study as well as in those of others is perhaps due to the instability of R plasmids in this organisms. Thus this study shows that *V. parahaemolyticus* strains can undergo spontaneous mutation to 0/129 resistance.

The authors are grateful to Prof. H. P. C. Shetty and Dr R. R. Colwell for helpful discussions. This work was supported in part by the United States Department of Agriculture under Co-operative Agricultural Research Grant Program (PL-480).

1. Collier, H. O. J., Cambell, N. R. and Fitzgerald, M. E. H., *Nature* (*London*), 1950, **165,** 1004.
2. Shewan, J. M., Hodgkiss, W. and Liston, J., Nature (*London*), 1954, **173,** 208.
3. Davis, C. H. G. and Park, R. W. A., *J. Gen. Microbiol.*, 1962, **27,** 101.
4. Bian, N. and Shewan, J. M., In: *Identification methods for microbiologists*, Part B (eds.), B. M. Gibbs and D. A. Shapton, Academic Press, London, 1968, p. 79.
5. Sundaram, S. P. and Murthy, K. V., *FEMS Microbiol. Lett.*, 1983, **19,** 115.
6. Arai, T., Hamashima, H. and Hasegawa, H., *Chemotherapy* (In Japanese), 1983, **31,** 517.
7. Matsushita, S., Kudoh, Y. and Ohashi, M., *Microbiol. Immunol.*, 1984, **28,** 1159.
8. Arai, T., Hamashima, H. and Hasegawa, H., *Microbiol. Immunol.*, 1985, **29,** 103.
9. Joseph, S. W., Colwell, R. R. and Kaper, J. B., *CRC Crit. Rev Microbiol.*, 1982, **10,** 77.
10. Aoki, T., Egusa, S. and Arai, T., *Antimicrob. Agents Chemother.*, 1974, **6,** 534.
11. Aoki, T., Kitao, T., Watanabe, S. and Takeshita, S., *Microbiol. Immunol.*, 1984, **28,** 1.
12. Hedges, R. W. and Jacob, A. E., *J. Gen. Microbiol.*, 1975, **89,** 383.
13. Hedges, R. W., Vialard, J. L., Pearson, N. S. and O'Grady, F., *Antimicrob. Agents Chemother.*, 1977, **11,** 585.
14. Kanoli, T., Yokoto, T., Yamamoto, T. and Kuwahara, S., *Proc. 14th Joint Conf. US-Japan Co-op. Med. Sci.*, Cholera Panel, 1978, p. 200.
15. Kuwahara, S., Yokoto, T. and Kanoli, T., *Proc. 15th Joint Conf. US-Japan Co-op. Med. Sci.*, Cholera Panel, 1979, p. 237.
16. Rahal, K., Gerbaud, G. and Bouanchand, D. M., *Ann. Microbiol.* (*Inst. Pasteur*), 1978, **1294,** 409.
17. Guerry, P. and Colwell, R. R., *Infect. Immun.*, 1977, **16,** 328.
18. Hayashi, F., Harada, K., Mitsuhashi, S. and Inoue, M., *Microbiol. Immunol.*, 1982, **26,** 479.
19. Nakajima, T., Suzuki, M., Harada, K., Inoue, M. and Mitshuhashi, S., *Microbiol. Immunol.*, 1983, **27,** 195.
20. Yokoto, T., Kasuga, T., Kaneko, M. and Kuwahara, S., *J. Bacteriol.*, 1972, **109,** 440.
21. Yokoto, T. and Kuwahara, S., *Antimicrob. Agents Chemother.*, 1977, **11,** 13.

15 April 1986

MICROBIAL ENZYMES IN THE PROCESSING OF OILSEEDS

SATYAVEER BHATNAGAR and
B. N. JOHARI

Department of Microbiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar 263 145, India.

OILSEEDS and oil industry in India are of utmost importance because of their usefulness. Usually oil is extracted by the Soxhlet extraction procedure[1]. It has recently been reported that the recovery of oil from oilseeds can be increased by addition of microbial enzymes[2]. Enzymatic ability of thermophilic moulds is much superior by way of their greater thermostability and better production under nutritional conditions on a comparative basis with mesophiles[3]. Because of this higher ambient temperature (45–60°C), industrial processes are beset with less contamination and cooling costs.

The extraction procedures including Soxhlet do not allow complete recovery of oil from the seeds. The commercial recovery procedures also have similar difficulties. During the course of deterioration of oilseeds, viz. castor, sunflower, soybean and cotton by thermophilous moulds it was observed that the amount of soxhlet extractable oil increased in some cases. Since these moulds are known to secrete extracellular polysaccharases, proteases and lipases[3–5] the enzymic processing of oilseeds was studied. Available literature suggests that the mechanism of release of extra oil is based on the hydrolysis of proteins[2].

