCCGGGCCU	UGUACACACC
A nidulans	UUCGGAUUGC	ACUCUGCAAC	UCGAGUGCAU	GAAGUUGAA	UCGCUAGUAA	UCGUGGAUAA	GCAUGCCAGC	GUGAAUACCU	UCCCGGGCCU	UGUACACACC
R quintana	GCCCUCACAC	CCAUGGGAGU	UGGUUUUACC	CGAAGGUGCU	GUGCUAAGC.G	CAA.GGAGGCA	GGCAACACAG	GUAGGUGCAG	CGACUGGGG	GAAGUCGUAA
A tumefaciens	GCCCUCACAC	CCAUGGGAGU	UGGUUUUACC	CGAAGGUGCU	GUGCUAAGC.G	CAA.GGAGGCA	GGCAACACAG	GUAGGUGCAG	CGACUGGGG	GAAGUCGUAA
E coli	GCCCUCACAC	CCAUGGGAGU	UGGUUUUACC	CGAAGGUGCU	GUGCUAAGC.G	CAA.GGAGGCA	GGCAACACAG	GUAGGUGCAG	CGACUGGGG	GAAGUCGUAA
B subtilis	GCCCUCACAC	CCAUGGGAGU	UGGUUUUACC	CGAAGGUGCU	GUGCUAAGC.G	CAA.GGAGGCA	GGCAACACAG	GUAGGUGCAG	CGACUGGGG	GAAGUCGUAA
A nidulans	GCCCUCACAC	CCAUGGGAGU	UGGUUUUACC	CGAAGGUGCU	GUGCUAAGC.G	CAA.GGAGGCA	GGCAACACAG	GUAGGUGCAG	CGACUGGGG	GAAGUCGUAA
R quintana	CAAGGUAGCC	GUAGGGGAAC	CUGUGGUGG	AUACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU
A tumefaciens	CAAGGUAGCC	GUAGGGGAAC	CUGUGGUGG	AUACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU
E coli	CAAGGUAGCC	GUAGGGGAAC	CUGUGGUGG	AUACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU
B subtilis	CAAGGUAGCC	GUAGGGGAAC	CUGUGGUGG	AUACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU
A nidulans	CAAGGUAGCC	GUAGGGGAAC	CUGUGGUGG	AUACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU	UACCCUCCUUU

Fig. 1. Sequence alignment for the 16S rRNA of *R. quintana* and other eubacteria. *Rochalimaea quintana* strain Fuller DNA was cleaved with Bgl II restriction endonuclease, and the resulting fragments were inserted into the Bam HI site of phage lambda L47 by standard techniques (16). We selected a fragment of approximately 10 kilobase pairs, using radioactively labeled *R. quintana* 16S rRNA as a hybridization probe. Appropriate fragments were subcloned into phage M13 mp8 and mp9, and the single-stranded phage DNA's were used as templates for dideoxy sequencing of the ribosomal RNA gene (17). Both the usual M13 primer and primers specific for ribosomal RNA genes were used (7). At least 85 percent of the sequence was determined for both DNA strands. The alignment procedure was that used by Yang *et al.* (7).

Table 2. The relationship of *R. quintana* to selected members of the α subdivision of the purple eubacteria (11). The ribonuclease T1-type (G ending) oligonucleotide segments contained in the *R. quintana* sequence have been compared to their counterparts determined directly from the 16S rRNA's of various α purple bacteria (11). The total number of bases in oligonucleotides (hexamer and larger) of identical composition in any pair of these catalogs is shown. The numbers in parentheses are these counts divided by 3.09; this normalization brings most of the entries in the α -2 group to values near 100 and thereby facilitates their comparison. For the α -1 (seven species) and α -3 (five species) subgroups of the α subdivision (11), the counts are presented as average values between each of these groups and individual species of the α -2 subgroup. Genus abbreviations not listed in Table 1 are as follows: *Rh.*, *Rhizobium*; *Rps.*, *Rhodopseudomonas*, and *Rm.*, *Rhodomicrobium*.

