exhibited higher antioxidant activity than either the component erythrocytes or plasma.

It is quite interesting that RBC stroma increased autoxidation rate significantly.

These results confirm the earlier reports[6] which showed that soluble fractions of mammalian tissue and serum show significant inhibitory activity towards autoxidation of L-ascorbic acid. Human serum has been shown to contain powerful antioxidant activity towards unsaturated lipids[1,2], catecholamines[3] and bilirubin[5] and the protective component of the human serum against lipid autoxidation has been identified[10] as ceruloplasmin. However, our unpublished observations indicate that both Cu^{++} ions and ceruloplasmin enhanced the ascorbic acid autoxidation. The nature of the protective factor, in blood, is therefore still not clear and work is in progress to identify this factor.

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1. Barber, A. A., *Arch. Biochem. Biophys.*, 1961, **92**, 38.
2. Kartha, V. N. R., Vijayan, E. and Krishnamurthy, S., *Indian. J. Biochem. Biophys.*, 1978, **15**, 111.
3. Bloch, W., *Helv. Physiol. Pharmacol. Acta.*, 1948, **6**, 122.
4. Monder, C., Williams, J. N., Jr. and Waisman, H. A., *Arch. Biochem. Biophys.*, 1958, **75**, 46.
5. Barac, G. and Roseman, R. J., *Wash. Acad. Sci.*, 1946, **36**, 296.
6. Mishra, C. P. and Kovachich, *Neurosci. Lett.*, 1983, **43**, 103.
7. Dodge, J. T., Mitchell, C. and Hanahan, D. J., *Arch. Biochem. Biophys.*, 1963, **100**, 119.
8. Roe, J. H. and Kuether, C. A., *J. Biol. Chem.*, 1943, **147**, 399.
9. Reinhold, J. G., In: *Standard methods of clinical chemistry* (ed.) M. Reiner, Academic Press, New York and London 1983, Vol. 1, p. 88.
10. Al-Timini, and Dormandy, T. L., *Biochem. J.*, 1977, **168**, 233.

A SCREENING MEDIUM FOR L-ASPARAGINASE PRODUCTION BY *ERWINIA CAROTOVORA* AND *PSEUDOMONAS PUTIDA*

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BROOM[1] presented evidence to show that the enzyme L-asparaginase present in guinea pig serum was responsible for the antitumor properties which could suppress some transplanted lymphomas of mice and rats. Subsequent findings[2] indicated that the antitumor activity of L-asparaginase obtained from *E. coli* was quite similar to guinea pig serum. This opened up the possibility of large scale microbial production of the enzyme for clinical trials. An attempt was made to develop a reasonably simple medium with minimal amounts of yeast extract and tryptone (the basis being glucose and asparagine) which proved very useful for screening the two genera *Pseudomonas* and *Erwinia*. The latter genus was chosen since it produced tumor active enzyme and the former as a control.

Cultures: Fiftytwo strains of *E. carotovora* and 46 strains of *P. putida* were used in this study. The former were isolated from plants and the latter from soil. All these strains were tested for L-asparaginase activity.

Medium and cultivation conditions: The medium developed was a simple salt solution that contained (g/l); $HO_2CCH_2C(OH)(CO_2H)\ CH_2CO_2Na$, 0.5; $MgSO_4.7H_2O$, 1.0; NaCl, 1.0; glucose, 5.0; K_2HPO_4, 0.7; KH_2PO_4, 1.05; NH_4NO_3, 0.5; yeast extract, 0.5; tryptone 0.5; L-asparagine 5.0; The final pH was adjusted to 5.53–5.56. The medium (80 ml) was distributed in 500 ml flasks and sterilized at 115°C for 20 min. The flasks were inoculated with Tryptone glucose yeast extract agar (TGY agar) cultures of the strains grown for 18–20 hr at 28°C and incubated at 37°C with shaking (220–230 rpm) for 16–46 hr. The enzyme activity was determined after harvesting the flasks according to the method of Wriston[3]. Results of activity were calculated to International Units (I.u.).

Results and discussion

Out of the 46 strains of *P. putida* tested, only one strain NBPP-18 showed activity for L-asparaginase upto 2.00 I.u/ml. The final pH was 8.8 after 42 hr of fermentation (table 1). The minimum and maximum

Table 1. *Enzyme activities of some of the promising strains of Pseudomonas putida and E. carotovora.*
(enzyme activity in I.u/ml)

Strain No.	Age (hr)	pH	*P. putida*	*E. carotovora*
NBPP-13	40	8.96	1.250	—
NBPP-16	42	8.76	1.336	—
NBPP-18	42	8.80	2.016	—
NBPP-20	42	8.81	1.393	—
NBPP-31	16	7.61	—	1.350
NBEC-1	16	8.19	—	1.276
NBEC-20	16	8.10	—	1.466
NBEC-25	16	7.85	—	1.228
NBEC-24	16	7.97	—	1.283

pH during this period was 7.34 and 9.14 respectively.

In *E. carotovora*, one strain of NBEC-20 showed enzyme activity up to 1.47 I.u./ml. (table 1). Here also, the pH went up to the alkaline side, 8.1 in 16 hr, but the enzyme activity was lost on prolonged cultivation (beyond 24 hr). On the other hand, in *P. putida* there was no loss of enzyme activity up to a maximum period of 46 hr. In view of this the fermentation was terminated by 16–20 hr and harvested. The common observation in both organisms was that the pH profile showed a tendency to rise to the alkaline side. There was no correlation of extreme pH increase and enzyme production in both strains.

The advantage of the present medium for primary screening of the bacterial strains for L-asparaginase production over the TGY medium includes the utility of the former for diverse genera like *Pseudomonas* and *Erwinia*. Secondly, the present medium is composed of simple salts with low quantities of rich nutrients like yeast extract and tryptone unlike that of TGY medium where both ingredients are in much larger quantities. The latter properties could serve as a model if scale-up studies for large scale production of L-asparaginase are to be carried out.

A somewhat similar medium for L-asparaginase production by *P. boreopolis* was reported by Smirnova *et al*[4] where in the enzyme activity was 0.47 I.u./ml. But the present medium with *P. putida* gave much higher enzyme activities—0.6 to 2.0 I.u./ml, the carbon source in both cases was glucose.

By employing the rich medium of TGY, L-asparaginase activity with *E. carotovora* was earlier reported[5] to be 2 I.u. whereas with the present relatively simple medium, 1.47 I.u. of enzyme activity could be obtained with the same organism.

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1. Broom, J. D., *Nature* (*London*), 1961, **191,** 1114.
2. Mashburn, L. T. and Wriston, J. C., *Arch. Biochem. Biophys.*, 1963, **105,** 450.
3. Wriston, J. C., *Methods Enzymol.*, 1970, **17,** 732.
4. Smirnova, I. P., Zamin, V. A. and Berezov, T. T., *J. Appl. Biochem. Microbiol.*, 1976, **12,** 8.
5. Nathan, G. E. and Goory, R., *Ferment. Technol. Today*, 333–338 and *Proc. IVth International Fermentation Symposium*, 1972.

MORPHOLOGICAL VARIATIONS IN *EPIDERMOPHYTON FLOCCOSUM*

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PLEOMORPHISM, described as the variation in phenotypic expression of micro-organisms, is a phenomenon encountered commonly in dermatophytes[1, 2]. This has been attributed to the difference in the culture media or fungal strains themselves[2]. Although several reports are available on the pleomorphism in fungi, it has not been adequately explained. In our work on dermatophytes, on strain of *Epidermophyton floccosum* (BMS 14) showing considerable variations morphologically