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27 March 1985; accepted 4 September 1985

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.

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	*R. quintana*	*A. tumefaciens*	*E. coli*	*B. subtilis*	*A. nidulans*
R. quintana	- - - -	94	6	10	17
A. tumefaciens	7.1	- - - -	8	4	11
E. coli	20.5	20.2	- - - -	27	46
B. subtilis	20.0	20.4	21.1	- - - -	63
A. nidulans	21.3	21.7	21.9	19.0	- - - -

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* 16*S* 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.

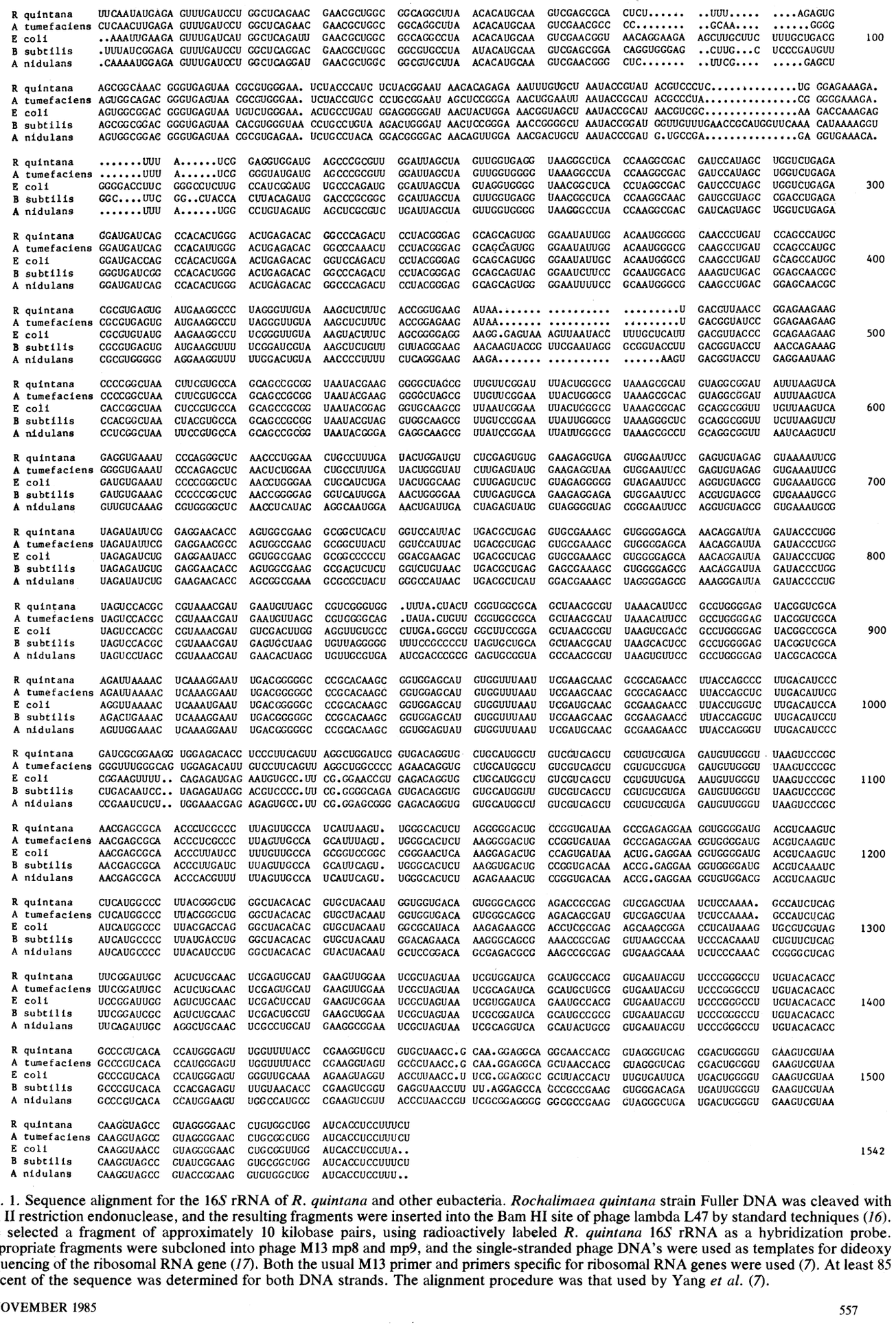

Fig. 1. Sequence alignment for the 16*S* rRNA of *R. quintana* and other eubacteria. *Rochalimaea quintana* strain Fuller DNA was cleaved with Bgl II restriction endonuclease, and the resulting fragments were inserted into the Bam HI site of phage lambda L47 by standard techniques (*16*). We selected a fragment of approximately 10 kilobase pairs, using radioactively labeled *R. quintana* 16*S* 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 16*S* 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	*R. quintana*	*A. tumefaciens*	*Rh. leguminosarum*	*Rps. palustris*	*Rps. viridis*	*Rm. vannielii*
R. quintana	--------					
A. tumefaciens	362(117)	--------				
Rh. leguminosarum	377(122)	440(142)	--------			
Rps. palustris	293 (95)	294 (95)	329(106)	--------		
Rps. viridis	334(108)	321(104)	306 (99)	308(100)	--------	
Rm. vannielii	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* 16*S* rRNA sequences prompts an investigation of the possible relationships of *R. quintana* to other species in the α subdivision. Partial 16*S* 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 pathogens 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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18. Supported by grants to C.R.W. from the National Aeronautics and Space Administration and the National Science Foundation, and by the Naval Medical Research and Development Command, research projects 3M162770A870.AE.307 and MR0000101.1327 (E.W.)

5 April 1985; accepted 5 September 1985

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