EXTRACELLULAR PRODUCTION OF L-METHIONINE BY *BACILLUS MEGATERIUM* B71 ISOLATED FROM SOIL

SWAPAN K. ROY, A. K. MISHRA and GEETA NANDA

Department of Microbiology, Bose Institute, Calcutta 700 009, India.

THE discovery of microbial production of glutamic acid[1] accelerated the researchers to produce other amino acids by the use of microorganisms. This process potentially produces the biologically active L-form of the amino acids. Attempts have been made to get the L-methionine, an essential amino acid, by microbial means. Usually, it is difficult to obtain a wild strain capable of excreting significant amounts of L-methionine, which is biosynthesized *via* several alternate and highly branched pathways under strict feedback regulation[2–6]. Hence, a mutant with genetically altered regulatory mechanism could be useful for the production of this amino acid. Here, we report for the first time a soil isolated strain of *Bacillus megaterium* B71 capable of excreting L-methionine by a chemically defined fermentation medium[7].

The organism grew well in nutrient broth under shaking condition (120 rpm) at 30°C and attained mid-log phase of growth after 5 hr of incubation. It also multiplied without growth factors in a basal minimal medium[8]. The morphological, cultural and biochemical studies suggested the strain as *B. megaterium* according to Bergey's Manual of Determinative Bacteriology, 8th Edition. The strain was further confirmed from the Commonwealth Mycological Institute (Kew, Surrey, England). During screening, the serial number of the strain was B71. So, it was designated as *B. megaterium* B71 after its identification.

For fermentation studies, the bacterial suspension (1.2×10^8 cells/ml) was prepared in sterile saline using the mid-log phase culture. The inoculum (5.0%, V/V) was added to 20 ml of fermentation medium contained in 100 ml Erlenmeyer flasks, which were incubated at 30°C under shaking condition (120 rpm) for 120 hr. The fermented broth was collected at 24 hr interval, and L-methionine in cell-free broth was detected by paper chromatography using the solvent system: *n*-butanol-formic acid-methanol-water (4:1:2:0.5). The production of the amino acid was also confirmed[9,10] from the growth (figure 1) of methionine auxotroph of *E. coli* (obtained from Dr Barbara J. Bachmann, *E. coli* genetic stock center, Yale University, New Haven, USA). Both the chemical assay[11] and the rapid microbioassay[12] indicated that *B. megaterium* B71 accumulated maximum L-methionine (72 μg/ml) in the fermented broth after 96 hr. Attempts are being made to isolate an ethionine-resistant mutant of *B. megaterium* B71. Initially, we observed that the growth of the organism in (1:1) diluted basal minimal medium could be inhibited by 50 μg/ml DL-ethionine (Sigma Chemical Co., USA), and it was restored by 5 μg/ml L-methionine (figure 2). After isolation of ethionine-resistant mutant, it will be improved by

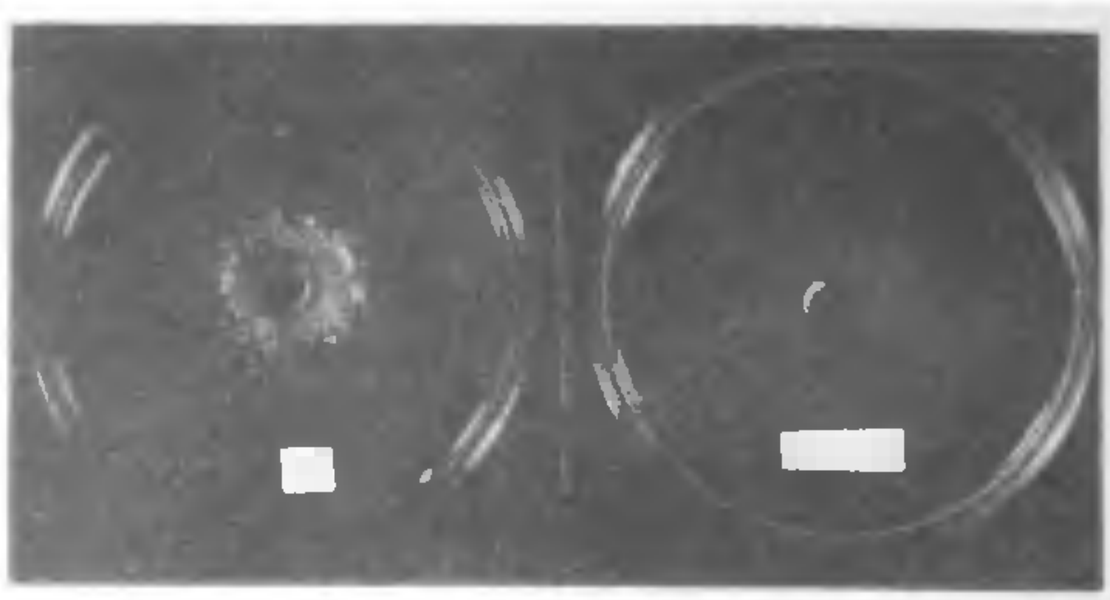

Figure 1. Growth of methionine auxotroph of *E. coli* in plate B71, but no growth of the same in control plate.

