Vol. 207, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 6, 1995 Pages 223-230

NUCLEOTIDE SEQUENCE AND EXPRESSION OF UDP-GLUCOSE DEHYDROGENASE GENE REQUIRED FOR THE SYNTHESIS OF XANTHAN GUM IN *XANTHOMONAS CAMPESTRIS*

Cheng-Sheng Lin[1], Nien-Tsung Lin[2], Bih-Ying Yang[2,#] Shu-Fen Weng[1] and Yi-Hsiung Tseng[1,*]

[1]Institute of Molecular Biology and [2]Department of Botany, National Chung Hsing University, Taichung 402, Taiwan, Republic of China

Received December 19, 1994

SUMMARY *Xanthomonas campestris* pv. *campestris*, producing large amounts of exopolysaccharide xanthan gum, has a mucoid phenotype. Strain SD7 was a non-mucoid mutant deficient in UDP-glucose dehydrogenase. A DNA fragment able to complement the mutation of SD7 was cloned from the parental wild-type strain Xc11. Sequence analysis of the region required for the complementation revealed an open reading frame which could encode a polypeptide of 445 amino acids with a calculated molecular weight of 48,432, a size similar to that of the product produced by maxicell. The amino acid sequence had significant homology to that of the GDP-mannose dehydrogenase from *Pseudomonas aeruginosa*.

The gram-negative plant pathogenic bacterium *Xanthomonas campestris* pv. *campestris* synthesizes great amounts of an exopolysaccharide, xanthan gum, rendering the colonies mucoid (1). Xanthan is a high molecular weight heteropolymer which has various applications in oil drilling, food, agriculture and industry as a thickening, emulsifying and suspending agent (2). It consists of a cellulosic (1->4)-β-D-glucose backbone with a trisaccharide side chain (mannose-glucuronic acid-mannose) attached to alternate glucose residues, with some of the mannose residues being acetylated or pyruvylated at specific sites (3). Biosynthesis of xanthan starts with the synthesis of sugar nucleotide precursors, followed by assembly of the pentasaccharide repeating unit from UDP-glucose, UDP-glucuronic acid and GDP-mannose on an isoprenoid glycosyl carrier, acetylation and pyruvylation of the mannose residues,

Present address: Center of Marine Biotechnology, Maryland Biotechnology Institute, Baltimore, MD 20212.

* To whom correspondence should be addressed.

Abbreviations: dCTP, deoxycytidine triphosphate; GDP, guanosine diphosphate; IPTG, isopropyl-β-D-thiogalactopyranoside; NAD, nicotinamide adenine dinucleotide; SDS, sodium dodecyl sulfate; UDP, uridine diphosphate.

0006-291X/95 $5.00

polymerization of the pentasaccharide repeating units, and finally secretion of the polymeric molecules into the medium (4). Defects in any of these steps can cause the failure of xanthan synthesis, resulting in non-mucoid or low mucoid phenotype. To date, mutations affecting mucoid phenotype of *X. campestris* have been mapped to at least four different regions of the chromosome (5, 6, 7, 8, 9, 10), and several of the genes involved in the synthesis of xanthan have been characterized (8, 11). In this study, we identified a DNA fragment which possessed ability to restore mucoid phenotype to a non-mucoid mutant. The results of DNA sequence analysis and enzyme assay suggested that it was the gene coding for UDP-glucose dehydrogenase required for the formation of UDP-glucuronic acid, one of the precursors for xanthan biosynthesis.

MATERIALS AND METHODS

Bacterial strains, media and cultivation conditions. Strain Xc11, isolated in Taiwan, was a wild-type strain producing great amounts of xanthan gum rendering the colonies slimy (12). Strain SD7 was a non-mucoid mutant isolated from Xc11 by nitrous acid mutagenesis (13). *Escherichia coli* DH1 was the host for gene cloning (14). Luria broth and L agar (15) were the general-purpose media for liquid and plate culture, respectively. *X. campestris* was grown at 28 °C and *E. coli* at 37 °C. Liquid cultures were incubated in a rotary shaker with vigorous shaking (280 rpm).

