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Procaryote Phylogeny IV: Concerning the Phylogenetic Status of a Photosynthetic Bacterium

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Summary. The 16S ribosomal RNA (30S subunit) of *Rhodopseudomonas spheroides* has been characterized in terms of T1 ribonuclease digestion products. This "fingerprint" ultimately permits the placement of *R. spheroides* into a detailed procaryotic phylogenetic tree. Given the number of major procaryotic lines that have been characterized in these terms to date, one can tentatively place the Athiorhodaceae closer to the Vibrio-Enteric group than to the Bacillaceae or Cyanophyta.

Key words. *Rhodopseudomonas spheroides* - 16S Ribosomal RNA - T1 Ribonuclease Digest - Phylogeny - Evolution.

INTRODUCTION

Although it was once generally accepted that the first living systems were heterotrophic (and so catalyzed the degradation of their environment), there are now suggestions from the micro-fossil record that some very early cells had photosynthetic capabilities (Schof & Barghoorn, 1967). This reinforces the theoretical argument that primitive living systems had to contribute molecular complexity *to*, as well as extract it *from*, their environment, if evolution were to progress beyond the very simplest "living" forms.

While extant living forms might not provide clues to the nature of the very first living system, it seems reasonable that a comprehensive Procaryote phylogeny would at least reveal whether or not the common ancestor(s) of *today's* Procaryotes was itself photosynthetic.

Traditionally the photosynthetic bacteria are divided into two distinct groupings, the bluegreen algae (Cyanophyta)

on one hand, and the photochemolithotrophs (Thiorhodaceae, Athiorhodaceae, Chlorobacteriaceae) on the other. The blue-greens are generally considered to be rather distinct from all other Procaryotes; and the remaining photosynthetic procaryotes, although viewed as true bacteria, do not give clear evidence of their relationship to nonphotosynthetic procaryotes.

The present communication concerns the phylogenetic status of the Athiorhodaceae - represented by *Rhodopseudomonas spheroides*, as determined by the method of oligonucleotide fingerprinting of (16S) ribosomal RNAs. (Sogin, S. et al., 1972; Woese et al., 1974; Zablen et al., 1975; Doolittle et al., 1975).

MATERIALS AND METHODS

Rhodopseudomonas spheroides strain NCIB 8253, obtained from Dr. J. Lascelles, was grown aerobically at 37 °C in Sistrom's medium with 0.01 M Tris (hydroxymethyl) amino methane buffer, pH 7.2, replacing the phosphate buffer, and to which a phosphate "free" supplement of yeast extract-peptone was added (Sogin, M. et al., 1972). To 30 ml log phase cultures $^{32}PO_4$ was added to a final concentration of 0.5 mc/ml. Approximately three generations (4 to 5 hrs) later a culture was harvested by centrifugation and the pellet washed in 0.1 M KCl in a 0.01 M Tris-HCl buffer, pH 7.4. Cells were resuspended in 1.0 ml of a 0.005 M Tris-HCl buffer, pH 7.4, containing 0.05 M KCL, 0.001 M $MgCl_2$, 1 mg/ml Mackaloid (Baroid Corp.) and 2µg/ml polyvinyl sulfate (PVS).

Cells were ruptured by two serial passages through a French press at 10,000 p.s.i., and cell debris removed by centrifugation. Ribosomal subunits were isolated from the resulting supernatant by zone centrifugation - using a 5%-20% (w/w) sucrose gradient in the above Tris-HCl buffer (minus Makaloid and PVS) in a SW25.1 Spinco rotor.

The appropriate ribosomal subunit peak fractions were pooled, dialysed to remove sucrose, and RNA extracted by the phenol method (Kirby, 1956). 16S rRNA was subsequently further purified by passage over a Whatman CF-11 cellulose column (Zablen, Ph.D. thesis, University of Illinois, 1975).

T1 ribonuclease digests of the RNA were done and analysed by the method of Sanger et al. (1965) as modified by Sogin & Woese (mss. in preparation; Uchida et al., 1974).

Secondary analysis of the primary T1 ribonuclease digest "fingerprint" pattern (i.e. Fig.1) followed the procedures described fully in Uchida et al. (1974). Briefly these in-

Fig.1. Two dimensional electrophoretic analysis of a T1 ribonuclease digest of *R.spheroides* 16S ribosomal RNA by the method of Sanger and co-workers (1965) as modified by Sogin & Woese (mss. in preparation)

volved characterizing each oligomer spot on the primary pattern in terms of the secondary digestion products produced by pancreatic and U2 ribonucleases, followed where necessary by similar analysis in turn of the resulting secondary digestion products.

Oligomers were quantitated by scintillation spectrometry of the individual spots on the primary fingerprint.

RESULTS AND DISCUSSION

"Fingerprinting" ribosomal RNAs (by the method of Sanger and coworkers, 1965) has proven to be an effective approach to establishing phylogenetic relationships. Not only can the "fine structure" of phylogenetic trees - i.e. that within

Table 1.