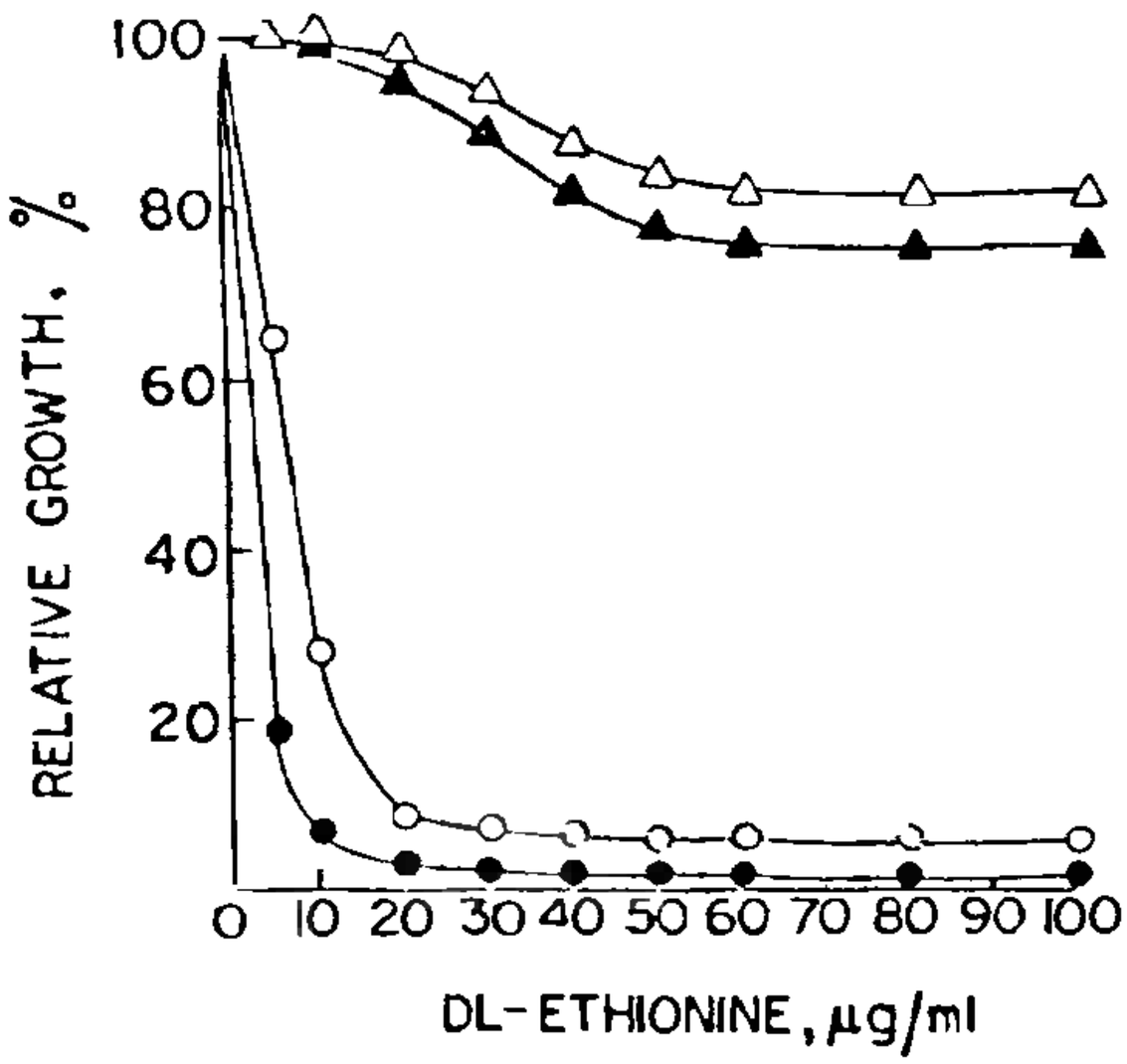

Figure 2. Effect of DL-ethionine on the growth of *B. megaterium* B71. Cultivation was carried out at 30°C for 24 hr (●, without L-methionine; ▲, with 5 μg/ml L-methionine), and for 48 hr (○, without L-methionine; △, with 5 μg/ml L-methionine).

other mutagens to increase the production of L-methionine.

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RAPID GERMINATION OF COTTON AND OKRA POLLEN ON AN ARTIFICIAL MEDIUM

P. A. PHILOMENA and B. V. DAVID
Fredrick Institute of Plant Protection and Toxicology, Padappai 601 301, India.

THE literature on the germination of pollen (in general) is extensive[1]. However, a review of the literature reveals that artificial germination of cotton pollen (*Gossypium hirsutum* L.) is extremely difficult[2]. Banerji[3] tried several techniques such as cold drawn castor oil, cane sugar solution, glucose, agar-agar-sucrose combinations etc. with little success; Iyengar[4] failed to observe *in vitro* pollen germination and hence resorted to *in vivo* method. This paper presents a simple and rapid method, which is a modification of Taylor's[2] and Barrow's[5] methods for *in vitro* germination of cotton pollen. The same medium was also found equally good for okra pollen germination (figure 1 A–D).

