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DNA-DNA Solution Hybridization Studies of the Bacterial Symbionts of Hydrothermal Vent Tube Worms (*Riftia pachyptila* and *Tevnia jerichonana*)

DEEANNE B. EDWARDS† AND DOUGLAS C. NELSON*

Department of Microbiology, University of California, Davis, California 95616

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The giant tube worm, *Riftia pachyptila* (phylum Vestimentifera), is known only from four widely separated sulfide-rich deep-sea hydrothermal vent systems. This invertebrate is nourished by intracellular, chemoautotrophic bacterial symbionts which reside in a specialized trophosome tissue. The symbiont has not been cultured independently and is believed to be acquired de novo by host larvae of each generation. In the current study, *R. pachyptila* symbiont DNA was purified from the two most distant sites on the basis of its difference in density versus host DNA. These two standards were hybridized against trophosome DNAs of 13 individuals from the Guaymas Basin, Galapagos Rift, and 13°N vents. This indicated that all *R. pachyptila* symbionts are conspecific and that the variability in DNA-DNA hybridization (relative binding ratio [RBR]) was comparable within or between widely separated vents. The symbiont of another tube worm, *Tevnia jerichonana*, was found to be the same as that of *R. pachyptila*, the first case in which distinct hosts possess the same sulfur bacterial symbiont. By contrast, *Lamellibrachia* sp. (same class as *T. jerichonana*) showed insignificant RBR with the *R. pachyptila* symbiont. DNA derived from solely eucaryotic tissue of *R. pachyptila* showed a surprisingly high RBR (20 to 50) with density-separated DNA standards. With DNAs obtained from physically separated symbionts, independent solution hybridization experiments confirmed the above-described conclusions. Possible explanations for this host-symbiont homology are discussed.

A variety of invertebrates living in dense populations at deep-sea hydrothermal vents are known to derive much or all of their nutrition from symbiotic, chemoautotrophic sulfur bacteria (reviewed in references 8 and 15). The giant tube worm, *Riftia pachyptila*, was the first discovered and is still the most distinctive of these animals, reaching up to 1.5 m in length and almost 4 cm in diameter. Adults of this species have no mouth, gut, or anus and are classified as belonging to the phylum Vestimentifera, along with a few other deep-sea tube worm species (18).

R. pachyptila contain a spongy trophosome tissue which comprises approximately 50% of the host's body volume and spans about 75% of its length. Studies by Cavanaugh et al. (7) and Felbeck (13) showed that the tissue was the locus of the densely packed chemoautotrophic procaryotic symbionts (approximately 4×10^{10} cells per g [wet weight]). It has been established that these symbionts are contained within host bacteriocytes (6). Furthermore, the symbionts contain intracellular (periplasmic) globules of elemental sulfur, presumably derived from partial oxidation of hydrogen sulfide, their major electron donor. The mechanism by which young tube worms acquire symbionts is unclear. Symbionts have not been observed in unfertilized eggs or spermatozoa (5, 7, 20).

Recent anatomical studies led to the conclusion that juveniles (and possibly larvae) of *R. pachyptila* and another vestimentiferan genus, *Ridgeia*, transiently possess a ciliated duct that can be traced from an external opening to the vicinity of what will (has) become the trophosome (19, 20). This, coupled with the lack of evidence for transovarian acquisition, led to the hypothesis that the *Riftia* symbiont is acquired de novo via this duct by each generation. It was further hypothesized that when appropriate bacteria are encountered they colonize the nascent trophosome, possibly via phagocytosis by the bacteriocytes (20).

R. pachyptila is documented to occur only in four narrowly circumscribed locations which, in turn, are separated from each other by 1,000 km or more. These vent sites are located in the Eastern Pacific ranging from the equator to approximately 30°N (depth, 2,000 to 2,600 m). If symbionts are acquired de novo by each generation and if the inoculum source is other tube worms at the same site, genomic divergence between symbionts from widely separated vent sites could occur.

