Table 3 *Effect of ultraviolet exposure on the viability of phages*

Time of exposure (min)	Per cent survivors remaining after exposure				
	BS12	BS25	BS65	BS138	BS158
No exposure	100	100	100	100	100
5	98	95	98	97	98
10	95	93	96	92	93
15	81	83	83	84	82
20	72	74	72	71	72
30	51	49	51	48	47
40	31	32	30	30	31
50	13	15	13	12	11
60	0.1	0.4	1	0.5	0.1

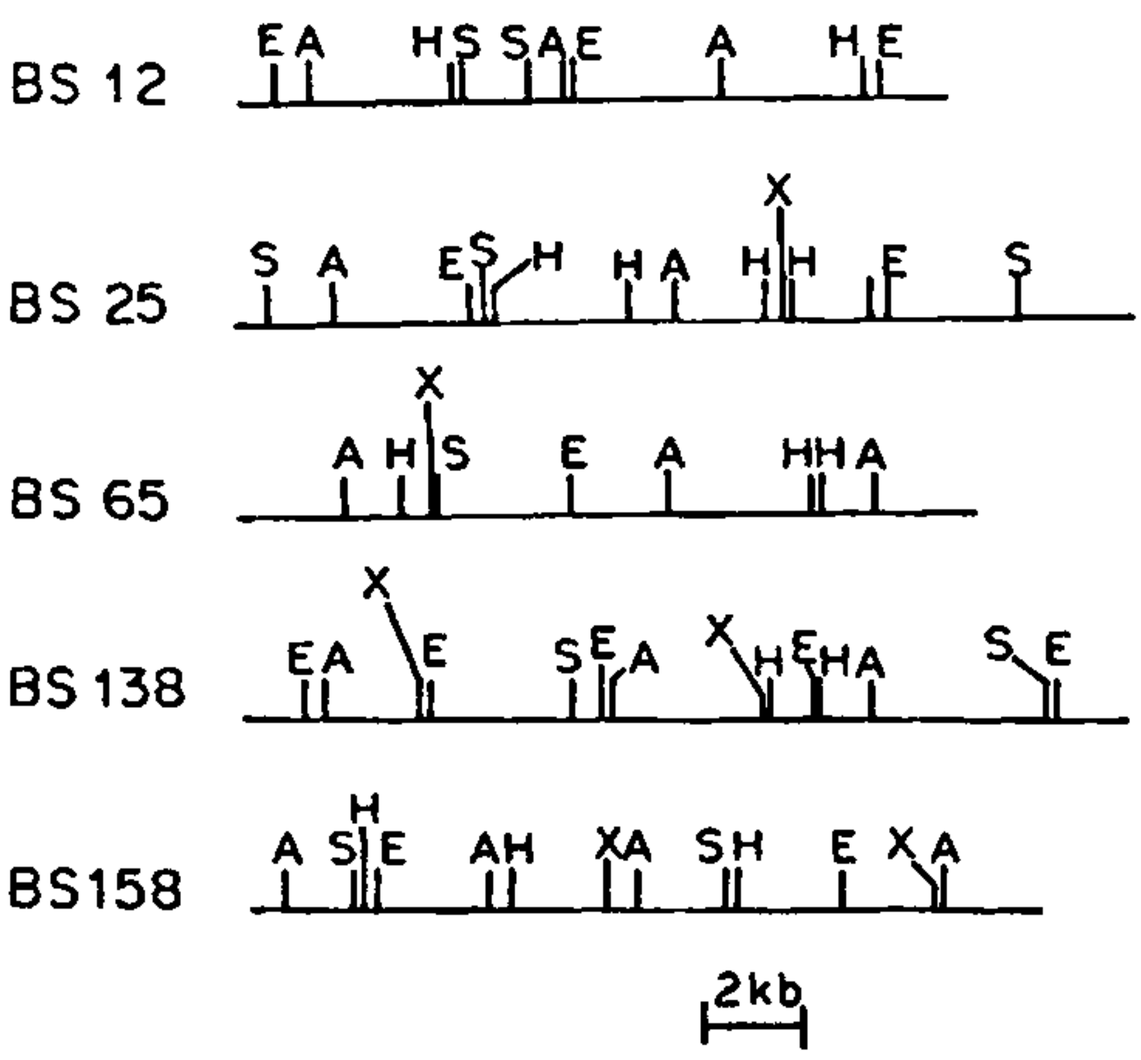

Figure 1. Restriction maps of *B. brevis* phages BS12, BS25, BS65, BS138 and BS158. E, *Eco*RI; A, *Ava*I; H, *Hind*III; S, *Sal*I; X, *Xho*I.

Labelling of phage DNAs using the Klenow fragment of DNA polymerase resulted in the labelling of only two *Eco*RII fragments (BS12 and BS138), two *Sal*I fragments (BS25), and two *Ava*I fragments (BS158 and BS65), all of which lie at the ends of the respective restriction maps (figure 1) indicating that the DNAs of these phages have 5′ protruding single-stranded ends. Further experimentation suggested that the single-stranded protrusions are complementary in base sequence but not palindromic.

28 September 1988; Revised 5 December 1988

1. Bradely, D. E., *Bacteriol. Rev.*, 1967, **31**, 230.
2. Charter, K. F., *Dev. Ind. Microbiol.*, 1980, **21**, 65.
3. Lomovskaya, N. D., Charter, K. F. and Mkrtumian, N. M., *Microbiol. Rev.*, 1980, **44**, 206.
4. Charter, K. F., Hopwood, D. A., Kaiser, T. and Thompson, C. J., *Curr. Top. Microbiol. Immunol.*, 1982, **96**, 69.
