Production of Glutamic Acid by *Arthrobacter globiformis*: Influence of Cultural Conditions

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ABSTRACT. *Arthrobacter globiformis* isolated from Burdwan soil excretes glutamic acid in a glucose mineral salt medium with suboptimal level of biotin. Glutamate begins to accumulate in the medium from the mid-exponential phase of growth and its excretion could be prolonged by adjustment of pH to the neutral range. Among the different carbon and nitrogen sources tested glucose (8 %) and ammonium nitrate (0.53 %), respectively, were found to be most suitable. Molasses could not be used as a substitute for glucose even if antibiotics or Tween 80 are incorporated in the medium. Bacitracin (1 μg/mL) stimulated glutamate excretion. A temperature of 28 °C and an inoculum dose of 4 % were optimal for production. Under optimal conditions, in the flask culture the isolate excreted 16.1 g glutamic acid per litre in 120 h. Glutamic acid isolated from the fermented broth was found to be pure L-diastereomer.

Since the isolation of *Micrococcus glutamicus* from nature (Kinoshita et al. 1957) numerous surveys of microorganisms capable of producing extracellular amino acids different geographical areas were made and rewarded by the isolation of an array of microorganisms producing glutamic acid and other amino acids. The ability to excrete glutamate, though not restricted to any particular group of microorganisms (Kinoshita et al. 1969), is generally displayed by the following genera of bacteria: *Micrococcus*, *Brevibacterium*, *Corynebacterium*, *Arthrobacter* and *Microbacterium* (Kinoshita and Tanaka 1972). A number of species of the genus *Arthrobacter*, viz. *A. globiformis* (Veldkamp 1963; Kosinikiesz 1973), *A. aminofaciens* (Mogi et al. 1967), *A. citreus*, *A. paraffineus* (Tanaka et al. 1960) and *A. mysorens* (Nanda et al. 1971), have been reported to produce glutamic acid.

The present paper reports the production of glutamate under different cultural conditions by *A. globiformis* isolated in the course of a brief survey.

MATERIALS AND METHODS

Organism, medium and cultivation. The glutamate-producing *A. globiformis* strain 3514 was isolated (Roy and Chatterjee 1982) from Burdwan soil following

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bioautographic technique (Udaka 1960). Stock culture of the organism was maintained on agar slants supplement with biotin (0.1 μg/L). The basal medium (Robinson 1964) for fermentation experiments contained (g/L): glucose 10, KH₂PO₄ 3, Na₂HPO₄ 6, NH₄Cl 2, MgSO₄·7H₂O 0.2, NaCl 5 and 1 mg each of CaCl₂·2H₂O, FeSO₄·7H₂O, MnCl₂, (NH₄)₂MoO₄. Stock solutions of vitamins were prepared and sterilized separately through a bacterial filter. Stock solutions of different carbon sources, antibiotics etc. were made separately and pH was adjusted to 7.0. Fermentation experiments were carried out in 250-mL Erlenmeyer flasks taking 50 mL basal medium supplemented with biotin (0.1 μg/L). Aqueous suspension of bacteria (cell concentration 50/mL) made from a 24-h-old slant, was used as inoculum. After inoculation the flasks in triplicate were incubated at 30 ± 1 °C on a rotary shaker (amplitude 30 mm, frequency 2 Hz), with 2 % (V/V) inoculum. Bacterial growth was measured turbidimetrically in an EEL photoelectric colorimeter at 540 nm.

Analytical methods. The cell-free culture filtrate was analyzed for glutamic acid residual sugar and pH. Glucose was estimated by the 3,5-dinitrosalicylic acid method (Bernfeld 1955). Glutamic acid was estimated by a microbiological assay using glutamate auxotroph of *E. coli* K12 coupled with quantitative paper chromatography. For quantitative determination 10 μL of desalted culture filtrate was spotted in triplicate on a Whatman No. 1 chromatographic paper. The paper also contained a spot of authentic sample of L-glutamic acid. It was run along the fibre direction with 1-butanol-acetic acid-water (4 : 1 : 5, upper layer) and air-dried. The spots were developed with 0.1% ethanolic ninhydrin. The spots were cut out, eluted overnight in the dark with 5 mL of 70 % ethanol and absorbance of the colour was determined at 540 nm. The amount of glutamate was extrapolated from a calibration curve prepared similarly with different amounts of authentic L-glutamate.

RESULTS

Selection of a suitable medium. The organism was grown in five different synthetic media (Davis and Mingioli 1950; Makula and Finnerty 1968; Robinson 1964; Tanaka et al. 1967; Tokoro et al. 1970). The isolate 3514 was capable of growth and glutamate production in all media (Table I). Robinson's medium (1964) supported good growth and the amount of glutamate excreted was higher than in other media. This medium was, therefore, selected for further fermentation experiments.

Changes in culture medium during fermentation. These changes were studied at 6-h intervals. Growth was rapid and entered into stationary phase in about 30–36 h of incubation (Fig. 1). During this period about 60 % of glucose was consumed and the pH of the medium gradually shifted towards acid side. From 18 h on, glutamate began to be excreted. Glucose was continuously consumed even though there was no active growth. The amount of glutamate accumulated was highest at 42–48 h of fermentation.