Materials. Restriction endonucleases, Klenow, T4 DNA ligase, and IPTG were purchased from Promega Corporation (Madison, Wisconsin). α-[^{32}P]-dCTP and [^{35}S]-methionine were obtained from Amersham Life Sciences (England, UK). UDP-glucose and GDP-mannose were purchased from Sigma Chemicals.

DNA techniques. Plasmid DNA preparation, restriction digestion, ligation of DNA, preparation of α-[^{32}P]-labelled probe, and DNA hybridization were done by following the procedures described by Maniatis *et al.* (14). Agarose gel electrophoresis was performed in TAE buffer, pH 7.9 in 0.7% agarose (14). For complementation tests, plasmid pSD701 was mobilized into SD7 by conjugation (16), and recombinant plasmids containing subfragments of the pSD701 insert were transferred by electroporation (17).

Marker exchange. To knock out the wild-type UDP-glucose dehydrogenase gene, a kanamycin cartridge (18) was inserted into the restriction sites in the 2.8-kb *Eco*RI-*Xho*I fragment cloned in pUC18 (19) and then introduced into the wild-type Xc11 cells by electroporation (17). The disrupted versions were then recombined into the chromosome by double crossing-over, generating mutations at various sites. A double crossing-over event was verified by DNA hybridization showing that only the Km^r gene, but not the vector, was associated with the chromosome.

Nucleotide sequence analysis. The DNA fragments to be sequenced were cloned into M13mp18 and M13mp19, and amplified in *E. coli* JM101 (19). Nested deletion clones were generated by the non-random deletion method of Dale *et al.* (20). The nucleotide sequences of both strands were determined by the dideoxy chain termination method of Sanger *et al.* (21). Nucleotide sequences were analyzed by using Release 6.01 of PC/GENE (IntelliGenetics). Tfasta (22) was used to search in the Genetics Computer Group (GCG) for homologous sequences.

Maxicell expression. Expression vector pCKR101 (from Dr. Dahlquist, R.) carrying *tac* promoter was used for maxicell expression in *E. coli* LCD44 (23). Preparation of maxicell, induction by IPTG (5 mM), and labelling the protein with [^{35}S]-methionine were carried out as described by Sancar *et al.* (24). The protein products were subjected to SDS-polyacrylamide gel electrophoresis (25) followed by autoradiography.

Enzyme assay. Cell-free extract was prepared by disrupting the cells harvested from overnight cultures with sonicator followed by centrifugation (12,000 x g, 30 min) to remove unbroken cells and cell debris. UDP-glucose dehydrogenase activity was assayed at 25 °C by measuring the rate of change of the A_{340} caused by the reduction of NAD (nicotinamide adenine dinucleotide), accompanying the oxidation of UDP-glucose to UDP-glucuronic acid. The assay mixture contained 0.2 M Tris-HCl, pH 8.5, 1 micromole of NAD, 0.5 micromole of UDP-glucose and crude extract (ca. 300 μg protein) in a total volume of 1 ml. One unit of UDP-glucose dehydrogenase is defined as the amount of enzyme that catalyzes the reduction of 1 micromole of NAD per min. Protein was determined by the method of Lowry (26) using crystalline bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Localization of the gene complementing the SD7 mutation.

Strain SD7 was a non-mucoid mutant isolated from the wild-type Xc11. Plasmid pSD701, cloned from a genomic bank of Xc11 (27) constructed by ligating *Eco*RI fragments into the mobilizable cosmid pLAFR1 (28), was able to restore the mucoid phenotype to SD7. Fig. 1A shows the restriction map of the pSD701 insert, an *Eco*RI fragment, which was estimated to be 5.3 kb in length. To localize the position of the gene complementing the SD7 mutation, different portions of the pSD701 insert were subcloned into the broad host range pRK415 (29) and electroporated into SD7. The results showed that clone pKA3 which contained the upstream *Eco*RI-*Xho*I fragment (2.8 kb) retained the complementation ability, being able to restore the mucoid phenotype to SD7 (Fig. 1B). To confirm these results, marker exchange