Because the symbionts of *R. pachyptila* have not been successfully cultivated apart from the host, standard phenotypic methods of classification are not applicable. Attempts at classification of the *Riftia* symbionts have used 5S (21, 29) and 16S (11) rRNA sequence analysis, which compares these bacteria with other known sequences in a data base. All of the vent symbionts studied thus far cluster most closely with other uncultured symbionts from vent and vent analog environments, and no free-living bacteria tested so far fall into this cluster. The degree of divergence within these symbionts is fairly large, i.e., comparable to the *Escherichia coli-Pseudomonas aeruginosa* separation.

The purpose of the current study was to use DNA-DNA hybridization to determine the degree of phylogenetic similarity among *R. pachyptila* symbionts from widely separated vent sites. Despite our inability to cultivate the symbiont apart from the host (16, 26), purified *R. pachyptila* symbiont DNA standards could be obtained. This was possible because (i) the symbiont DNA can be physically separated

* Corresponding author.
† Present address: Marine Biology Research Division (0202), Scripps Institution of Oceanography, University of California, La Jolla, CA 92093.

from host DNA because of its higher G+C content (25) and (ii) possession of high-density internal sulfur globules makes it possible to purify the symbiont physically from freshly homogenized trophosome tissue (10). Standard DNAs thus obtained were hybridized against trophosome (predominantly symbiont) DNAs obtained from distantly separated *R. pachyptila* individuals, as well as other vestimentiferan genera, to investigate symbiont similarities.

MATERIALS AND METHODS

Source of animals. Several specimens of *R. pachyptila* were collected from each of three hydrothermal vent sites located in the Eastern Pacific by using research submersibles. These included the Guaymas Basin site (Gulf of California; 27°N, 111°W), the 13°N site (East Pacific Rise; 13°N, 104°W), and the Galapagos Rift spreading center (0.5°N, 86°W). The depth of collection ranged from 2,000 to 2,600 m. The Guaymas Basin animals were collected by using *ALVIN* (February 1989), immediately frozen (−80°C) as intact worms, and later dissected on solid CO_2 as needed. Animals from other sites were dissected immediately after collection, and separate tissues were immediately frozen (−80°C). *Lamellibrachia* sp. was collected from the Louisiana Slope hydrocarbon seep (*JOHNSON SEA-LINK*, June 1987). *Tevnia jerichonana* and *R. pachyptila* were collected from the 13°N site (*NAUTILE*, October 1987), and *R. pachyptila* was also collected from the Galapagos site in April 1988 (*ALVIN*).

Isolation of high-molecular-weight standard DNA. High-molecular-weight DNAs were isolated from two *Riftia* specimens as the first step in making radioactive probes for hybridization experiments. A combination of the procedures of Lippke et al. (22) and Weeks et al. (32) was performed, with the following modifications. Trophosome tissue was lyophilized overnight, and 1.5 to 2.5 g was ground to a fine powder and dissolved in 15 ml of the following buffer: 5 M guanidine isothiocyanate–50 mM Tris (pH 7.6)–10 mM EDTA (disodium salt)–2% *N*-lauroylsarcosine sodium salt–140 mM 2-mercaptoethanol. The volume was brought to 32.2 ml with sterile distilled water, and then 1.0 ml of ethidium bromide (10 mg/ml) and 30.0 g of cesium chloride were added and mixed until dissolved. The DNA solution was spun in 40-ml quick-seal tubes (Beckman) in a Beckman VTi-75 ultracentrifuge rotor for 22 to 24 h at 180,750 × *g*.

The DNA band was removed, and the ethidium bromide was extracted with water-saturated *n*-butanol (24). To remove the cesium chloride, the DNA solution was dialyzed (molecular weight cutoff 8,000) against 4 liters of TE buffer (10 mM Tris, 2 mM EDTA, pH 7.5) overnight with three changes of buffer. The DNA was deproteinized with at least two extractions in phenol-chloroform-isoamyl alcohol (25:24:1 by volume), followed by one extraction in chloroform-isoamyl alcohol (24:1 by volume). The solution was dialyzed as before. The DNA (at this point, a mixture of host and symbiont DNAs) was then ethanol precipitated and suspended in sterile distilled water. The concentration was determined by fluorometry (Hoefer Scientific Instruments). To prepare radioactive symbiont probes, a sample (200 to 500 μg) of this DNA was brought to 8.25 ml with sterile water in a polyallomer centrifuge tube. Cesium chloride (10.45 g, high grade) was added to the tube and dissolved by repeated inversion. The tubes were spun in a Beckman 50 Ti ultracentrifuge rotor for 24 h at 110,850 × *g* and then for 48 h at 81,400 × *g*.