Isolates of *Aspergillus fumigatus*, *Humicola lanuginosa and Sporotrichum thermophile* were recovered from deteriorating oilseeds and maintained in YpSS medium[4]. For enzyme preparation[6], these were grown in YpSS broth in Erlenmeyer flasks for 8 days at 45°C. Culture filtrates were collected by

Table 1 *Yield of oil from oilseeds using microbial enzymes in the presence of hexane*

	% yield of oil from seeds*											
	Cotton			Sunflower			Soybean			Castor		
Enzyme conc. (per cent)	1	2	3	1	2	3	1	2	3	1	2	3
Enzyme source												
A. fumigatus	16.76	18 10	19.39	33.96	35.46	36 86	21.92	23.23	24.22	46 23	47 37	48.76
S. thermophile	16.96	18.31	19.16	34.00	35.21	36.22	21.78	22.92	24.11	46.21	47.72	48.93
H. lanuginosa-I	17.21	18.32	19.51	34.23	35.62	36.64	21.35	22.49	23.69	46.96	48.21	49.26
H. lanuginosa-II	16.31	17.52	18.96	34.31	35.32	36.54	21.23	22.52	23.50	47.10	48.32	49 06
Cellulase**	16.42	17.77	18.82	33.96	34.31	35 86	21.69	22.36	23.32	46.21	47.31	48.61
Hemicellulase**	16.10	17.72	18.77	33.49	34.52	35.75	21.47	22.35	23.12	46.36	47.52	48.42

*Yield of oil from seeds in control (%): cotton, 15.20; sunflower, 32.65; soybean, 20 33; castor, 45.50.
**Sigma Chemical Co., USA.

filtration through Whatman No. 1 paper and used as crude enzyme preparation. Cellulase and hemicellulase, purchased from Sigma Chemical Co., USA, were used in some experiments.

For oil extraction in the presence of crude/ purified enzymes, 1–3% levels were employed. Suitable aliquots of enzyme were added to the weighed and crushed oilseeds (10 g each) in 250 ml flasks. The reaction was carried out at 45 ± 2°C for 8 hr in the presence/absence of hexane. Oil was extracted in each case and yield calculated on per cent basis with comparison to controls that were run without addition of enzyme to the seeds.

From the data based on duplicate set of experiments it has been adduced that oil yield increased in most cases (table 1). The recovery of oil from cotton increased by 2–5% when an enzyme preparation from *H. lanuginosa* I was employed. Addition of crude enzyme from *A. fumigatus* improved oil recovery by 4·2% in sunflower; this preparation was also effective in better oil yield from soybean. Oil yield from castor was improved by an enzyme preparation from *H. lanuginosa* I. Commercially available preparations of cellulase and hemicellulase were slightly inferior to enzyme preparations from any of the four thermophilous moulds. Oil yield was greater in the presence of hexane than in samples without this solvent.

It would appear that solvent extraction of oil in the presence of enzyme(s) allows greater solubilization of plant tissue and proteins to which oil may remain bound even after the normal extraction procedures. Further studies on improved oil recovery and their fatty acid make up are currently underway. In view of the shortage of oilseeds, any improved technology with greater oil yield will be a welcome sign. This is a novel field of microbial biotechnological research which holds potential for commercial exploitation.

Financial support by ICAR, New Delhi is gratefully acknowledged.

1 January 1987; Revised 16 March 1987

1. Vogel, A. I., *Elementary practical organic chemistry*, Part I, Longmans, Green and Co. Ltd., London, 1966, p. 150.
2. Fullbrook, P. D., *J. Am. Oil. Chem. Soc.*, 1983, **60**, 476.
3. Johri, B. N., Jain, S. and Chouhan S., *Proc. Indian Acad. Sci. (Plant Sci.)*, 1985, **94**, 175.
4. Satyanarayana, T. and Johri, B. N., *Trop. Plant. Sci. Res.*, 1983, **1**, 137.
5. Macrae, A. R. and Hammond, R. C., *Biotech. Genetic. Eng. Rev.*, 1985, **3**, 193.
6. Satyanarayana, T. and Johri, B. N., *Curr. Sci.*, 1981, **50**, 680.

NEW APPROACHES TO REDUCE THE COST OF HYBRID SEED PRODUCTION: I USE OF HORMONES

N. L. BHALE, M. R. K. RAO and
R. K. DESHMUKH
Central Institute for Cotton Research, Nagpur 440 001, India.

HYBRID cotton has revolutionized cotton cultivation in our country and is a crucial factor in the