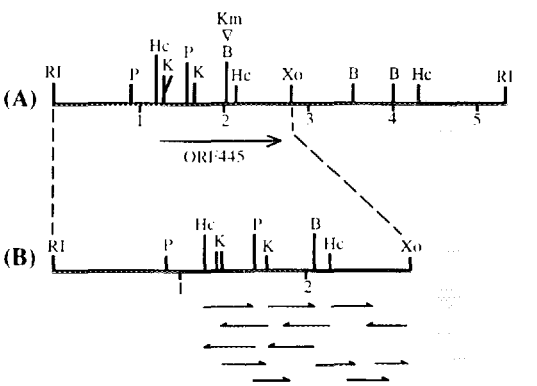

Fig. 1. Restriction maps of the 5.3-kb pSD701 insert (A), and the 2.8-kb pKA3 insert from *X. c.* pv. *campestris* 11. Symbol Km with a triangle pointing downwards represents the *Bam*HI site which was interrupted by a kanamycin cartridge followed by marker exchange to knock out the wild-type gene. The arrow under (A) is the position of ORF445, whereas the arrows under (B) indicate the strategy for sequencing the *Hin*cII-*Xho*I fragment. Abbreviations for restriction enzymes: B, *Bam*HI; Hc, *Hin*cII; K, *Kpn*I; P, *Pst*I; RI, *Eco*RI; Xo, *Xho*I.

was carried out to knock out the wild-type gene. It was found that insertion of a kanamycin cartridge into the unique *Bam*HI site of the *Eco*RI-*Xho*I fragment followed by recombining the disrupted gene into the Xc11 chromosome resulted in a mutation leading to non-mucoid phenotype. These results indicated that the gene complementing the mutation of SD7 straddles the *Bam*HI site within the 2.8-kb *Eco*RI-*Xho*I fragment (Fig. 1).

DNA sequence analysis.