The gradients were fractionated from the bottom of the tube (5-drop fractions), and the DNA concentration of each fraction was determined by fluorometry. Appropriate fractions were pooled and dialyzed in a microdialysis apparatus, and the pooled fractions were subjected to thermal denaturation (see below) to analyze their purity. Pools showing only high-G+C content DNA (symbiont DNA) were then used for radiolabeling. The size of this purified standard DNA was determined by using 0.5% agarose gels to be in the range of 10 to 25 kb. A more detailed account of all of the methods used may be found elsewhere (12).

Extraction of test DNAs used in hybridizations. The DNA extraction procedures of Herdman et al. (14) were used. Samples (1 to 2 g [wet weight]) of the frozen bacterial pellet, frozen trophosome tissue, or lyophilized and ground vestimentum tissue were added to 15 ml of lysis mixture. These suspensions were passed twice through a French pressure cell (110 MPa). The DNA was then purified by partial deproteinization, followed by binding to hydroxylapatite and repeated washings. Final dialysis and dilution into 0.02 M NaCl–1.0 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer achieved a concentration of about 1.0 mg/ml as determined by fluorometry. All test DNAs were thermally denatured (see below) to estimate the percentages of symbiont and host DNAs. With 2.0% agarose gels, the average fragment size for each test DNA was determined to be in the range of 400 to 600 bp.

Thermal denaturation of DNA. For thermal denaturation of DNA, the protocol of Nelson et al. (25) was used, with some exceptions. All DNA was exhaustively dialyzed in 0.1× SSC (1× SSC consists of 0.15 M sodium chloride and 0.015 M sodium citrate). A single batch of 10× SSC was the source of all of the 0.1× SSC used for dialysis, and care was taken to minimize variability in making dilutions. Each DNA solution (0.3 ml; 1.0 to 2.0 absorbance units) was melted in a sealed cuvette by using a Gilford Response II spectrophotometer equipped with a thermoprogrammer. A reference cuvette containing 0.1× SSC was included in each melt, as well as a standard test DNA from *Pseudomonas putida* ATCC 12633. The maximum of the first derivative of the optical density at 260 nm versus temperature was used to calculate the midpoint melting temperature (T_m) of the thermal denaturation curve. Data were collected in 0.1°C increments, and the rate of heating was approximately 0.4°C/min. The equation $\%G+C = 2.44(T_m - 53.9)$ (reference 23) was used to determine the G+C content from the T_m. The value for each *Riftia* sample was adjusted by using the value of 63.0 mol% G+C for *P. putida* (27).

Radioactive labeling of probes. The symbiont standard DNAs were radioactively labeled with [^{35}S]dCTP by using a commercial nick translation kit (Amersham). The quantity of DNA labeled was 1 to 2 μg, but the kit instructions were modified by adding 300 pmol of unlabeled dCTP per 100 μl of reaction mixture, as this was found to increase the length of the resultant probes and improve the hybridization results. The reaction mixture was incubated at 15°C for 1.5 h. The reaction was then stopped by adding EDTA to 25 mM and deproteinizing it with an equal volume of phenol-chloroform-isoamyl alcohol. The organic layer was back extracted with 30 μl of buffer, and the pooled aqueous layers were extracted with chloroform-isoamyl alcohol. The label was then run through a spun column (Evergreen) of Sephadex G-50 (Sigma) previously equilibrated with 0.02 M NaCl–1.0 mM HEPES for 2 min at maximum speed on an IEC clinical centrifuge. The eluate (probe) was collected and

counted for total and trichloroacetic acid (TCA)-precipitable counts as detailed below. The specific activity obtained was 10^7 to 10^8 cpm/μg of DNA, and at least 90% was TCA precipitable.