Oligomer composition	# in *R.spheroides*	Enterics	Bacilli	*Anacystis*
G isoplith				
CCCG	2 1/2			
CACG	2			
CCAG	3			
ACCG	1			
CAAG	4			
ACAG	1			
AACG	2			
AAAG	4			
C*CGCG[1]	1	1	1	1
C**CCCG[1]	1	1	1	1
*CAACG	1	1	1	1
CCCCG	2	O	O	O
CCCAG	1	1	1	1
CAACG	1	1	2	1
ACACG	1	1	1	1
CAAAG	1	O	O	O
ACCACG	1	O	O	O
CACAAG	1	1	1	1
CAAACAG	1	1	1	O
CACACAG	1	O	O	O
AAC(A,C)CAG	1	O	1	1
(CAA,CCCA)CG	1	O	O	1
UG isoplith				
UCCG	2			
CUCG	2			
CCUG	4			
UACG	1-2			
(CU)AG	5-4			
CAUG	4			
ACUG	3			
AUCG	6			
AAUG	4			
AUAG	1			
UCCCG	1/2	1	1	1
CUCAG	1	2	1	1
CCAUG	1	1	O	1

Table 1 (continued)

Oligomer composition	# in *R.spheroides*	Enterics	Bacilli	*Anacystis*
CAUCG	1	0	0	0
ACCUG	1	0	1	0
UAACG	1-2	1	1	1
UCAAG	1	1	0	1
AUCAG	2	1	1	1
AAUCG	1	1	1	1
AACUG	1	2	0	1
UAAAG	2	3	2	1-2
ACAUG	1	0	0	0
AUAAG	1	0	0	0
AAUAG	1	0	1	0
UCCACG	1	1	1	0
ACUACG	1	0	0	0
UAAACG	1	1	1	1
UAACAG	1	1	0	0
AAUACG	1	1	1	1
AAACUG	1	1	1	1
ACAAUG	1	0	0	0
**AACCUG	1	1	0	0
*UAACAAG	1	1	1	1
CAACUCG	1	1	1	1
CCACACUG	1	1	1	1
CCAACCCUG	1	0	0	0
UACACACCG	1	1	1	1
(CUA,CA,CA)CG	1	1	1	1
(CCA,CA,CA)UG	1	0	0	0
CCUACCAAG	1	0	1	1
AAACCUCCG	1	0	0	0
AAAUCCCAG	1	0	0	0
ACACACCUAACG	1	0	0	0
U2G isoplith				
UUCG	4			
UCUG	3			
CUUG	0-1			
AUUG	2			
CUCUG	1	0		0
CCUUG	1	1	1	1
UCUAG	1/2	0	0	0
UUAAG	1	2-3	2	2
UAAUG	1	1	0	0

Table 1 (continued)

Oligomer composition	⧺ in *R.spheroides*	Enterics	Bacilli	*Anacystis*
AUUAG	1	2	2	2
AAUUG	2	1	1	1
*AUUAG	1	O	O	O
UUCCCG	1	1	1	1
UUCCAG	1	O	O	O
UCUCAG	1	O	O	O
CCUUAG	1	O	O	O
AUCCUG	1	O	1	1
UAAUCG	1	1	1	1
AUACUG	1	1	O	O
AUCUAG	1	O	O	O
ACUUAG	1	O	O	O
AUUAAG	1	O	O	O
AUAAUG	1	O	O	O
CAUUCCG	1	O	O	O
CUCACUG	1	O	O	O
CAUUCAG	1	O	1	O
UAAUACG	1	1	1	1
AAUUCCG	1	O	O	O
AAAUUCG	1	O	O	O
AUACCCUG	1	1	1	O
CUACAAUG	1	1	1	O
UAAUACCG	1	O	O	O
AUCCAUAG	1	O	O	O
(C)CCCUUACG	1	1	O	O
CUAACUCCG	1	1	O	O
(CCUCUA,CA)G	1	O	O	O
AC(UC)CUACG	1	1	1	1
[(UC)A,CA]CCAUG	1	1	O	O
CUCAACCCUG	1	O	O	O
CCUAACACAUG	1	1	O	O
U3G isoplith				
UUUG	3	2-3	2-3	2
UCUCUG	1	O	O	O
CCUUUG	1	O	O	O
AUCUUG	1	O	O	O
CCCUUUG	1	O	O	O
CUCUUAG	1	O	O	O
AUAUUCG	1	O	O	O

Table 1 (continued)

Oligomer composition	# in *R.spheroides*	Enterics	Bacilli	*Anacystis*
(UC)C(UC)AUG	1	O	O	O
AAUUACUG	1	1	O	O
AAUCUUAG	1	O	O	O
UUCUACCCG	1	O	O	O
UUAAAACUCAAAG	1	O	O	O
UAUCCCAAAAUAAG	1	O	O	O
U4G isoplith				
UUAUUCG	1	O	O	O
AUUUAUCG	1	O	O	O
UUCCCUUAG	1	O	O	O
UUCAACUUG	1	O	O	O
AU(UC)U(UC)AG	1	O	O	O
AACCUUAC(C_{1-2})UUG	1	O	O	O
U5G isoplith				
UUUAAUUCG	1	1	1	1

[1] Sequence determined by Fellner (1969) - preliminary sequence characterization in this laboratory indicates it to be incorrect (Zablen, unpublished results).

