

Numerical Classification of Phototrophic Nonsulfur Bacteria

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수리분류학적 방법에 의한 비유황 광합성 세균 분류

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A total of 10 main characters of 16 species of family *Rhodospirillaceae* were phenetically and cladistically analyzed by Farris' method. The obtained phenogram and cladistic tree were compared with Bergey's manual and other papers. The results supported that the system of 5 subgroups (genera) is available in family *Rhodospirillaceae* and indicated that close affinities between *Rhodospirillum tenue* and *Rhodopseudomonas gelatinosa* and between *Rhodomicrobium vannielii* and other species of genus *Rhodopseudomonas* were proved.

Phototrophic nonsulfur purple bacteria (family *Rhodospirillaceae*) are the most diverse group of bacteria comprising species with widely different morphologies, internal structures and carotenoid compositions (Trüper *et al.*, 1978). All this while, primarily morphological properties have been used to differentiate among the four genera of the *Rhodospirillaceae* (Pfennig and Trüper, 1971), Morphological, cytological and physiological properties, as well as recently accumulated data on the similarities of macromolecular structures show that the existing genera comprise groups of species with widely differing properties, which do not warrant classification with the same genus. Therefore, we believe that a rearrangement of the species and genera of the purple nonsulfur bacteria is urgently needed to reach a better arrangement between the taxonomic units and the established similarities of the species. The principles of numerical taxonomy are well established, and several hundred publications concerning the application of these techniques to bacterial classification and identification have

been published since the feasibility of the application of computer methods to the numerical taxonomy of bacteria became evident in 1957. A great content of taxonomic information may be readily and objectively assessed by numerical techniques.

In this study, a total of 10 main characters of the *Rhodospirillaceae* were phenetically and cladistically analyzed by Farris' method. The obtained phenogram and cladistic tree were compared with Bergey's manual and other papers. Our results agree to other phylogenetic papers based on the data of analysis of cytochrome and DNA-rRNA hybridization (Schwartz *et al.*, 1978; Seewaldt *et al.*, 1982; Woese *et al.*, 1982; Ludwig *et al.*, 1983).

MATERIALS AND METHODS

All kinds of characters quoted from Pfennig (1978), Trüper *et al.* (1978) and Ambler *et al.* (1981). Table 1 is the distribution of character state in the *Rhodospirillaceae*, table 2 is the raw data matrix of 10 main characters, and table 3 is data of electron

Table 1. Distribution of character state in the *Rhodospirillaceae*.

| Character | Character state | | | | |
|---------------------------------------|----------------------------------|---------------------------|---------------------|----------------------|----------|
| | 1 | 2 | 3 | 4 | 5 |
| I. Cell shape | sphere | ovoid | rod | circle | spiral |
| II. Cell length | 2 μm > | 2 - 4 μm | 4 - 7 μm | 7 - 10 μm | |
| III. Intracytoplasmic membrane system | tubes | polar stack | stacks | vesicles | |
| IV. Growth condition | strict anaerobic | | microaerobic | | aerobic |
| V. Growth factor | 3 < | 2 | 1 | none | |
| VI. Motility | none | | | have | |
| VII. Cell multiplication | binary | | budding | | |
| VIII. Predominant carotenoid | diketo-tetrahydro-spirilloxantin | 1, 2-dihydro-neurosporene | spheroidenone | spirilloxantin | lycopene |
| IX. Sporelike apparatus | none | | have | | |
| X. Bacteriochlorophyll b | have | | none | | |

donors and carbon sources used by the *Rhodospirillaceae*. The program WAGTREE for cladistic analysis was obtained from Dr. John D. Lundberg, Department of Zoology, Duke University, N.C., U.S.A. It had been originally programmed to in the IBM and was slightly modified to run in the DEC-11 750 computer. The program CLINK for phenetic analysis was made by Dr. Sang-Tae Lee, Department of Biology, Sung Kyun Kwan University, Suwon, Korea. In this analysis the hypothetical ancestor was assumed to have only the primitive state in all characters.