Formation of DNA duplexes. The hybridization procedure used was a combination of those of Crosa et al. (9) and Johnson et al. (17). Reassociation reactions were done in 0.3-ml microvials (Wheaton) with sleeve-type serum stoppers, taking care to keep the vials completely submerged. Each vial contained 30 μg of test DNA (unlabeled homologous, heterologous, or control DNA) and 30 ng of labeled standard DNA in 0.42 M NaCl–1.0 mM HEPES buffer (pH 7.0). The total volume was 0.1 ml. A self-reassociation control, in which only labeled DNA was added, was included for each experiment. These reactions were performed in silanized vials to prevent loss of counts due to DNA adhesion to the glass. Vials were well mixed and completely submerged in a boiling water bath for 8 min and then incubated at 74°C (T_m − 25°C for the *R. pachyptila* symbiont) with very slow shaking. Incubation proceeded for 28 h to a C_0t of approximately 100 (3).

Preparation and titration of S1 nuclease. S1 nuclease (143 U/μl; Sigma) from *Aspergillus oryzae* was diluted to 20 u/μl in sterile water. Various concentrations were tested at 50°C for 20 min to optimize enzyme action as judged by effects on both double- and single-stranded labeled DNAs. Calf thymus DNA (30 μg) was used in place of trophosome DNA to simulate actual hybridization conditions and procedures (see below). The concentration of 2.0 nominal units/μg of DNA was optimal, digesting >90% of the single-stranded DNA while leaving intact >90% of the double-stranded DNA. The enzyme was aliquoted and frozen at −80°C with no loss of activity. Each new batch of S1 nuclease was optimized in the same manner.

S1 nuclease reaction. Glass test tubes (12 by 75 mm), each containing 1.0 ml of S1 buffer (50 mM sodium acetate–0.3 M sodium chloride–0.5 mM zinc sulfate [pH 4.6]) (17), were kept on ice. Sheared, denatured calf thymus DNA (20.0 μg) was added. The contents of each hybridization vial (at hybridization temperature) was transferred to the S1 buffer with rinsing of the sides of the hybridization vial to remove any condensation. After the tubes were mixed well, duplicate 10-μl samples were applied to GFC filters (Whatman) to measure total radioactivity. Duplicate 10-μl samples without S1 were TCA precipitated (24). The hybridization mixtures were then digested with S1 endonuclease at 50°C for 20 min and placed on ice to stop the digestion. Duplicate 10-μl samples with S1 were TCA precipitated. Each TCA-precipitated sample was collected onto a 25-mm-diameter GFC filter and washed four times with 2.5 ml of ice-cold 5% TCA containing 200 μM thymine (Sigma). This was followed by a 2-ml acetone wash to remove water (2). Filters were then counted with a liquid scintillation spectrophotometer in 10 ml of cocktail (Aquasol II).

RBR calculation. The degree of homology was estimated by the relative binding ratio (RBR), which was calculated for each hybridization as follows. The counts with S1 were corrected by subtracting the counts attributable to self-reassociation of the probe, and this value was divided by the counts without S1 to yield a percentage. The percentage for each heterologous reassociation was then normalized to the corrected homologous reassociation value (taken as 100).

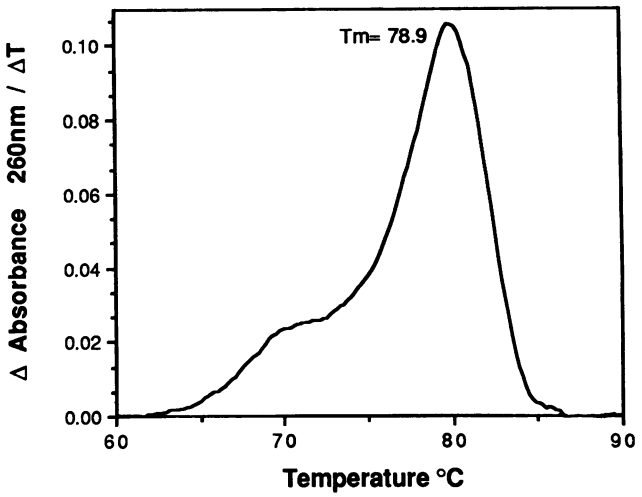

FIG. 1. Thermal denaturation (in 0.1× SSC) of a typical test DNA extracted from *R. pachyptila* trophosome tissue containing a mixture of host and symbiont DNAs. The first derivative of the A_{260} with respect to temperature is plotted versus temperature. The peak in the first-derivative curve defines the T_m of the dominant DNA (symbiont), and the shoulder represents a lower concentration of lower-G+C content (host) DNA.