Catalog of oligomer sequences, tetramers and larger, generated by T1 ribonuclease digestion of *R.spheroides* 16S ribosomal RNA.

The methods of Uchida et al. (1974) have been used to determine the sequences of the individual spots shown in Fig.1.

The table also indicates whether or not the *R.spheroides* sequences are found in (most) Enterics (Uchida et al., 1974; Zablen et al., 1975; Woese et al., 1974; Zablen Ph.D. thesis, Univ.of Illinois, 1975), in (most) *Bacilli* (Pechman et al., unpublished; Stahl, unpublished; Bonen, unpublished, Sogin & Woese, mss. in prep.) and in *Anacystis* (Doolittle et al., 1975).

An asterisk over a base indicates post-transcriptional modification.

families or genera - be established, but the extent of conservation found in ribosomal RNA primary structures is sufficient to permit the establishment ultimately of more distant relationships that could span the entire Procaryote Kingdom (Sogin, S. et al., 1972; Doolittle et al., 1975).

A typical two dimensional electrophoretic fingerprint for a T1 ribonuclease digest of the 16S rRNA of *R.spheroides* is shown in Fig.1. Table 1 catalogs the oligomer sequences found therein, and indicates as well, the occurrence (or lack thereof) of the *R.spheroides* oligomers in the 16S rRNAs of three other groups of procaryotes, the Enterics, the Bacilli, and the blue-green algae (as represented by *Anacystis nidulans*).

The table already shows considerable conservation of the larger oligomers among all four procaryotic groups, which conservation by any reasonable account must represent sequence homology. There are over a dozen oligomers (hexamers and larger) common to all four groups.

None of the four groups of organisms is very closely related to any of the others, however. (An example of closely related organisms being the Enterics, and Vibrios, whose 16S rRNA fingerprints resemble one another to a much higher degree than that seen in Table 1; Zablen, Ph.D. thesis, University of Illinois, 1975; Woese et al., unpublished results.) Yet these four procaryotic fingerprints are very unlike the eucaryote (i.e. yeast) 18S rRNA fingerprint, which shows almost no large oligomers in common with procaryote 16S rRNAs (Woese et al., unpublished). Even at this initial stage, certain general trends in procaryotic phylogenetic relationships seem to be emerging - which should become increasingly apparent as more organisms are characterized in these terms.

If one looks for large (normal) oligomers common just to pairs of organisms, one finds an insignificant number of examples for all pairs *except Rhodopseudomonas* - Enteric and *Bacillus* - *Anacystis* - see Table 2.[1] This trend is also seen in terms of the post-transcriptionally modified sequences. $\overset{**}{A}ACCUG$ and (probably) $U\overset{*}{C}ACACCAUG$ characterize *Rhodopseudomonas* and the Enterics, while Bacillaceae and *Anacystis* share a modified sequence not found in the other two groups (Doolittle et al., 1975). [It is interesting to note that $\overset{**}{A}ACCUG$ has been implicated in Kasugamycin sensitivity (Helser et al., 1972); and while *R.spheroides* and Enterics are sensitive to the antibiotic, *Bacilli* and *Anacystis* are not (Chao & Woese, unpublished).] The coincidences in post-transcriptionally modified sequences are individually more significant than those involving normal oligomers; in these cases not only are the sequences common, but so must be the modifying enzyme systems and certain local nucleic acid conformations as well.

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[1] We will not discuss the probable significance of oligomers common to three but not the fourth group of organisms at this time.

Table 2.

I. Found in all	13
II. Found in three of the four	
A. except in *Rhodopseudomonas*	0
B. except in Enterics	3
C. except in Bacilli	0
D. except in *Anacystis*	4
III. Found in pairs of organisms only	
A. *Rhodopseudomonas* - Enteric	6
B. *Rhodopseudomonas* - Bacilli	1
C. *Rhodopseudomonas* - *Anacystis*	1
D. Enteric - Bacilli	0
E. Enteric - *Anacystis*	1
F. *Bacillus* - *Anacystis*	4

Distribution of common oligomers (unmodified hexamers and larger) among the four groups of organisms. (Doolittle et al., 1975; Uchida et al., 1974; Pechman & Stahl, unpub.)

Therefore, we can tentatively conclude that the Enteric-*Vibrio* group and the Athiorhodaceae are on one side of a very primitive phylogenetic divide, while Bacillaceae and Cyanophyta are on another. Clearly far more work of this kind will be required before the detailed nature of these very primitive evolutionary junctures will come into clear focus.

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