5. Lomovskaya, N. D., Sladkoya, I. A., Klochkova, O., Chinenova, T. A., Mkrtuniam, N. M. and Orekov, A. V., *Overproduction of Microbial Products*, (eds) V. Krumphazl, B. Sikyta and Z. Vanek, Academic Press, New York, 1982, p. 395.
6. Verma, M., Siddiqui, J. Z. and Chakravorty, M., *Biochem. Int.*, 1985, **11**, 179.
7. Verma, M. and Siddiqui, J. Z., *Biochem. Int.*, 1986, **12**, 167.
8. Adams, M. H., *Ann. N.Y. Acad. Sci.*, 1953, **56**, 442.
9. Adams, M. H., *Bacteriophages*, Interscience Publishers, New York, 1959, p. 1.
10. Joshi, A., Siddiqui, J. Z., Rao, G. R. K. and Chakravorty, M., *J. Virol.*, 1982, **41**, 1038.
11. Guay, R., Ackermann, H. W. and Murthy, M. R. V., *CRC Handbook of Microbiology*, CRC Press, Florida, 1981, p. 731.
12. Isogai, T., Takahashi, H. and Saito, H., *Agric. Biol. Chem.*, 1980, **44**, 2425.
13. Chung, S. T., *Gene*, 1982, **17**, 239.

PLASMID-ENCODED PRODUCTION OF HYDROCARBON-SOLUBILIZING FACTOR BY *PSEUDOMONAS* PG–I

SWARANJIT S. CAMEOTRA* and H. D. SINGH
Biochemistry Division, Regional Research Laboratory, Jorhat 785 006, India
**Present address: Institute of Microbial Technology, 177-G, Industrial Area, Chandigarh 160 002, India*

ISOLATION of a polymeric hydrocarbon-solubilizing substance produced by *Pseudomonas* PG-I during growth on liquid and solid hydrocarbons has been reported previously[1,2]. Production of extracellular alkane-solubilizing factor by the yeast *Endomycopsis lipolytica* YM has also been reported[3]. It was shown that hydrocarbon solubilization by solubilizing factors could fully account for substrate uptake and growth of the organisms on hydrocarbons.