RESULTS

All test DNA samples were melted to confirm that each consisted of predominantly symbiotic DNA (75 to 90%). The thermal denaturation plot of a typical test DNA (Fig. 1) shows a major peak that corresponds to a T_m of 78.9°C and a G+C content of 61.0 mol% (uncorrected). The small shoulder represents the host DNA, which has a T_m roughly 8 to 10°C lower than that of the symbiont DNA. Thermal denaturation of DNA extracted from *Riftia* vestimentum (host tissue only) showed a single peak with a T_m of approximately 70°C (data not shown). These observations are consistent with previous results from the 21°N vent site (25).

Comparisons of the G+C contents of the symbiont DNA in the different test DNAs (different *R. pachyptila* individuals) revealed only minor differences within or between hydrothermal vent sites. Average values (±standard deviations) obtained were as follows: Guaymas Basin, 58.9 (±0.55; n = 5) mol%; 13°N site, 59.1 (±0.54; n = 4) mol%; and Galapagos, 59.3 (±0.60; n = 4) mol%. Although these results are similar to the range of 57.0 to 59.3 mol% reported previously (25), the current data should be regarded as definitive since they are based on a larger sample size and were always normalized to a standard DNA. The G+C contents of the tissues of several host *R. pachyptila* specimens (vestimentum and gonadal tissue) were also determined, and these averaged 34.9 mol% G+C.

Preparation of the two symbiont DNA standards was initiated by density gradient separation of bulk trophosome-derived DNA into putative symbiont and host DNAs. This is shown for the Galapagos *R. pachyptila* standard in Fig. 2, as two distinct peaks corresponding to DNAs of different densities. Previous studies by Nelson et al. (25) indicated that the higher-density DNA was that of the symbiont. The fraction of trophosome DNA possessing procaryotic renaturation kinetics confirmed that this was the case in the

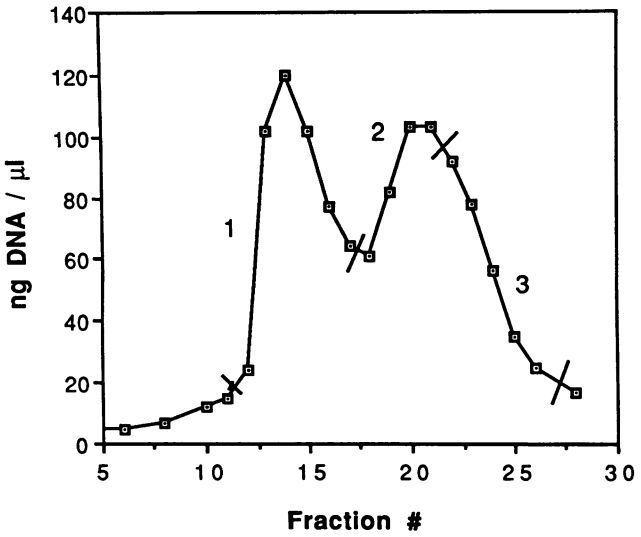

FIG. 2. Demonstration of separation of *R. pachyptila* host and symbiont DNAs by fractionation on a CsCl gradient. Fraction 5 corresponds to the bottom of the tube. The fractions between the slashes were combined (pools 1 to 3). The starting material was trophosome from Galapagos worm 2012-4.