The nucleotide sequence of the *Hinc*II-*Xho*I fragment from the pSD701 insert was determined following the strategy shown in Fig. 1, and totally 1,557 nucleotides were revealed.

```
                                                    ****
GTTGACGTTCGATGTCGAACTGATGGGTATCCTTCCCTGATCCCCCACAGGAGTACCCCC    60
ACCCATGCGAGTTGCGATCTTTGGTACCGGCTATGTTGGTCTTGTCACCGGTACCTGTCT   120
    M  R  V  A  I  F  G  T  G  Y  V  G  L  V  T  G  T  C  L
GGCGGAAGTAGGTCATCACGTTATCTGCGTGGATATCGACCAGGCGAAAGTTGATGGTCT   180
 A  E  V  G  H  H  V  I  C  V  D  I  D  Q  A  K  V  D  G  L
CAATCGTGGGGTGATTCCCATCTATGAACCCGGCCTGGAGCCGATGGTGAAAGCCAACCA   240
 N  R  G  V  I  P  I  Y  E  P  G  L  E  P  M  V  K  A  N  H
CGCCTCTGGCCGGTTGCGCTTCACCACCGACGCGGCCGAGGCGATTGCGCACGGCGAAAT   300
 A  S  G  R  L  R  F  T  T  D  A  A  E  A  I  A  H  G  E  I
CACCTTCATCGCCGTGGGCACGCCGCCGGACGAGGACGGCAGCGCCGACCTGCAGTACGT   360
 T  F  I  A  V  G  T  P  P  D  E  D  G  S  A  D  L  Q  Y  V
GCTGGCGGTTGCGCGCACCGTGGGCCGTCATATCGACGGGCCGTCGGTGATCGTCAATAA   420
 L  A  V  A  R  T  V  G  R  H  I  D  G  P  S  V  I  V  N  K
GTCCACGGTGCCGGTCGGTACCGCCGACAAGGTGCGCGCGGCCATCCAGGAAGAACTGGA   480
 S  T  V  P  V  G  T  A  D  K  V  R  A  A  I  Q  E  E  L  D
TGCACGCGGCGTGGACCATGAGTTCGACGTGGTTTCCAACCCTGAGTTCCTCAAGGAAGG   540
 A  R  G  V  D  H  E  F  D  V  V  S  N  P  E  F  L  K  E  G
CGACGCGGTGGCCGACTGCATGCGCCCGGACCGGATCGTGATCGGGGCCAAGAAGCCGGC   600
 D  A  V  A  D  C  M  R  P  D  R  I  V  I  G  A  K  K  P  A
AGCGATTGCACGCATGCGCCGTCTGTATGCCCCGTTCAACCGCAACCGCGATCGCATCGT   660
 A  I  A  R  M  R  R  L  Y  A  P  F  N  R  N  R  D  R  I  V
GGAAATGGATGTGCGCTCGGCCGAGCTGACCAAGTACGCGGCCAACGCGATGCTGGCGAC   720
 E  M  D  V  R  S  A  E  L  T  K  Y  A  A  N  A  M  L  A  T
CAAGATCAGTTTCATGAACGAGATCGCCAACATTGCCGAACGCGTCGGTGCGGACGTCGA   780
 K  I  S  F  M  N  E  I  A  N  I  A  E  R  V  G  A  D  V  E
GCATGTGCGTAACGGCATTGGTTCGGATCCGCGCATTGGCTGGCACTTCATCTATCCGGT   840
 H  V  R  N  G  I  G  S  D  P  R  I  G  W  H  F  I  Y  P  V
GCCGGCTACGGCTCGTGCTTCCCGAAGATGTGCAGGCGCTCGCAAGACCGCGCACGAGTA   900
 P  A  T  A  R  A  S  R  R  C  A  G  A  R  K  T  A  H  E  Y
CGGCATGCAGCCGACCCTGCTCAACGCGGTGGAAAGCGTCAACAACGCGCAGAAGGGCA   960
 G  M  Q  P  T  L  L  N  A  V  E  S  V  N  N  A  Q  K  G  H
TCTGTTCGAACTGGTGCAGCGTCACTATGCCGATGGCAACGGCAGCATCGCCGGCAAGAC  1020
 L  F  E  L  V  Q  R  H  Y  A  D  G  N  G  S  I  A  G  K  T
CTTTGCGGTGTGGGGCCTGGCGTTCAAGCCCAATACCGATGACATGCGTGCAGCCTCCAG  1080
 F  A  V  W  G  L  A  F  K  P  N  T  D  D  M  R  A  A  S  S
CCGTCGCCTGATGGCCAGTTGTGGGAAGCCGGTGCCAAGGTGCGCGCGTACGACCCCGAA  1140
 R  R  L  M  A  S  C  G  K  P  V  P  R  C  A  R  T  T  P  K
GCCACGCACGAAGCCAAGCGCATCTTCGGCGAGCGCGACGACCTGGCGTTCTGCGACGAC  1200
 P  R  T  K  P  S  A  S  S  A  S  A  T  T  W  R  S  A  T  T
GCCTTCGCCGCACTGGAAGCGCCGACGCGCTGGTCGTGGTCACCGAGTGGAAGCAGTTCC  1260
 P  S  P  H  W  K  R  R  R  A  G  R  G  H  R  V  E  A  V  P
GCAGCCCGGATTTCGGCAAGATCAAGCAGGCTCTGAAGGACGACGTCGTGTTCGACGGCC  1320
 Q  P  G  F  R  Q  D  Q  A  G  S  E  G  R  R  R  V  R  R  P
GCAACCTGTACGACCCGCAGGAGATCGAAGCCGCAGGTCTGCGTACTACGCCATCGGACG  1380
 Q  P  V  R  P  A  G  D  R  S  R  R  S  A  Y  Y  A  I  G  R
AGGCCGTTCGCTGCATGCATGAGCAACTCTCGCCGCGCGACCAGGAACTGGAAGCGCGGT  1440
 G  R  S  L  H  A
TGGTGGAACTGGAAACGCGCCTCTCTTTTCAGGAGCAGGCGCTGACCGAACTGAGTGAAC  1500
GCGTCTAGCCGTTTGACGGGCGCCCGCAATGCCGAATTGATCCGCCACCTGCTCGAG     1557
```

