Porphobilinogen-accumulation by a porphyrin auxotrophic strain of *Bacillus subtilis*

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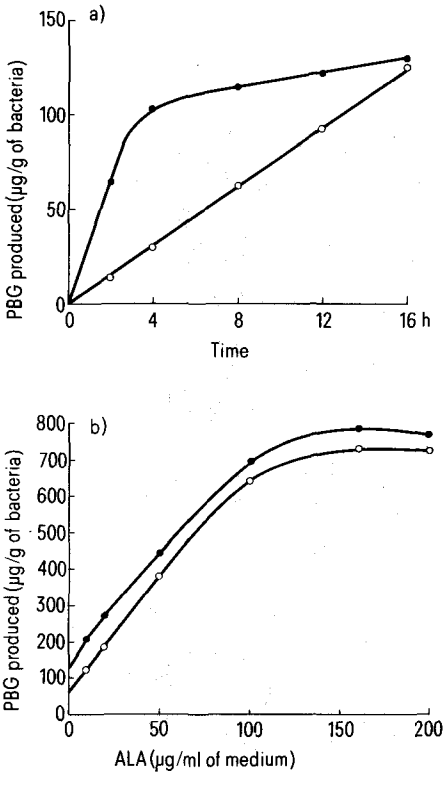
Summary. The amount of porphobilinogen accumulated by a *Bacillus subtilis hem*C mutant strain is dependent on time and exogenic aminolevulinic acid addition. *hem*C mutant seems to be suitable for porphobilinogen production on a large scale.

Several porphobilinogen (PBG)-accumulating mutants have been described in bacteria[2-5]. Berek et al.[4] briefly characterized the porphyrin auxotrophic strains of *Bacillus subtilis*. The porphyrin mutants of *B. subtilis* can utilise only the first [aminolevulinic acid (ALA)] and the last (haem) products from their environment. The *hem*C mutants are deficient in urophorphyrinogen I synthase (EC. 4.3.1.8) activity and accumulate PBG. The location of the *hem*C gene on the *B. subtilis* chromosome has been determined[6]. It is also known that the strains belonging to this mutant group are unable to synthetise vitamin B_{12}, because the branch in the porphyrin and corrinoid synthetic pathways occurs after the synthesis of PBG, and uroporphyrinogen I synthase is necessary for the synthesis of both[7].

Materials and methods. The PBG-accumulating mutant strain of *B. subtilis* strain 168 *trp*C, designated as strain II/33 (*hem*C), was used[4]. The chemically defined medium gGM[8] supplemented with tryptophan and cysteine (50 µg/ml), or HCA medium[9] (gGM supplemented with tryptophan, cysteine, 2.5 µg of haemin/ml, and 1-2 mg of bovine serum albumin/ml) were used. Bacteria were grown in HCA medium at 37 °C under continuous shaking. After reaching OD 0.5 at 620 nm the culture was centrifuged, washed in gGM medium and centrifuged again. The pellet was resuspended in the same volume of gGM (with tryptophan and cysteine) or HCA medium for growth experiments. PBG was assayed in the supernatant as described by Mauzerall and Granick[10].



Porphobilinogen production by a *hem*C mutant of *B. subtilis* growing in HCA (○) or gGM (●) medium for different times (a), or in the presence of different amounts of ALA (b) (cultivation time was 8 h). Yields of PBG were expressed in µg/g (wet weight) of bacteria.

Results and discussion. Figure a shows the amounts of PBG accumulated in the supernatants of the media in which the bacteria were grown for different times. When HCA medium was used, at the beginning there was very little PBG production. We assume this was caused by feed-back inhibition of haemin in the medium. Later, this inhibitory effect gradually decreased in proportion to the decrease of haemin concentration. Bacteria growing in gGM medium for 4 h accumulated a larger amount of PBG than in HCA medium after 12 h, although these bacteria grew very slowly and for a very limited time in gGM medium because it did not meet the growth requirements of *hem* mutants. In the other experiment the cultivation time was 8 h uniformly, and different amounts of ALA were added to the cultures (figure b).

There was a very small difference between the quantities of PBG accumulated in the 2 different media in the presence of a given amount of ALA. Approximately the same difference was observed without ALA addition (see figure a at 8 h). The addition of ALA to the medium did not enhance the accumulation of PBG in the case of the parent strain. Sasarman et al.[5] showed that the addition of ALA had no effect on PBG-accumulating *Salmonella typhimurium* strains. Mutants of *Rhodopseudomonas spheroides* behaved like our mutants in this respect[2]. *hem*C mutants of *B. subtilis* seem to be suitable for PBG production on a large scale, but preliminary experiments showed a lower yield than in the case of *Propionibacterium shermanii*[11]. We intend to study the regulation of porphyrin biosynthesis using this mutant.

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2 T. Hatch and J. Lascelles, Archs Biochem. Biophys. *150*, 147 (1972).
3 K. Powell, R. Cox, M. McConville and H.P. Charles, Enzyme *16*, 65 (1973).
4 I. Berek, A. Miczák, I. Kiss, G. Ivánovics and I. Durkó, Acta microbiol. hung. *22*, 157 (1975).
5 A. Sasarman, M. Desrochers, S. Sonea, K.E. Sanderson and M. Surdeanu, J. gen. Microbiol. *94*, 359 (1976).
6 I. Berek, A. Miczák, and G. Ivánovics, Molec. gen. Genet. *132*, 233 (1974).
7 A. Miczák, J. Bacter. *131*, 379 (1977).
8 T. Anderson and G. Ivánovics, J. gen. Microbiol. *49*, 31 (1967).
9 E. Marjai, I. Kiss and G. Ivánovics, Acta microbiol. hung. *17*, 133 (1970).
10 D. Mauzerall and S. Granick, J. biol. Chem. *219*, 435 (1956).
11 G. Müller, Z. Naturf. B. *27*, 473 (1972).