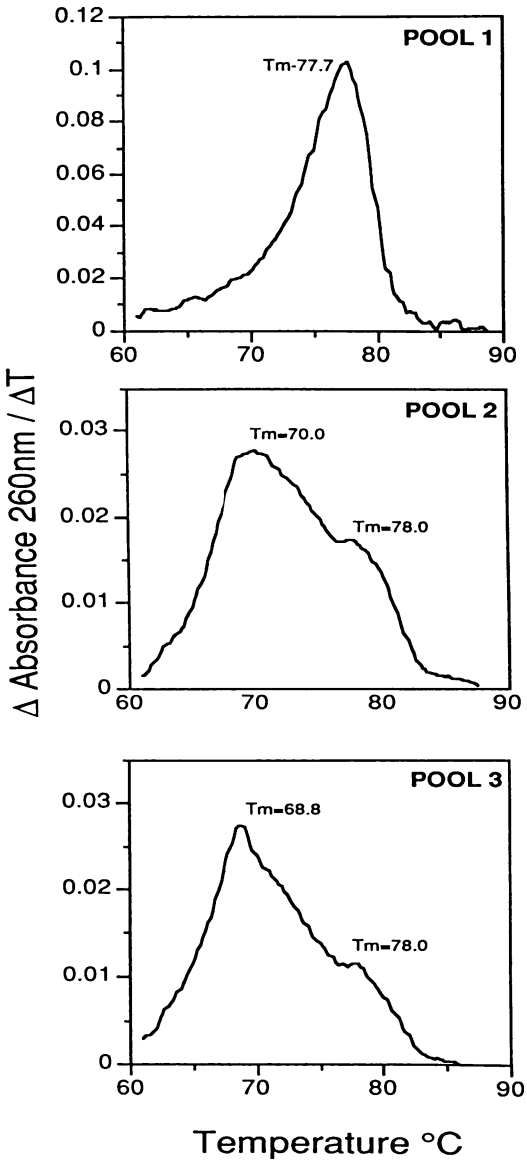

FIG. 3. Thermal denaturation of pools shown in Fig. 2. The graphs are first-derivative plots. The peak corresponds to the T_m of the predominant DNA (Fig. 1).

current study (data not shown). The slash marks indicate the fractions that were combined to generate pools 1 to 3.

Thermal denaturation was used to assess the purity of these pools. Figure 3 shows a thermal denaturation plot of the DNA from pool 1, the Galapagos standard DNA which was subsequently nick translated. The single peak shows a T_m of 77.7°C, which corresponds to a G+C content of 58.1 mol%, very similar to that of the symbiont DNA (25). Pools 2 and 3 each contained a mixture of two DNAs but were predominantly host rather than symbiont DNAs. The results for the Guaymas Basin standard (specimen GB1966-7) were very similar (data not shown).

The results of the DNA-DNA hybridization experiments are summarized in Table 1. For the two *Riftia* symbiont DNA standards hybridized with test DNAs from the various vent sites, the RBRs revealed only limited variation. RBRs ranged from 84.2 to 101.7 with the Guaymas Basin standard and from 85.6 to 102.4 with the Galapagos standard. The average data show patterns which are consistent with both standards and suggest that homology is, at most, slightly higher when the test DNA is from the same site as the standard.

RBR data are also summarized for DNAs from other sources. Low-homology controls, including bacterial (*Escherichia coli* or *P. putida*) or calf thymus DNA, showed RBRs of 2 to 13 at 74°C (T_m − 25°C for symbiont DNA). DNAs derived from *Riftia* vestimentum or gonadal tissue, both of which are presumed to contain only host DNA, were tested in several hybridizations. The resulting RBR values of 20 to 50 were surprisingly high.

The DNAs of other symbiont-containing tube worms gave the following results. The trophosome DNA of *T. jerichonana*, a member of a different genus within the phylum Vestimentifera, showed an RBR of approximately 80 with either *R. pachyptila* symbiont standard. This finding of high homology was supported by the very similar G+C contents of the *T. jerichonana* and *R. pachyptila* symbionts (60.5 and 59.1 mol%, respectively). On the other hand, the trophosome DNA of *Lamellibrachia* sp. (another vestimentiferan worm) showed insignificant hybridization (RBR = 11) with the *Riftia* symbiont. The G+C content of the *Lamellibrachia* symbiont (53.1 mol%) was also significantly different from that of the symbiont of *R. pachyptila*. The determination of which DNA was that of the symbiont was accomplished for *Riftia* as discussed above. For the *Tevnia* symbiont, the conclusion was based on the high RBR with the *Riftia* symbiont. In the case of *Lamellibrachia* sp., assignment of the higher G+C content to the symbiont (host, approximately 38 mol% G+C) was based on relative abundance; the DNA was extracted from trophosome material, which should contain mainly symbiont DNA. For all three worm species, the host G+C contents were within the known range for invertebrates (35 to 50 mol%; reference 30).