Fig. 2. Sequence of the 1,557-nucleotide *Hinc*II-*Xho*I fragment containing the ORF445 from *X. c.* pv. *campestris* 11. The coding region is indicated by the amino acids (one-letter code). The possible ribosome-binding sites are indicated by asterisks, and the conserved glycine motif is underlined. The nucleotide sequence has been deposited in the EMBL Data Library under accession No. X79772.

Computer search on this fragment showed a possible open reading frame (ORF445) straddling the *Bam*HI site. This ORF starts with ATG at position 65 and ends at position 1,400, having capacity to encode a polypeptide of 445 amino acids with a calculated molecular weight of 48,432 (Fig. 2). There is a 5'-AGGA-3' at 12 nucleotides upstream of the start codon which is consensus to a ribosome binding site (30) (Fig. 2). No sequences consensus to a promoter were found.

The G+C content of ORF445 is 63% which is within the normal range observed for *Xanthomonas* (63 to 69%) (1). In agreement with the high G+C content is the preference for G or C to be used as third base of codons (73%).

Comparisons of the nucleotide sequence.

The deduced amino acid sequence of the ORF445 product showed strong homology with that of the GDP-mannose dehydrogenase from *Pseudomonas aeruginosa* (31). Through an overlap of 349 amino acids, 34.7% identity was found (Fig. 3).

It has been reported that many dehydrogenases possess a glycine motif with the sequence -G-X-G-X-X-G- at their NH_2-termini for binding to the coenzyme NAD (32). A consensus

```
ORF445   MRVAIFGTGYVGLVTGTCLAEVGHHVICVDIDQAKVDGLNRGVIPIYEPG
         MR::IFG GYVG V :.CL:. GH:VI VD:. :K:D :N:G  PI EPG
algD     MRISIFGLGYVGAVCAGCLSARGHEVIGVDVSSTKIDLINQGKSPIVEPG

ORF445   LEPMVKANHASGRLRFTTDAAEAIAHGEITFIAVGTPPDEDGSADLQYVL
         LE::::..:.:GRL. TTD  .A: :::::FI VGTP:..:G. DL Y:
algD     LEALLQQGRQTGRLSGTTDFKKAVLDSDVSFICVGTPSKKNGDLDLGYIE

ORF445   AVARTVG---RHIDGPSVIVNKSTVPVGTADKVRAAIQEELDARGVDHEF
         :V R.:G   R: ... .:V :STV  GT.::V  :: E: .:: .: :F
algD     TVCREIGFAIREKSERHTVVVRSTVLPGTVNNVVIPLIEDCSGKKAGVDF

ORF445   DVVSNPEFLKEGDAVADCMRPDRIVIGAKKPAAIARMRRLYAPFNRNRDR
         :V :NPEEL:E:.A: D.  P ..VIG. . .: . :  :Y  :    ..
algD     GVGTNPEFLRESTAIKDYDFPPMTVIGELDKQTGDLLEEIYREL---DAP

ORF445   IVEMDVRSAELTKYAANAMLATKISFMNEIANIAERVGADVEHVRNGIGS
         I: ..V  AE:.KY: N.  A:K::F NEI:NIA. VG.D  :V.: I
algD     IIRKTVEVAEMIKYTCNVWHAAKVTFANEIGNIAKAVGVDGREVMDVICQ

ORF445   DPRIGWHFIYPVPATARASRRCAGARKTAHEYGMQPTLLNAVESVNNAQK
         D.::.    Y  P: A ::. C      A .Y  ::: L:. ::: .: .
algD     DHKLNLSRYYMRPGFAFGGS-CLPKDVRALTY--RASQLDVEHPMLGSLM

ORF445   GHLFELVQRHYADGNGSIAGKTFAVWGLAFKPNTDDMRAASSRRLMASCG
            : VQ: : D  .S ..:. :: GL:FK:.TDD:R.::   L .
algD     RSNSNQVQKAF-DLITSHDTRKVGLLGLSFKAGTDDLRESPLVELAEMLI

ORF445   KPVPRCARTTPKPRTKPSASSASATTWRSATTPSPHWKRRRAGRGHRVEA
               ..:    .  ::... . .. . S:   .  ..   . :.
algD     GKGYEFRIFDRNVEYARVHGANKEYIESKIPHVSSLLVSDLDEVVASSDV

ORF445   VPQPGFRQDQAGSEGRRRVRRPQPVRPAGDRSRRSAYYAIGRGRSLHA
         :   .  :  .:  .:. .  :      :  .   :A  .
algD     LVLGNGDELFVDLVNKTPSGKKLVDLVGFMPHTTTAQAEGICW
```

