References

1. Sprent, J.I. The Biology of Nitrogen-Fixing Organisms. p. 2, 13 England: Mc Graw-Hill Book Co. Ltd. (1979)

2. Barraquio, W.L., and Watanabe, I. "Occurence of aerobic N_2-fixing bacteria in wetland and dryland plants." Soil Sci. Plant Nutr. 27(1981): 121-125.

3. Simasatitkul, C. and Boonjawat, J. "N_2-fixing bacteria in the rice rhizosphere." The Abstract of the Fifth International Conference on Global Impacts of Applied Microbiology. 21-26 Nov. (1977): 30.

4. Harinasut, P. "Isolation and characterization of some nitrogen fixing aerobic diazotrophic bacteria from rice rhizosphere." Thesis, Department of Biochemistry, Chulalongkorn University (1981)

5. Boonjawat, J. and Limpananont, J. "Associative N_2-fixation between Klebsiella sp. and rice (Oryza sativa L.)" In Nitrogen fixation research progress p. 372, ed. by H.J. Evans, P.J. Bottomley and W.E. Newton, Martinus Nijhoff Publishers. (1985)

6. Watanabe, I. and Barraquio, W.L. "Low level of fixed nitrogen required for isolation of free living N_2-fixing organisms from rice roots." Nature 277 (1979): 565-566.

7. Barraquio, W.L., et al. "Population of aerobic heterotrophic nitrogen-fixing bacteria associated with wetland and dryland rice." Appl. Environ. Microbiol. 42 (1982): 124-128.

8. Dommergues, Y.R., et al. "Non-symbiotic nitrogen fixation in the rhizosphere of rice, maize and different tropical grasses." Soil Biol. Biochem. 5 (1973): 83-89.

9. Watanabe, I., et al. "Nitrogen-fixing (acetylene reduction) activity and population of aerobic heterotrophic nitrogen-fixing bacteria associated with wetland rice." Appl. Environ. Microbiol. 37 (1979):813-819.

10. Watanabe, I. and Lin, C. "Response of wetland rice to inoculation with Azospirillum lipoferum and Pseudomonas sp." Soil Sci. Plant Nutr., 30 (1984): 117-124.

11. Nayak, D.V. and Rajaramamohan Rao, V. "Nitrogen fixation by Spirillum sp. from rice roots." Arch. Microbiol. 115 (1977): 359-360.

12. Ladha, J.K., Barraquio, W.L. and Watanabe, I. "Isolation and identification of nitrogen-fixing Enterobacter cloacae and Klebsiella planticola associated with rice plants." Can. J. Microbiol. 29 (1983): 1301-1308.

13. Watanabe, I., Barraquio, W.L. and Daroy, M.L. "Predominance of hydrogen-utilizing bacteria among nitrogen-fixing bacteria in wetland rice root." Can. J. Microbiol. 28 (1982): 1051-1054.

14. Barraquio, W.L., Ladha, J.K. and Watanabe, I. "Isolation and identification of N_2-fixing Pseudomonas associated with wetland rice." Can. J. Microbiol. 29 (1983): 867-873.

15. Tarrand, J.J., Krieg, N.R. and Dobereiner, J. "A taxonomic study of the Spirillum lipoferum group, with descriptions of a new genus, Azospirillum gen. nov. and two species, Azospirillum lipoferum (Beijerinck) comb. nov. and Azospirillum brasilense sp. nov." Can. J. Microbiol. 24 (1978): 967-980.

16. Ladha, J.K., Barraquio, W.L. and Watanabe, I. "Immunological techniques to identify Azospirillum associated with wetland rice." Can. J. Microbiol. 28 (1982): 478-485.

17. Thomas-Bauzon, D., Weinhard, P., Villecouart, P. and Balandreau, J. "The spermosphere model. I. Its use in growing, counting, and isolation N_2-fixing bacteria from the rhizosphere of rice." Can. J. Microbiol. 28 (1982): 922-928.

18. Klucas, R. "Nitrogen fixation by Klebsiella grown in the presence of oxygen." Can. J. Microbiol. 18 (1972): 1845-1850.

19. Haahtela, K., Kari, K. and Sundman, V. "Nitrogenase activity (acetylene reduction) of root-associated, cold-climate Azospirillum, Enterobacter, Klebsiella and Pseudomonus species during growth on various carbon sources and at various partial pressures of oxygen." Appl. ENviron. Microbiol. 45 (1983): 563-570.