Previous 16S rRNA sequence analysis (11) concluded that

TABLE 1. Summary of RBRs at 74°C[a] for *Riftia* symbiont and other DNA samples

Test DNA source	Avg RBR ± SD with the following source of standard DNA[b]:	
	Guaymas Basin worm 1966-7	Galapagos worm 2012-4
R. pachyptila trophosome from:		
Guaymas Basin[c] (n = 5)	95.2 ± 3.85	93.7 ± 2.11
13°N[c] (n = 4)	92.3 ± 8.11	93.8 ± 5.65
Galapagos[c] (n = 4)	92.8 ± 7.46	97.0 ± 4.77
R. pachyptila 1966-7 vestimentum	21.1	51.4
R. pachyptila 214-6 gonadal tissue	33.3	28.9[d]
Lamellibrachia sp.	11.9[d]	11.0[d]
T. jerichonana	76.0	88.6[d]
Thiomicrospira sp. strain L-12	<0[d]	
Calf thymus	5.2	10.0
P. putida	9.0	13.4
E. coli	1.9	

[a] T_m − 25°C for average moles percent G+C of *Riftia* symbiont DNA.
[b] Averages of two RBR determinations for each DNA sample, except as otherwise noted.
[c] Each average is based on the indicated number of individuals from each vent site. Duplicate RBRs were usually determined for each test DNA. See reference 12 for the entire set of data.
[d] Single sample.

the free-living bacterial relative (among those sequenced) closest to the *Riftia* symbiont is *Thiomicrospira* sp. strain L-12. In this study, its DNA showed an RBR of 0 in hybridization with *Riftia* symbiont DNA (Table 1).

Selected hybridizations were also performed at the more stringent temperature of 84°C (T_m − 15°C for the *Riftia* symbiont). The resulting RBRs were very similar to those obtained at T_m − 25°C (Table 1).

DISCUSSION

The current study indicates that earlier findings regarding the DNA base composition of *R. pachyptila* host and symbiont DNAs (25) can be extended to the four distantly separated vent sites where this tube worm has been found. These sites include the three of this study and the 21°N site of the earlier report.

The more specific approach taken in the current study showed that the RBRs ranged from 84.2 to 102.4, regardless of the geographical source of the symbiont and regardless of the radioactive standard DNA used. Wayne et al. (31) state that the phylogenetic delineation of any bacterial species is best achieved by DNA reassociation experiments and that strains with 70% or greater DNA-DNA relatedness constitute a single species. Therefore, it can be concluded that the symbionts of *R. pachyptila* from the three vent sites studied are all members of the same bacterial species. It follows that the association between the host and symbiont is highly specific. Any further consideration or explanations of this symbiosis should take these facts into account.

There do appear to be possible strain level dissimilarities within the *Riftia* symbionts of any given vent system. No strong trends are apparent with regard to degree of relatedness and distance from the source of the labeled standard DNA. In other words, Guaymas Basin *Riftia* symbionts are only slightly more closely related, on average, to the Guaymas Basin standard than to the Galapagos standard. In support of this, the two reciprocal samples, in which each standard was hybridized with the complementary test DNA of the other standard, still showed high homology.

Although the 21°N vent site was not included in this study, we postulate that its *Riftia* symbiont belongs to the same bacterial species. This conclusion was reached because the populations of the current study surround the 21°N site and show no suggestion of symbiont discontinuity. Previous studies of *Riftia* symbiont DNA (25), involving only samples from 21°N, gave DNA base compositions consistent with those of the current study.