Fig. 3. Comparison of the deduced amino acid sequence of ORF445 from *X. c.* pv. *campestris* 11 and that of the *Pseudomonas aeruginosa* GDP-mannose dehydrogenase (*algD*). Two dots indicate a conservative substitution, and one dot indicates a less conservative substitution.

sequence -G-T-G-Y-V-G- was found between positions 7 and 12 in the predicted product of Xc11 ORF445 (Fig. 3).

Maxicell expression.

Maxicell expression using the 2.8-kb *Eco*RI-*Xho*I fragment as the template produced a polypeptide with a molecular weight of about 50 kDa, as visualized in the autoradiogram after SDS-polyacrylamide gel electrophoresis, which is in agreement with the molecular size predicted from the nucleotide sequence (Fig. 4).

Enzyme activity of the UDP-glucose dehydrogenase.

The UDP-glucose dehydrogenase activity measured was 3.14 and 0.16 units per mg protein in the wild-type Xc11 and the non-mucoid mutant SD7, respectively. When plasmid pKA3, carrying the 2.8-kb *Eco*RI-*Xho*I fragment, was introduced into SD7, the enzyme activity was elevated by about 1.7-fold higher than the level in Xc11. Activity against GDP-mannose (0.5 micromole), in substitution for UDP-glucose as the substrate, was similar in both strains Xc11 and SD7 (1.7 versus 2.1 units per mg), suggesting the possibility that other dehydrogenase against GDP-mannose might be operative in *X. campestris*.

In conclusion, we propose ORF445 to be the UDP-glucose dehydrogenase gene of *X. campestris*, based on i) deficiency of enzyme activity against UDP-glucose in the non-mucoid mutant SD7 and simultaneous recovery of the enzyme activity upon cloning of the wild-type gene, ii) restoration of mucoid phenotype to SD7 by the wild-type gene resulting from the normal synthesis of xanthan, which requires UDP-glucuronic acid as a precursor, and iii) the amino acid sequence homology to that of the *P. aeruginosa* GDP-mannose dehydrogenase.

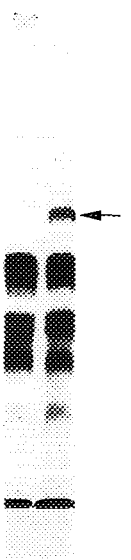

Fig. 4. Expression of a 50-kDa product (indicated by an arrow) encoded by the ORF445 from *X. c.* pv. *campestris* 11 in *E. coli* maxicell. Lanes: left, uninduced; right, induced with 5 mM IPTG.

ACKNOWLEDGMENTS

This work was supported by grant No. NSC83-0211-B005-041 from National Science Council of Republic of China. We thank Dr. C. N. Sun for reading the manuscript.