RESULTS AND DISCUSSION

Fig. 1 is a phenogram by program CLINK based on the data of table 2. In this phenogram, the *Rhodospirillaceae* subdivide into 5 subgroups: Group

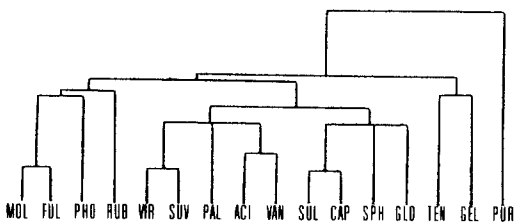


Fig. 1. Phenetic analysis of 16 species of the *Rhodospirillaceae*. The phenograms were resulted from using different similarity measures and clustering methods (Euclidean distance-average linkage).

I is defined by *Rhodospirillum molischianum*, *Rosp. fulvum*, *Rosp. photometricum*, and *Rosp. rubrum*. Group II contains *Rhodopseudomonas viridis*, *Rops. sulfoviridis*, *Rops. palustris*, *Rops. acidophila*, and *Rhodomicrobium vannielii*. Group III contains *Rhodopseudomonas sulfidophila*, *Rops. capsulata*, *Rops. sphaeroides*, and *Rops. globiformis*. Group IV is defined by *Rops. gelatinosa* and *Rosp. tenue*. Group V is defined *Rhodocyclus purpureus*.

This results are different from classical taxonomic group (Pfennig, 1978; Trüper *et al.*, 1981) at three points.

1) *Rhodospirillum tenue* and *Rhodopseudomonas gelatinosa* belong to same subgroup. A relatedness of *Rosp. tenue* and *Rops. gelatinosa* has been suggested and experimental evidence for such a relatedness is available now in the form of 16s RNA catalogues, cytochrome c_{551} sequence and lipopolysaccharide analysis (Pfennig, 1977; Gibson *et al.*, 1979; Weckesser *et al.*, 1979; Dickerson, 1980). The present result supported this papers.

2) The close affinity between genus *Rhodomicrobium vannielii* and species of genus *Rhodopseudomonas* was proved. *Rhodomicrobium vannielii* is the only species with the ability to produce exosporelike, moderately heat-resistance cysts. And this motile cells are peritrichously flagellated,

Table 2. Raw data matrix of 16 species of family *Rhodospirillaceae*.

| Species Character | Character states | | | | | | | | | |
|----------------------|------------------|----|-----|----|---|----|-----|------|----|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| ANC | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| PUR | 4 | 2 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 3 |
| VAN | 2 | 1 | 3 | 3 | 4 | 4 | 3 | 4 | 3 | 3 |
| ACI | 3 | 2 | 3 | 3 | 4 | 4 | 3 | 1 | 1 | 3 |
| CAP | 3 | 2 | 5 | 5 | 1 | 4 | 2 | 3 | 1 | 3 |
| GEL | 3 | 1 | 1 | 5 | 2 | 4 | 1 | 3 | 1 | 3 |
| GLO | 1 | 1 | 5 | 3 | 2 | 4 | 1 | 1 | 1 | 3 |
| PAL | 3 | 1 | 3 | 5 | 2 | 4 | 3 | 4 | 1 | 3 |
| SPH | 1 | 1 | 5 | 5 | 1 | 4 | 1 | 3 | 1 | 3 |
| SUL | 3 | 1 | 5 | 5 | 1 | 4 | 2 | 3 | 1 | 3 |
| SUV | 3 | 1 | 3 | 3 | 1 | 4 | 3 | 2 | 1 | 1 |
| VIR | 3 | 1 | 3 | 3 | 2 | 4 | 3 | 2 | 1 | 1 |
| FUL | 5 | 2 | 4 | 3 | 3 | 4 | 1 | 5 | 1 | 3 |
| MOL | 5 | 3 | 4 | 3 | 3 | 4 | 1 | 5 | 1 | 3 |
| PHO | 5 | 4 | 4 | 3 | 1 | 4 | 1 | 5 | 1 | 3 |
| RUB | 5 | 4 | 5 | 5 | 3 | 4 | 1 | 4 | 1 | 3 |
| TEN | 5 | 2 | 1 | 5 | 4 | 4 | 1 | 5 | 1 | 3 |

ANC : Ancestor

- Rhodocyclus purpureus*
- Rhodomicrobium vannielii*
- Rhodopseudomonas acidophila*
- " *capsulata*
- " *gelatinosa*
- " *globiformis*
- " *palustris*
- " *sphaeroides*
- " *sulphidophila*
- " *sulfoviridis*
- " *viridis*
- Rhodospirillum fulvum*
- " *molischianum*
- " *photometricum*
- " *rubrum*
- " *tenu*

other species have polar or subpolar to lateral flagellation. But this species divides by budding cell multiplication with 4 species of genus *Rhodopseudomonas*. And no cell properties are different from other species.