The RBRs between the putatively purified DNA of the *Riftia* symbiont (procaryotic) and the eucaryotic host tissues, (vestimentum and gonadal tissue) were surprisingly high (20 to 50; Table 1). There are two possible explanations for this unexpected homology. (i) Consider the separation of the symbiont DNA standards from host DNA based on density differences. Any host-derived DNA fragments of the higher G+C content range would have been included in the presumptively pure symbiont DNA standard. These sequences are then postulated to hybridize with the host portion of the test DNA derived from trophosome (10 to 25% eucaryotic). (ii) It is also possible that the symbiont and host share some highly conserved DNA sequences and thus show homology when hybridized. A preliminary test of this hypothesis was performed as follows. It was possible to obtain a small amount of the *R. pachyptila* symbiont purified at sea from fresh trophosome material by using Percoll gradients (standard method; 10). Because this method uses the very different density of sulfur globule-containing symbionts to separate them from host cells, it minimizes the possibility of copurification of host and symbiont DNAs. The DNA of these physically purified symbionts was then extracted and used as the labeled standard DNA in additional hybridizations. The few experiments possible because of limited material confirmed that *R. pachyptila* host-derived DNA (vestimentum and gonadal tissue) had an RBR of 20 to 40. The features of Table 1 pertaining to *R. pachyptila* trophosome DNA (all three sites) and procaryotic and eucaryotic negative controls were also confirmed. This supports the suggestion of DNA sequences shared between the host and symbiont.

All organisms currently living as intracellular symbionts existed outside the cell during some previous evolutionary stage (28). Recent reports by Jones and Gardiner (19, 20) suggest that *R. pachyptila* acquires its symbionts from the surrounding seawater de novo with each generation by using a transient digestive tract found only in juvenile worms. The results of the current study show that if this theory is true, acquisition of symbionts is not a random process. It appears that only a certain bacterial species is capable of colonizing the trophosome and maintaining a successful symbiotic existence with the tube worm. Whether that particular bacterium is broadly available and picked up by young worms after settling at a particular vent or acquired at the vent of origin by larvae, which are thereafter dispersed to different vent sites, is not known. No free-living, unicellular, colorless sulfur bacterium which resides in the same phylogenetic grouping with the *R. pachyptila* symbiont (11) and also deposits intracellular sulfur globules has ever been isolated. Thus, the symbiont may not be viable for long outside the host trophosome environment, or it may be significantly modified once taken up by the host.

The results obtained with *T. jerichonana*, another vestimentiferan worm so far found only at the 13°N vent site

along with *R. pachyptila*, are interesting in this connection. This is the first case demonstrated in which two different host genera contain the same endosymbiotic sulfur bacterial species (9a, 11). A transient digestive tract has been found in some other vestimentiferan species but has yet to be looked for in *T. jerichonana*. A fine-scale genetic analysis of symbionts in both host species collected from adjacent and distant populations could go far in testing whether the de novo acquisition theory is viable.

It has been asserted that the phylogenetic relationships of symbionts parallel those of their hosts (1). If this is true, the evolutionary relationships among *R. pachyptila*, a *Lamellibrachia* sp., and *T. jerichonana* should be reexamined. Currently, *T. jerichonana* and a *Lamellibrachia* sp. are placed in one class within the phylum Vestimentifera and *R. pachyptila* is placed in a separate one (18). On the basis of this postulated phylogeny of hosts, it is counterintuitive that only *R. pachyptila* and *T. jerichonana* have closely related symbionts.

Allozyme variability, as studied by Bucklin (4), suggested that there was little genetic variation in host enzymes between samples from the Galapagos and 21°N sites. The results of the current study parallel her finding of little variability of host alleles. The most convincing explanation offered for lack of host differentiation is that there was not time for accumulation of mutations to occur because the extinction and reestablishment of populations must be very rapid given the short lifespan of hydrothermal vents. *R. pachyptila* is one of the more cosmopolitan of the symbiont-containing vent animals. Therefore, it seems reasonable that the lack of genetic diversification observed in the host should extend to the symbiont. This does not, however, help to clarify the mode of transmission of the symbiont.

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