REFERENCES

1. Bradbury, J.F. (1984) In: Bergey's Manual of Systematic Bacteriology, Vol. 1 (N.R. Krieg, Ed.), pp. 199-210. Williams & Wilkins, Baltimore.
2. Kennedy, J.F., and Bradshaw, I.J. (1984) In: Progress in Industrial Microbiology, Vol. 19 (Bushell, M.E., Ed.), pp. 319-371. Elsevier, Amsterdam.
3. Jansson, P.E., Keene, L., and Lindberg, B. (1975) Carbohydr. Res. 45, 275-282.
4. Ielpi, L., Couso, R.O., and Dankert, M.A. (1993) J. Bacteriol. 175, 2490-2500.
5. Barrere, G.C., Barber, C.E., and Daniels, M.J. (1986) Int. J. Biol. Macromol. 8, 372-374.
6. Harding, N.E., Cleary, J.M., Cabanas, D.K., Rosen, I.G., and Kang, K.S. (1987) J. Bacteriol. 169, 2854-2861.
7. Hote, B., Rath-Arnold, I., Puhler, A., and Simon, R. (1990) J. Bacteriol. 172, 2804-2807.
8. Koplin, R., Arnold, W., Hotte, B., Simon, R.,Wang, G., and Puhler, A. (1992) J. Bacteriol. 174, 191-199.
9. Thorne, L., Tansey, L., and Pollock, T.J. (1987) J. Bacteriol. 169, 3593-3600.
10. Vanderslice, R.W., Doherty, D.H., Capage, M.A., Betlach, M.R., Hassler, R.A., Henderson, N.M., Ryan-Graniero, J., and Tecklenberg, M. (1990) In: Biomedical and Biotechnological Advances in Industrial Polysaccharides (V. Crescenzi, I.C.M. Dea, S. Paoletti, S.S. Stivala, and I.W. Sutherland, Ed), pp. 145-156. Gordon and Breach Science Publishers, NY.
11. Marzocca, M.P., Harding, N.E., Petroni, E.A., Cleary, J.M., and Ielpi, L. (1991) J. Bacteriol. 173, 7519-7524.
12. Yang, B.-Y., and Tseng, Y.-H. (1988) Bot. Bull. Acad. Sinica. 29, 93-99.
13. Yang, B.-Y. (1985) Master thesis, National Chung Hsing University, Taichung, Taiwan, ROC.
14. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
15. Miller, J.H. (1972) Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
16. Yang, B.-Y., Tsai, H.-F., and Tseng, Y.-H. (1988) Chinese J. Microbiol. Immunol. 21, 40-49.
17. Wang, T.-W., and Tseng, Y.-H. (1992) Lett. Appl. Microbiol. 14, 65-68.
18. Prentki, P., and Krisch, H.M. (1984) Gene 29, 303-313.
19. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene 33, 103-119.
20. Dale, R.M.K., McClure, B.A., and Houchins, J.P. (1985) Plasmid 13, 31-40.
21. Sanger, F., Nicklen, S., and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
22. Pearson, W.R., and Lipman, D. J. (1988) Proc. Natl. Acad. Sci. USA 85, 2444-2448.
23. Li, S.J., and Cronan, J.E., Jr. (1992) J. Biol. Chem. 267, 855-863.
24. Sancar, A., Hack, A.M., and Rupp, W.D. (1979) J. Bacteriol. 137, 692-693.
25. Laemmli, U.K. (1970) Nature 227, 680-685.
26. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.

27. Yang, B.-Y., Tsai, H.-F., and Tseng, Y.-H. (1987) In: Proceedings of the ROC-USA Agricultural Biotechnology Workshop, Taipei, Taiwan, 6-10 April 1987. pp.229-243. National Science Council, Taipei.
28. Friedman, A.M., Long, S.R., Brown, S.E., Buikema, W.J., and Ausubel, F.M. (1982) Gene 18, 289-296.
29. Keen, N.T., Tamaki, S., Kobayashi, D., and Trollinger, D. (1988) Gene 70, 191-197.
30. Shine, J., and Dalgarno, L. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 1342-1346.
31. Deretic, V., Gill, J.F., and Charkrabarty, A.M. (1987) Nucleic Acid Res. 15, 4567-4581.
32. Wierenga, R.K., and Hol, W.G.J. (1983) Nature (London) 302, 842-844.