3) In the genus *Rhodopseudomonas* two main groups can be discriminated. This result also had been mentioned by other paper (Pfenning, 1977). Pfenning's first group which includes *Rhodopseu-*

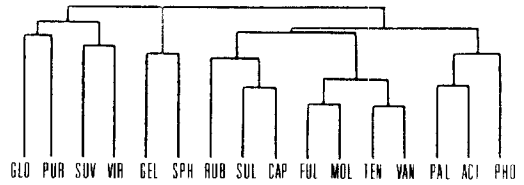


Fig. 2. Complete linkage of lattice distance matrix by program CLINK based on the data of table III.

domonas palustris, *Rops. viridis*, *Rops. acidophila*, *Rops. sulfoviridis*, and *Rops. blastica* is characterized by the budding type of cell multiplication and an intracytoplasmic membrane system consisting of membranes parallel to and underlying the cytoplasmic membrane. Second group is *Rops. capsulata*, *Rops. sphaeroides*, *Rops. sulfidophila*. These organisms have a short-rod to coccus shapes, intracytoplasmic membranes of the vesicular type and carotinoids of the spheroidene group (Trüper, 1978). Only *Rops. globiformis*, present result disagree to Pfenning's system.

Fig. 2 showed complete linkage of lattice distance matrix by program CLINK based on the data of table II. This phenogram showed random distribu-

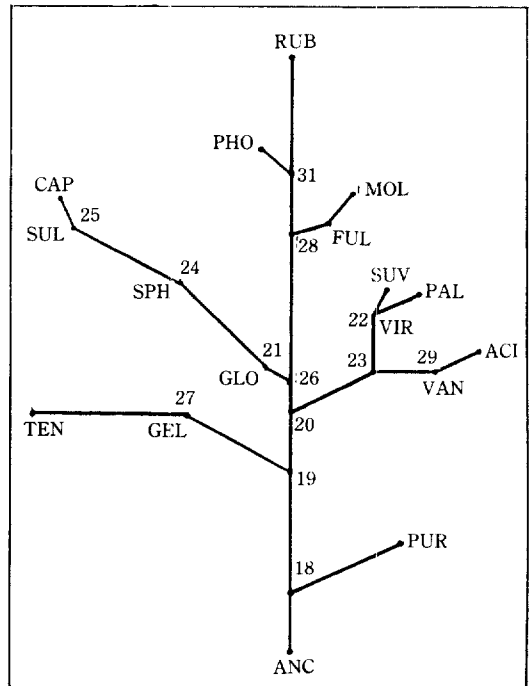


Fig. 3. Cladogram made by program WAGTREE based on the data of table II.

Table 3. Raw data matrix of carbon sources of 16 species of family *Rhodospirillaceae*.