Many reports have appeared on the specific action

of sodium dodecyl sulphate (SDS) in the removal (curing) of bacterial plasmids[4-7]. This communication reports that synthesis of hydrocarbon-solubilizing factor in *Pseudomonas* PG–I is plasmid-dependent, as plasmid freed organism failed to utilize alkanes. This barrier could be overcome by the use of artificially emulsified substrate or exogenous addition of the solubilizing factor.

The characteristics and conditions for culture of *Pseudomonas* PG–I were described previously[1,2]. Hexadecane-emulsifying and solubilizing factor (PG–I $ESFC_{16}$) was isolated from cell-free broth obtained from a culture of *Pseudomonas* PG–I on *n*-hexadecane by precipitation with chilled acetone[1].

For plasmid curing by SDS, 50 ml of mineral medium was inoculated with a loopful of *Pseudomonas* PG–I culture, *n*-hexadecane was added as sole carbon source for 12 h and the culture shake-incubated. SDS was added to a final concentration of 800 μg/ml and growth allowed to continue for another 12 h. Serial dilution up to 10^{-7} was carried out and 0.1 ml was plated on mineral medium. For development of colonies, plates were incubated at 37°C in an atmosphere of liquid petroleum gas–air mixture (1:3). Colonies that did not grow on liquid *n*-hexadecane were carefully selected. *Pseudomonas* PG–I was also grown on 50 ml minimal medium containing 1 g sodium acetate. Five ml of the culture was retransferred to fresh medium and allowed to grow for a further period of 24 h. The isolation pattern was followed as before except that colonies were plated on nutrient agar medium.

For growth on liquid petroleum gas (LPG) agar–mineral medium slants were placed inside an evacuable gas-tight container containing LPG–air mixture (1:3) at 37°C for 2 days. For growth on hydrocarbon vapours, slants containing inoculum at the top were utilized. The liquid hydrocarbons (0.3 ml) were injected to the bottom of the test tubes using a syringe with a long needle. Tubes were incubated at 37°C for 2 days. The alkane solution at the bottom and the inoculum at the top of the slant were separated by about 3 inches in all the cases and growth occurred only by virtue of utilization of the alkane vapour.

The plasmid-cured strain failed to grow on liquid *n*-hexadecane presented in its natural physical form (figure 1A). But when the alkane was artificially emulsified and solubilized very good growth was observed, almost to the level of the native strain. As described previously[2], such artifically emulsified and solubilized hydrocarbon in shake-incubation could