Species	<i>R. quin-</i> <i>tana</i>	<i>A. tume-</i> <i>faciens</i>	<i>Rh. legu-</i> <i>minosarum</i>	<i>Rps.</i> <i>palustris</i>	<i>Rps.</i> <i>viridis</i>	<i>Rm. van-</i> <i>nielii</i>
<i>R. quintana</i>	-----					
<i>A. tumefaciens</i>	362(117)	-----				
<i>Rh. leguminosarum</i>	377(122)	440(142)	-----			
<i>Rps. palustris</i>	293 (95)	294 (95)	329(106)	-----		
<i>Rps. viridis</i>	334(108)	321(104)	306 (99)	308(100)	-----	
<i>Rm. vanniellii</i>	309(100)	303 (98)	295 (95)	284 (92)	337(109)	
α -1	265 (86)	247 (80)	247 (80)	226 (73)	267 (86)	252 (81)
α -3	248 (80)	263 (85)	272 (88)	238 (77)	250 (81)	230 (74)

The close relationship between the *R. quintana* and *A. tumefaciens* 16S rRNA sequences prompts an investigation of the possible relationships of *R. quintana* to other species in the α subdivision. Partial 16S rRNA sequences, in the form of oligonucleotide catalogs, exist for over 350 eubacterial species; 100 of these are from the purple bacteria (11, 12), and 21 from the α subdivision (11). This partial sequence information permits a localization of the phylogenetic position of *Rochalimaea* within the α subdivision, and it rules out previously suspected relationships to organisms such as *Xanthomonas* and *Legionella* (12, 13).

Table 2 shows the similarity of *R. quintana* to representatives of the three subgroups of the α subdivision, α -1, α -2, and α -3. [Similarity here is defined in terms of oligonucleotide catalogs, that is, as the number of bases in oligonucleotides (hexamer and larger) that are common to each pair of catalogs (11).] It can be seen that *R. quintana* is a member of the α -2 subgroup and within that unit clusters specifically with the agrobacteria and rhizobacteria.

It is interesting that the closest relatives of *R. quintana* and the rickettsia itself are all associated somehow with eukaryotic cells, often intracellularly (14). This correlation is perhaps made more significant by the finding that the mitochondrion too seems to have originated from within the α subdivision (9).

The fact that *R. quintana* groups with bacteria that are associated with plants, not with animals, suggests that rickettsiae may have arisen as plant-associated bacteria, possibly plant pathogens, and through the plant-insect bridge may have evolved to be associated with mammals. The resemblance of some plant patho-

gens to rickettsiae (3) may turn out to be more than superficial, and the finding of agrobacteria in human clinical specimens now takes on new significance (15).

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Coronary Vasoconstrictor Effects of Atriopeptin II

Abstract. Atrial natriuretic peptides lower arterial pressure, cardiac filling pressure, and cardiac output. In isolated, Langendorff-perfused guinea pig hearts, atriopeptin II, the 23-amino acid atrial natriuretic peptide, is also a potent coronary vasoconstrictor. The median effective dose for atriopeptin II in guinea pig hearts is 26 nanomoles, the threshold constrictor dose is 5 nanomoles, and flow nearly ceases at a dose of 100 nanomoles in perfused hearts at constant pressure. Similar concentrations of atriopeptin II also cause coronary vasoconstriction in rat and dog heart preparations. The disulfide bridge is necessary for vasoconstrictor activity; reduction of this bridge abolishes the activity, as it does the other biological activities of atrial natriuretic peptides.

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Mammalian cardiac atria contain secretory granules. Peptides associated with granule-rich fractions have been isolated from atria that produce natriuresis and diuresis, relax vascular and intestinal smooth muscle, and lower arterial pressure (1). Several of these peptides have been purified, their amino acid sequences have been determined, and they have been synthesized (2). Because atrial extract and atrial natriuretic peptides (ANP) relax vascular smooth muscle, it is reasonable to assume that they lower arterial pressure by relaxation of arteriolar (resistance vessel) smooth muscle (2). However, we have discovered that the pure 23-amino acid ANP