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Production of Methionine and Glutamic Acid from *n*-Alkanes by *Serratia marcescens* var. *kiliensis*

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ABSTRACT. A hydrocarbon-utilizing *Serratia marcescens* var. *kiliensis* grew and accumulated methionine and glutamic acid in a synthetic medium with hydrocarbon as sole carbon source. *n*-Hexadecane and ammonium phosphate were found as the most suitable carbon and nitrogen sources, respectively. Optimum pH for growth and methionine production was 7.2, and that for glutamic acid accumulation was 7.4. Yeast extract significantly stimulated growth and amino acid production and could be substituted by cyanocobalamine. Benzylpenicillin, Tween 80, SDS or EDTA did not increase amino acid production. Under optimal cultural conditions in the laboratory the organism produced 1.68 g of glutamic acid and 0.78 g of methionine per litre.

Although microbial production of various amino acids is well known, there is an overall dearth of reports concerning extracellular accumulation of methionine by microorganisms (Dulaney 1967). Methionine has been reported to be produced rarely in appreciable quantities from *n*-alkanes by microorganisms (Kvasnykov *et al.* 1969; Abbott and Gledhill 1971). Production of methionine by a mutant of *Micrococcus glutamicus* has been reported by Banik and Majumdar (1974, 1975).

In the course of a survey on the production of amino acids by microorganisms (Ghosh and Banerjee 1982*a*) using hydrocarbon substrates a red-coloured gram-negative small rod obtained from a soil sample of Burdwan was found to produce glutamic acid and methionine. The organism was provisionally identified as *Serratia marcescens* subsp. *kiliensis* (Ghosh and Banerjee 1982*b*). The present paper deals with the effect of various cultural conditions on production of methionine and glutamic acid using hydrocarbons as sole carbon source.

MATERIALS AND METHODS

Media and substrates. The culture of the organism was maintained on agar slants containing (g/L): agar 20, polypeptone 10, yeast extract 5, glucose 5 (pH 7.0). The fermentation was composed of (g/L): $(NH_4)_2HPO_4$ 2, KH_2PO_4

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2, Na_2HPO_4 2, $MgSO_4.7H_2O$ 0.1, $MnSO_4.4H_2O$ 0.01, $ZnSO_4.7H_2O$ 0.01, hydrocarbon 20; pH 6.8—7.0. Hydrocarbons used as carbon sources were *n*-hexane, *n*-heptane, *n*-tetradecane, *n*-hexadecane, kerosene, diesel oil and straight-run gas oil. The latter contained 45 % aromatic hydrocarbons and 55 % paraffins (*V*/*V*). Vitamins, yeast extract and hydrocarbons were filter-sterilized using a Jena G 5 sintered glass filter. Yeast extract was added at

TABLE I. Effect of hydrocarbon source on growth and amino acid production

Hydrocarbon 2 %, *V*/*V*	Growth g/L	Extracellular amino acids, mg/L	
		glutamic acid	methionine
n-Hexane	0	0	0
n-Heptane	0	0	0
n-Tetradecane	1.22	320	220
n-Hexadecane	1.57	420	350
Kerosene	0.88	240	200
Diesel	1.23	360	250
Straight-run gas oil	0.44	0	0

0.05, 0.1, 0.5 and 1 g/L concentration. Tween 80, ethylenediaminetetraacetic acid (disodium salt) were added to the medium prior to sterilization. The media were sterilized under 103 kPa pressure.

Cultivation. A loopful of cells was transferred from an agar slant to 25 mL of liquid medium in a 250-mL Erlenmeyer flask and incubated at 30 ± 2 °C on a rotary shaker (2 Hz) for 1 d. The cells were washed with sterile distilled water and suspended in 25 mL of water. An aliquot of 2.5 mL of the suspen-

TABLE II. Effect of nitrogen source on growth and amino acid production

Nitrogen source (424 mg N per L)	Growth g/L	Extracellular amino acids, mg/L	
		glutamic acid	methionine
$(NH_4)_2SO_4$	1.57	580	340
NH_4Cl	0.64	320	250
NH_4NO_3	1.71	270	280
$(NH_4)_2HPO_4$	2.25	750	420
KNO_3	1.76	260	280
$CO(NH_2)_2$	1.18	250	100

sion was inoculated into 25 mL of fermentation medium in a 250-mL Erlenmeyer flask and incubated on a shaker as before. Triplicate flasks were removed at desired intervals for estimation of growth and extracellular amino acids.

Estimation of amino acids and growth. After 3 d of growth methionine and glutamic acid were estimated in the culture filtrate by paper chromatography on Whatman no. 1 paper sheets by the descending method using phenol—water (5 : 1) in the presence of ammonia and sodium cyanide in the chamber.