20. Chargaff, E. In The Nucleic Acids, ed. by E. Chargaff & J.N. Davidson, vol. 1 p. 308, New York: Academic Press, (1955)

21. Schildkraut, C.L., Marmur, J. and Doty, P. "Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl." J. Mol. Biol. 4 (1962): 430-443.

22. Marmur, J. and Doty, P. "Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature." J. MOl. Biol. 5 (1962): 109-118.

23. Lutz, L.H. and Yayanos, A.A. "Spectrofluorometric Determination of bacterial DNA base composition." Anal. Biochem. 144 (1985): 1-5.

24. Britten, R.J., Graham, D.E. and Neufeld, B.R. "Analysis of repeating DNA sequences by reassociation." Methods in Enzymology 29 (1979): 363-397.

25. Brock, T.D. Biology of microorganisms. 2nd ed. p. 406, Prentice-Hall Inc., New Jersy. (1974).

26. Radloff, R., Bauer, W., and Vinogard, J. "A dye-bouyant density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in Hela cells." Proc. Natl. Acad. Sci. U.S.A. 57 (1967): 1514-1521.

27. Kado, C.I., and Liu, S.T. "Rapid procedure for detection and isolation of large and small plasmids." J. Bacteriol. 145 (1981): 1365-1373.

28. Smith, H.O. et al. "A restriction enzyme from hemophilus influenzae. I Purification and general properties." J. Mol. Biol. 51 (1970): 379-391.

29. Krieg, N.R. "Systematics". In Manual of method for general bacteriology p. 417-435, Washington: American Society for Microbiology (1981).

30. Marmur, J. "A procedure for the isolation of deoxyribonucleic acid from micro-organisms." J. Mol. Biol. 3 (1961): 208-218

31. ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ, ภาควิชาชีวเคมี คณะวิทยาศาสตร์ และศูนย์อณูพันธุศาสตร์-พันธุวิศวกรรมศาสตร์ มหาวิทยาลัยมหิดล. "เทคนิคการขยายยีนและตัดต่อยีน" การประชุมเชิงปฏิบัติการ (2528): 95-113.

32. Kramer, V.C., Nickerson, K.W. "Prevalence of extreme detergent resistance among the Enterobacteriaceae." Can. J. Microbiol. 30 (1984): 711-713.

33. Barraquio, W.L., Uozumi, T. and Beppu, T. "Plasmids in nitrogen-fixing bacteria isolated from rice rhizosphere." Annual

reports of International Center of Cooperative Research and Development in Microbial Engineering, Japan 4 (1981): 185-199.

34. Uozumi, T. et al. "Plasmids and nif genes in rhizobia and nitrogen-fixing bacteria in the rhizosphere of rice." Research work in the International Post-Graduate University Course in Microbiology. 1981: 314-319.

35. Berg, R.H. et al. "Biology of Azospirillum-sugarcane association: enhancement of nitrogenase activity." Appl. Environ. Microbiol. 39 (1980): 642-649.

36. Lowry, O.H. et al. "Protein measurement with the Folin-phenol reagent." J. Biol. Chem. 193 (1951): 265-275.

37. Markwell, M.A. et al. "Modification of the Lowry procedure to simplify protein determination in membrane with lipoprotein samples." Anal. Biochem. 87 (1978): 206-210.

38. Belozersky, A.N. and Spirin, A.S. In The Nucleic Acids, ed. by E. Chargaff and J.N. Davidson, vol. 3, p. 147. New York: Academic Press (1960)

39. Elmerich, C. "Molecular biology and ecology of diazotrophs associated with non-leguminous plants." Bio/Technology (1986): in press

40. Jonhson, J.L. and Cummins, C.S. "Cell wall composition and deoxyribonucleic acid somilarites among the anaerobic coryeforms, classical propionibacteria and strains of Arachnia propionica." J. Bacteriol. 109 (1972): 1047-1066.

41. Hill, L.R. et al. Poster Session Abstracts, Vth International Congress of Culture Collections, Bangkok, 1984. (42) Johnson, J.L. and Francis, B.S. "Taxonomy of the clostidia: ribosomal

ribonucleic acid homologies among the species." J. Gen. Microbiol. 8 (1975): 229-244.

43. Casse, F. et al. "Identification and characterization of large plasmid in Rhizobium Meliloti using Agarose electrophoresis." J. Gen. Microbiol. 113 (1979): 229-233.