| | PRU | VAN | ACI | CAP | GEL | GLO | PAL | SPH | SUP | SUV | VIR | FUL | MOL | PHO | RUB | TEN |
|---------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Acetic acid | 3 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Arginine | 1 | 1 | 1 | | | 1 | 1 | | 1 | 1 | | 1 | 1 | 1 | 3 | 3 |
| Asparatate | 1 | 1 | 1 | 3 | | 1 | 1 | | 2 | | | 2 | 2 | 2 | 3 | 1 |
| Benzoate | 3 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 1 |
| Butyrate | 1 | 3 | 3 | 3 | 2 | 1 | 3 | 2 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 |
| Caproate | 3 | 3 | 2 | 3 | 1 | 1 | 3 | 2 | 3 | | 1 | 3 | 3 | 1 | 3 | 3 |
| Caprylate | 1 | 3 | 1 | 3 | | 1 | 3 | 2 | 3 | | | 3 | 3 | 1 | | 3 |
| Citrate | 1 | 1 | 3 | 1 | 3 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Ethanol | 1 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2 |
| Formate | 1 | 1 | 2 | | 2 | 1 | 3 | | 3 | 1 | 1 | | | 1 | 1 | 1 |
| Fructose | 1 | 1 | 1 | 3 | | 3 | 1 | 3 | 2 | 3 | | 1 | 1 | 3 | 2 | 1 |
| Fumarate | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | | 3 | | 3 | 3 | 3 | 3 | 3 |
| Gluconate | | | | 1 | | 3 | | 3 | | | | | | | 1 | |
| Glucose | 1 | 1 | 2 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 2 | 1 | 3 | 1 | 1 |
| Glutamate | 1 | 1 | 1 | 3 | | 1 | 3 | | 3 | | 3 | | | 1 | 3 | |
| Glycerol | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 3 | | 3 | 1 | 1 | 1 | 3 | 1 | 1 |
| Glycolate | 1 | 1 | 2 | | | 1 | 3 | | | | | | 1 | 3 | | 1 |
| Lactate | 1 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 1 | 1 | 2 | 3 | 3 | 3 |
| Malate | 1 | 3 | 2 | | | 1 | 3 | | | 1 | | | 1 | | | 3 |
| Malonate | 1 | 1 | 1 | 1 | 2 | 3 | 1 | 3 | 1 | 1 | | 1 | 1 | 3 | 1 | 1 |
| Mannitol | 1 | 2 | 2 | | 2 | 1 | 1 | | 1 | | | | 1 | 1 | 2 | 1 |
| Methanol | 1 | 1 | 1 | 3 | | 1 | 1 | 2 | 3 | | | 3 | 3 | 2 | | 2 |
| Pelargonate | 1 | 3 | 3 | 3 | 2 | 1 | 3 | 2 | 3 | 1 | 1 | 3 | 3 | 2 | 3 | 3 |
| Propionate | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 3 | 3 |
| Pyruvate | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Succinate | 1 | 1 | 2 | 1 | 2 | 3 | 1 | 3 | 1 | 1 | | | 1 | 1 | 1 | 3 |
| Tartrate | 1 | 3 | 3 | 3 | | 1 | 3 | 2 | 3 | 1 | 1 | 3 | 3 | 3 | 3 | 1 |
| Valerate | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | | | 3 | 3 | 3 | 3 | 3 |
| Yeast extract | 1 | 3 | 3 | 3 | | 1 | 3 | | 3 | 3 | 3 | 1 | 1 | 3 | 3 | 3 |
| Thiosulfate | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 1 |

Symbols: 3=utilized; 2=poorly utilized; 1=not utilized; blank=not tested.

The data were collected from Hansen and Veldkamp(1973), Keppen and Gorlenko(1975), Pfennig(1974), and Pfennig and Trüper(1974).

tion of each species. The similarity of this phenogram is meaningless because they do not correlate with other properties of the organisms. In our opinion, the phenogram based on the data of electron donor and carbon source can not fit phylogentic relationship of bacteria. The minimum number of evolutionary steps of the resulted claditic tree (Fig. 3) was found to be 55. The cladistic tree

showed a great but differential similarity to the empirical systems of Imhoff *et al.* (1984). Furthermore, present result was supported by many other result; DNA-DNA and DNA-rRNA hybridization (de Bont *et al.*, 1981), DNA base composition (Mandel *et al.*, 1971), lipid composition analysis (Collins *et al.*, 1981), cytochrome pattern sequence analysis (Ambler *et al.*, 1981). In conclusion, present result

supported in general the system of 5 subgrouping of family *Rhodospirillaceae* and agreed with rearrangement of species, *Rhodopseudomonas gelatinosa* and *Rhodospirillum tenue* to same species (Imhoff

et al., 1984). But to establish fundamentals of a phylogenetic tree of this field, many studies of numerical taxonomic approach are needed.

적 요

비유황 광합성 세균(Family *Rhodospirillaceae*)에 속하는 16종에서 총 10개의 주요 형질을 선택하여 Farris의 방법에 따라 Phenetic 및 Cladistic 분석을 행하였다. 얻어진 phenogram과 cladistic tree를 Bergey's manual 및 다른 결과들과 비교하였다. 비유황성 광합성 세균들과 4 개 또는 5 개의 subgroup(genus)으로 나누어졌으며, 특히 *Rhodospirillum tenue*와 *Rhodopseudomonas gelatinosa*는 밀접한 유연관계가 있음을 나타내었고, 또한 *Rhodomicrobium vanielii*와 *Rhodopseudomonas* 속의 몇몇 종들 사이에는 큰 차이점을 보이지 않는 것으로 나타났다.

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