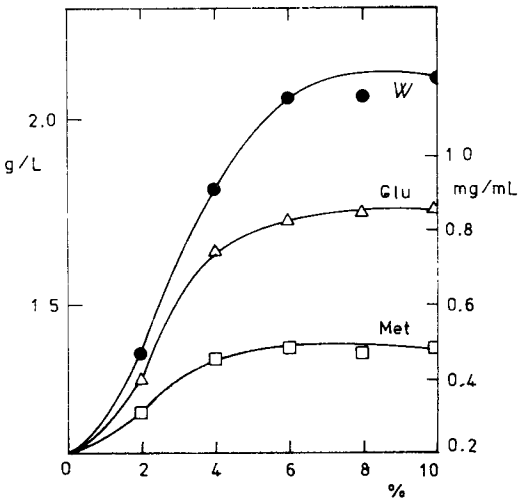

FIG. 1. Effect of the concentration of *n*-hexadecane (%) on growth (*W*, g/L) and amino acid yield (mg/mL) of *S. marcescens*.

The chromatograms were dried and sprayed with 0.3 % ninhydrin in 95 % ethanol. The spots were cut out and eluted with 70 % ethanol, filtered and the colour intensity was measured in EEL photoelectric colorimeter (*Evans Electroselenium Ltd.*, England) using a green filter. The amino acid concentration was determined from standard curves prepared with authentic samples. Growth was measured by the method of Ghosh and Banerjee (1981). Amino acids were identified according to Ghosh and Banerjee (1983).

RESULTS AND DISCUSSION

Effect of carbon source

Lower hydrocarbons fail to support growth of the organism (Table I); other hydrocarbons are utilized well as growth substrates. In general, there is a positive correlation between growth and amino acid yield. Straight-run gas oil supports growth weakly, but not amino acid accumulation. *n*-Hexa-

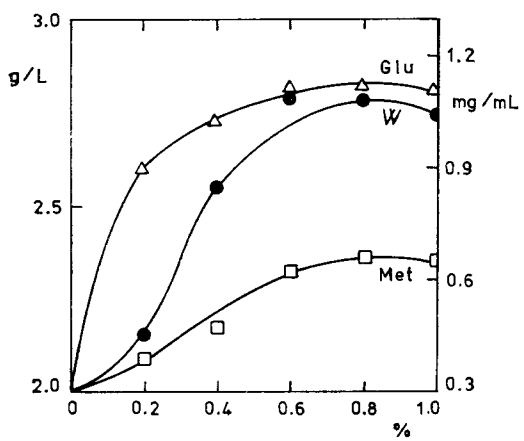

FIG. 2. Effect of the concentration of diammonium hydrogen phosphate (%) on growth (*W*, g/L) and amino acid yield (mg/mL) of *S. marcescens*.

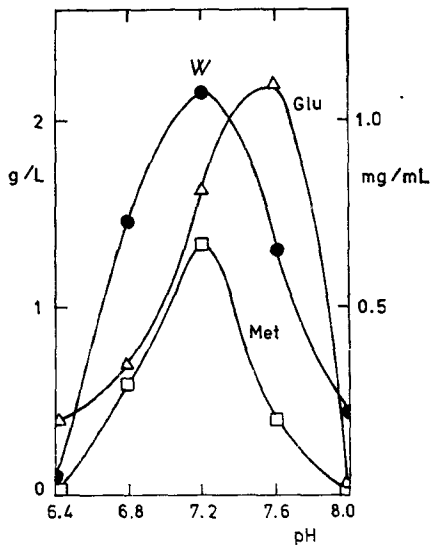

FIG. 3. Effect of pH on growth (*W*, g/L) and amino acid yield (mg/mL) of *S. marcescens*.

decane was found to be the best carbon source. When different levels of this hydrocarbon were added, it was observed that a concentration of about 6 % was optimal. At this concentration the production of glutamic acid increased by 100 % and methionine by 50 % over those at 2 % level (Fig. 1).

Lower hydrocarbons are toxic for most microorganisms probably due to their greater solubility in water, thereby exerting an adverse effect on the plasma membrane (Klug and Markoverz 1971). On the other hand, *n*-alkanes with chain lengths of C_{11} and C_{19} are generally suitable substrates for microbial growth (Wilkinson 1971; Walker 1973) and amino acid production (Abbott and Gledhill 1971). Straight-run gas oil which contains 45 % aro-