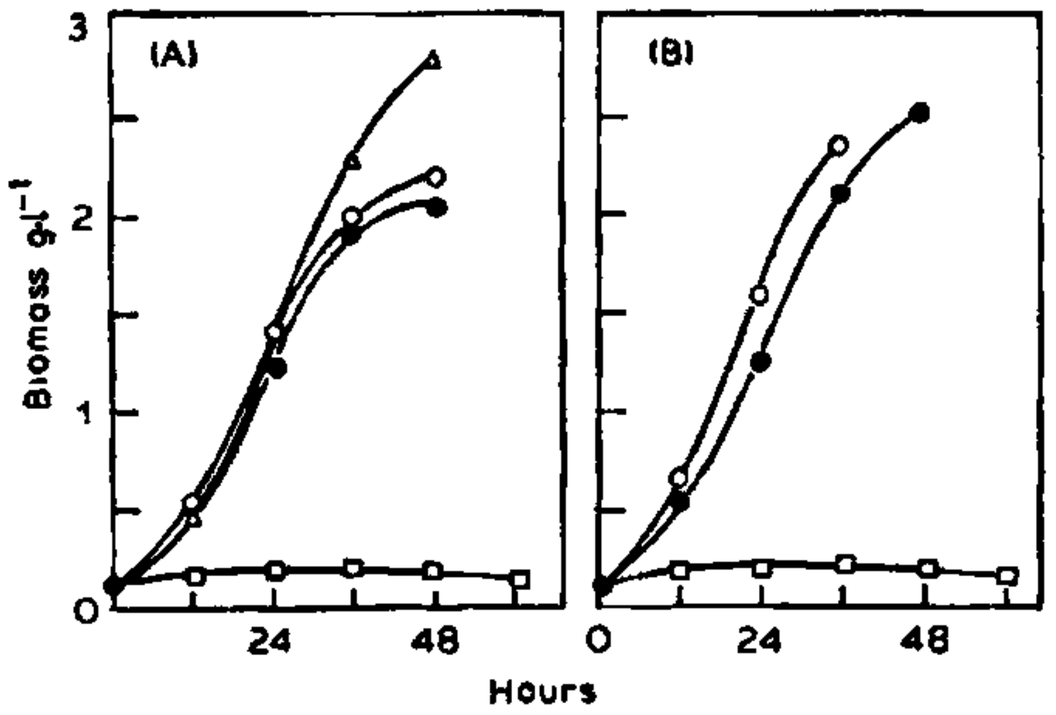

Figure 1. Growth of native and plasmid-cured *Pseudomonas* PG–I on various substrates. **A,** Native strain on liquid *n*-hexadecane (△), and plasmid-cured (SDS) strain on liquid *n*-hexadecane (□), liquid *n*-hexadecane + PG–I $ESFC_{16}$ (●), and emulsified *n*-hexadecane (○). **B,** *Pseudomonas* PG–I pregrown on sodium acetate, on liquid *n*-hexadecane (□), liquid *n*-hexadecane + PG–I $ESFC_{16}$ (●), and emulsified *n*-hexadecane (○).

generate more solubilized hydrocarbon, in addition to the solubilized hydrocarbon already present.

As shown in figure 1 PG–I $ESFC_{16}$ (100 mg%) stimulated growth of the plasmid-cured strain on liquid *n*-hexadecane. The findings suggest that with the loss of plasmid(s), the organism also lost its ability to produce solubilizing factor and was thus unable to utilize the hydrocarbon. It also appears (figure 1B) that *Pseudomonas* PG–I lost plasmid(s) during growth on sodium acetate. The plasmid curing appears to be permanent as the strain could not readapt to grow on liquid *n*-hexadecane even after prolonged incubation for 10 days.

Although the plasmid-cured strain could not utilize liquid *n*-decane, *n*-dodecane and *n*-hexadecane. good growth was obtained when these alkanes were presented in the vapour phase in a closed solid culture system. Similarly the strain could grow very well on LPG.

The results, that plasmid-cured cells could utilize gaseous hydrocarbons and artificially emulsified and solubilized alkanes for growth, indicate that the enzymatic mechanism for utilization of solubilized hydrocarbon was intact in the plasmid-cured cells. The obvious implication is that the synthesis of these enzymes is not determined by plasmids in this organism. In the dimorphic yeast *Saccharomycopsis lipolytica* the genetic determinants for alkane-

oxidizing enzymes were reported to be located on the chromosomes[8]. Chakraborty *et al.*[9] reported involvement of plasmids in degradation of hydrocarbons, including short-chain-length alkanes. Chakraborty[10] also documented plasmid-mediated degradation of simple aromatics.

1 August 1988; Revised 10 January 1989

1. Reddy, P. G., Singh, H. D., Pathak, M. G., Bhagat, S. D. and Baruah, J. N., *Biotechnol. Bioeng.*, 1983, **25**, 387.
2. Cameotra, Swaranjit, S., Singh, H. D., Hazarika, A. K. and Baruah, J. N., *Biotechnol. Bioeng.*, 1983, **25**, 2945.
3. Cameotra, Swaranjit, S., Singh, H. D. and Baruah, J. N., *Biotechnol. Bioeng.*, 1984, **26**, 554.
4. Inzuka, N., Nakamura, S., Inuzuka, M. and Tomoeda, M., *J. Bacteriol.*, 1969, **100**, 827.
5. Salisbury, V., Hedges, R. W. and Datta. N., *J. Gen. Microbiol.*, 1972, **70**, 443.
6. Hill, W. E. and Canlisle, L., *Appl. Environ. Microbiol.*, 1981, **41**, 1046.
7. Adachi, H., Nakano, M., Inuzuka, M. and Tomoeda, M., *J. Bacteriol.*, 1972, **190**, 114.
8. Bassel, J. and Ogrydzik, *Genetics of Saccharomycopsis lipolytica, with Emphasis on Genetics of Hydrocarbon Utilization in Genetics of Industrial Microorganisms*, (eds) O. K. Sebek and A. I. Laskin, American Society for Microbiology, Washington, 1979, p. 160.
9. Chakraborty, A. M., Chou, G. and Gunsalus, I. C., *Proc. Natl. Acad. Sci. USA*, 1973, **70**, 1137.
10. Chakraborty, A. M., *J. Bacteriol.*, 1972, **112**, 815