Pollen grains of *G. hirsutum* L. and *Abelmoschus esculentus* Moench. (L.) were seeded on two types of germination medium. The first one (solid medium) comprises of 3% bacto-agar, 0.04% each of boric acid and calcium nitrate, 0.07% of manganous sulphate and 25% sucrose. The second one (liquid medium) consists of all the other ingredients except bacto-agar. The agar based medium was sterilized in an autoclave at 1.5 kg/cm² pressure for 15 min and poured into

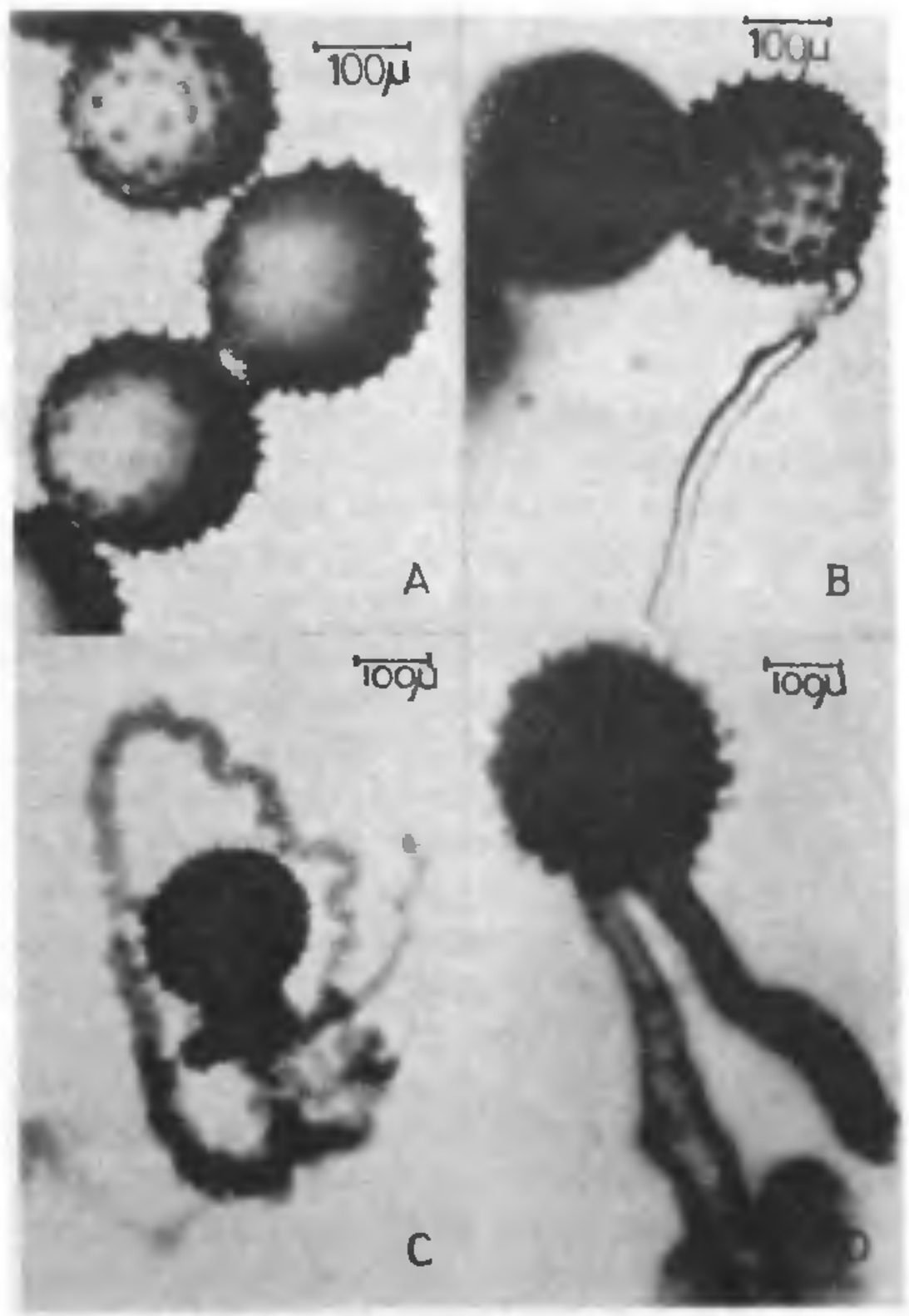

Figure 1. *In vitro* pollen germination in cotton and okra on an artificial culture medium. **A.** Ungerminated cotton pollen (×400). **B.** Germinated cotton pollen 2 min after contact with agar-based germination medium (×400). **C.** Germinated cotton pollen 15 min after contact with liquid culture medium (×320). **D.** Germinated okra pollen 10 min after contact with solid (agar-based) culture medium exhibiting twin pollen tubes (×320).