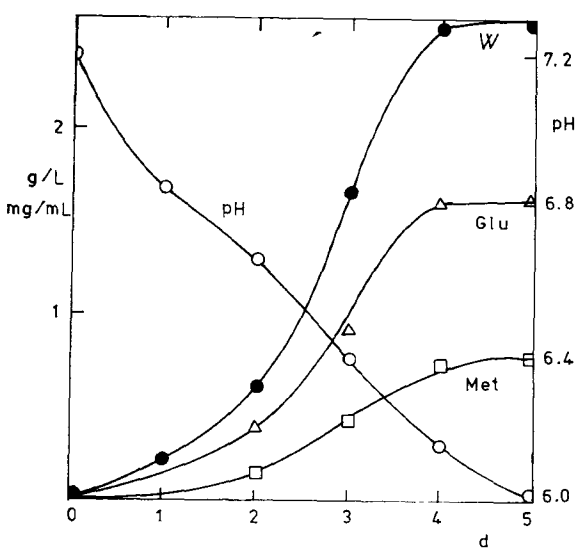

FIG. 4. Time course of the fermentation of *S. marcescens; W* — growth (g/L), Glu — glutamic acid (mg/mL), Met — methionine (mg/mL).

matics was found to be unsuitable as substrate, although Chatterjee (1980) reported it to be a satisfactory substrate for *Micrococcus luteus* and *M. varians*.

Effect of nitrogen source

Among the different nitrogen sources added to give 424 mg nitrogen per L in a N-free basal medium with 6 % *n*-hexadecane as carbon source, diammonium hydrogen phosphate was found to be most suitable for amino acid production (Table II). It produced the highest yield of amino acids at 0.6 to 0.8 % concentration (Fig. 2).

TABLE III. Effect of yeast extract supplementation on growth and amino acid accumulation

Yeast extract g/L	Growth g/L	Extracellular amino acid, mg/L	
		glutamic acid	methionine
0.0	1.91	950	560
0.05	2.30	1140	660
0.10	2.94	1450	750
0.50	3.00	1680	780
1.00	3.00	1610	780

Effect of yeast extract and vitamins

Yeast extract stimulated both growth and amino acid accumulation significantly (Table III). At the optimum concentration (0.01—0.05 %) it stimulated growth by about 58 %, glutamic acid production by about 77 % and methionine production by 40 %.

In an attempt to replace yeast extract by vitamins of the B-group it was observed that thiamin hydrochloride and cyanocobalamin exerted a definite stimulatory effect on growth and amino acid accumulation (Table IV). From the results it appears that the organism is somewhat deficient in the biosynthesis of these two vitamins in a hydrocarbon medium although a slight stimulatory effect of cyanocobalamin in two species of *Bacillus* producing glutamic acid and valine was noticed by Chattopadhyay and Banerjee (1978*a*,*b*), no pronounced effect of vitamin B_{12} on amino acid production has been reported so far.

Effect of some substances affecting permeability of cells

Among the agents used, penicillin was highly inhibitory even at 1 IU/mL, Tween 80 was also inhibitory although at much higher concentrations than penicillin. EDTA at 10 ppm concentration was slightly stimulatory, but inhibitory at higher levels (Table V).

Effect of pH

In a buffered medium (65 mM phosphate buffer) the optimum pH for growth was found to be between 7.0 and 7.2, that of glutamic acid production was 7.4 and of methionine production 7.0 to 7.2 (Fig. 3).

TABLE IV. Effect of B-vitamins on growth and amino acid production

Vitamin	Concentration	Growth g/L	Extracellular amino acids, mg/L	
			glutamic acid	methionine
None	—	1.18	240	180
Thiamin hydrochloride	10	1.67	280	300
	50	1.76	360	300
Pyridoxine hydrochloride	10	1.47	250	100
	100	1.32	230	100
4-Aminobenzoic acid	10	1.13	290	200
	100	0.93	180	220
Calcium panthotenate	10	1.76	200	0
	100	1.71	170	0
Nicotinic acid	10	0.98	170	0
	100	1.02	180	0
Riboflavin	10	0.93	240	100
	100	0.98	240	100
Cyanocobalamin	10	1.81	360	210
	100	1.96	520	240
Biotin	0.1	1.07	210	0
	1.0	0.98	200	0

TABLE V. Effect of penicillin, Tween 80 and EDTA on growth and amino acid production

Agent	Concentration	Growth g/L	Extracellular amino acids, mg/L	
			glutamic acid	methionine
None	—	1.96	1480	580
Penicillin (IU/mL)	1	0	0	0
Tween 80 (ppm, *V/V*)	100	1.51	1250	300
	500	0.59	740	0
	1000	0.39	100	0
EDTA (ppm, *W/V*)	10	2.20	1610	580
	100	1.96	1340	350
	1000	1.76	960	300

Time course of fermentation

The maxima of growth and amino acid production were reached after 4 d when the cultures were continuously shaken (Fig. 4). The bulk of amino acids was accumulated during the active growth phase and ceased with the onset of the stationary phase. During growth the pH value of the medium fell from 7.2 to 6.0.

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