44. Nutman, P.S. In The physiology of nodule formation in nutrition of legumes. ed. by E.G. Hallsworth p. 87-107 Scientific Publication, London. (1958).

45. Buchanan, R.E. and Gibbons, N.E. Bergey's Manual of Determinative bacteriology. 8th edition, U.S.A., Williams and Wilkina Company. (1974)

46. De bont, J.A.M. and Leijten, M.W.M. "Nitrogen fixation by hydrogen-utilizing bacteria." Arch. Microbiol. 107 (1976): 235-240.

47. Okon, Y., Albrecht, S.L. and Burris, R.H. "Methods for growing Spirillum lipoferum and for counting it in pure culture and in association with plants." Appl. Environ. Microbiol. 33 (1977): 85-88.

APPENDIX

Appendix 1 Statistical analysis of mole percentage of guanine plus cytosine content by F test with completely radomized design (unequal of N)

Table 1.1 Data taken from Table 4, summation of G + C content was listed as follow:

number of replication	G + C content (mol %)		
	Group I	Group II	Group III
1	54.9	68.8	64.2
2	56.4	70.8	65.2
3	54.7	69.4	
Total = T.j	166.0	209.0	129.4
Mean	55.33	69.67	64.7

504.4 = Tt

Total of all observation, Tt = 166.0 + 209.0 + 129.4 = 504.4

Correction term, CT $= \frac{Tt^2}{n}$

$= \frac{(504.4)^2}{8} = 31802.42$

$SS_{total} = \sum_{i=1}^{n} \sum_{j=1}^{k} X^2ij - CT$

$= (54.9)^2 + (56.4)^2 + \ldots + (65.2)^2 - 31808.42$

$= 319.76$

$SS_{treatment} = \sum_{j=1}^{k} \frac{T^2.j}{nj} - CT$

$$= \left(\frac{166}{3}\right)^2 + \left(\frac{209}{3}\right)^2 + \left(\frac{129.4}{2}\right)^2 - 31802.42$$

$$= 315.42$$

$$SS_{residual} = SS_{total} - SS_{treatment}$$

$$= 4.34$$

Table 1.2 ANOVA table for the randomized complete block design of G+C content.

Source of Variation	Degree of freedom[df.]	Sum of Square [SS.]	Mean Square [MS.]	F-ratio
treatments	2	315.42	157.71	181.69
residual	5	4.34	0.868	
Total	7	319.76		

Mean Square = Sum of Square/degree of freedom

$$F - ratio = \frac{MS\ treatments}{MS\ residual}$$

Tabular $F_{(2,5)} = 13.27 \quad (\alpha = 0.01)$

Since calculated F-ratio, 181.69 > 13.27

The mol % G + C were significantly different compared among groups of bacteria.

Data were paired and were subjected to further step of calculation using Duncan's new multiple range test.

Standard error of the mean, $S_{\bar{x}} = \sqrt{\text{error mean square/ri}}$

$$\text{error mean square/}_{ri} = \frac{\text{error some square}}{\text{df. of error X ri}}$$

$$= \sum_{j=1}^{k} \left[\sum_{i=1}^{n} Xij^2 - \frac{\left(\sum_{i=1}^{n} Xkj\right)^2}{r_i} \right] / ri$$

$$= \left[(54.9)^2 + (56.4)^2 + (54.7)^2 - \frac{(166)^2}{3}\right]/3 + \ldots$$

$$\ldots + \left[(64.2)^2 + (65.2)^2 - \frac{(129.4)^2}{3}\right]/2$$

$$= 1.53$$

$$S\bar{x} = \sqrt{\frac{1.53}{3}} = 0.553$$

At $\alpha = 0.05$, df. of error = 5, the significant studentized ranges (SSR) were as follow:

	p = number of means for range being tested	
SSR.	2	3
	3.64	3.74
LSR.	2.013	2.068

Least significant range (L.S.R.) = $SSR.XS\bar{x}$

Summation of the range of minimal to maximal values of data.

Group	I	III	II
Mean	55.33	64.70	69.67

Table 1.3 Statistical test for different pairs of mean by Duncan's new multiple range test.

Pair-being tested	Difference of mean	P	LSR.	Interpretation
Gr.I : GrII	14.34	3	3.396	SD+
Gr.II : Gr.III	4.97	2	3.15	SD+
Gr.I : GrIII	9.37	2	3.15	SD+

SD + = significant difference, SD - = no significant difference

BIOGRAPHY

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