BIOSYNTHESIS OF PYRIMIDINES DURING GERMINATION OF BLACK GRAM SEEDS: CHANGES IN THE LEVELS OF PARTICIPANT ENZYMES

H. ASHIHARA and Y. KAMEYAMA
Department of Biology, Faculty of Science, Ochanomizu University, 2-1-1, Otsuka, Bunkyo-ku, Tokyo, 112, Japan

DRAMATIC changes in rates of respiration, and synthesis and degradation of macromolecules, such as proteins, nucleic acids and starch, have often been observed in seeds during germination[1]. Nevertheless, little is known about the metabolism of pyrimidine nucleotides in germinating seeds. The present study was designed to determine the factors that affect the rate of biosynthesis of pyrimidine nucleotides during germination. We determined the levels of some key enzymes of the *de novo* and salvage pathways of biosynthesis of pyrimidine nucleotides, and followed changes in levels of 5-phosphoribosyl-1-pyrophosphate (PRPP) and ATP, donors of phosphoribosyl and phosphate groups, respectively, in the biosynthesis, in the cotyledons and embryonic axes of germinating black gram seeds.

Seeds of black gram (*Vigna mungo* L.) were sterilized and germinated on 0.55% agar gel in the dark at 27°C under aseptic conditions[2,3]. After a fixed length of time, cotyledons and embryonic axes were separated and analysed. Dry seeds were also used for the assays of enzymatic activities. Extraction of enzymes from seeds and seedlings was performed as described by Nobusawa and Ashihara[4]. The activities of enzymes were assayed radiochemically as described earlier[5-7]. For determination of levels of PRPP, samples (approximately 2 g fresh weight) were frozen rapidly with liquid N_2 and powdered with a chilled mortar and pestle. The powder was homogenized with 50 mM Tris–HCl buffer (pH 7.4) containing 2 mM NaEDTA and 100 mM KF in a glass homogenizer. The homogenate was centrifuged at 23,000 *g* for 20 min at 2°C. The resultant supernatant was immediately used for assays. The level of PRPP was determined radiochemically[8]. For determination of ATP, samples (approximately 0.5 g fresh weight) were frozen in liquid N_2 and homogenized with 6% perchloric acid in a glass homogenizer. After centrifugation, the acid extracts were neutralized with KOH, and levels of ATP were determined with a Packard Pico-Lite luminometer, Model 6100, using firefly luciferase and luciferin[9].

Changes in the fresh and dry weights and in the levels of DNA and RNA in pairs of cotyledons and in individual embryonic axes of black gram seeds during germination are essentially identical, as we found in earlier studies[2,10] (data not shown).

The levels of selected enzymes involved in the biosynthesis of pyrimidine nucleotides *de novo*, i.e. carbamoyl-phosphate synthetase (CPSase), aspartate transcarbamoylase (ATCase), orotate phosphoribosyl transferase (OPRTase) and orotidine-5′-monophosphate decarboxylase (ODCase); the level of two enzymes involved in the pyrimidine salvage pathway, i.e. uridine kinase (UKase) and uracil phosphoribosyltransferase (UPRTase); and the level of PRPP synthetase were determined in extracts of dry seeds