Polish Journal of Microbiology
2008, Vol. 57, No 4, 333–335

SHORT COMMUNICATION

Selective Isolation of *Bacillus thuringiensis* from Soil by Use of L-Serine as Minimal Medium Supplement

SYLWIA ANDRZEJCZAK* and ELŻBIETA LONC

Department of Microorganisms Ecology and Environmental Protection, Institute of Genetics and Microbiology, University of Wrocław, Wrocław, Poland

Received 20 June 2008, revised 4 August 2008, accepted 20 August 2008

A b s t r a c t

The influence of L-serine on the growth of different strains of the genus *Bacillus* was investigated. It has been observed that the addition of L-serine to minimal synthetic media results in an inhibition in the growth of certain strains of *Bacillus* spp. but not *B. thuringiensis.* Then L-serine-resistance phenomenon was used in isolation of *B. thuringiensis* strains from soil. An isolation method with media supplied with L-serine was compared to the previously applied procedure (isolation on nutrient agar). L-serine-selective medium appeared to be more effective in isolation of Bt strains.

K e y w o r d: *Bacillus thuringiensis*, L-serine resistance, *B. thuringiensis* selective isolation

Some amino acids *i.e.* valine (Leavitt and Umbarger, 1962), tyrosine (Beerstecher and Shive, 1947) or leucine (Washburn and Niven, 1948), preclude the growth of bacterial cells. L-serine has long been known to cause growth inhibition of *Escherichia coli* (Amos and Cohen, 1954), *Bacillus anthracis* (Gladstone, 1939), *Bacillus subtilis*, *Bacills megaterium*, *Bacillus pantothenticus*, *Bacillus mycoides* (Lachowicz *et al.*, 1996; Saito *et al.*, 2001) cultured in minimal medium. However data concerning one species – *Bacillus thuringiensis* are contradictory. In opposite to Lachowicz *et al.* (1996), where three tested *B. thuringiensis* strains were completely resistant to the inhibitory action of L-serine, Singer and Rogoff (1986) provided evidence that this amino acid inhibits *B. thuringiensis* growth. The aim of this study was to confirm L-serine-resistance phenomenon and utilization of L-serine in selective isolation of *B. thuringiensis* from environment.

Twenty-eight reference strains as well as environmental isolates of *Bacillus* spp. (Table I) were tested in this study. To examine the influence of L-serine on bacterial growth, the strains were cultured on synthetic medium (M9) composed of (g/l): $Na_2HPO_4 \times 7H_2O$ 6; KH_2PO_4 3; NaCl 0.5; NH_4Cl 0.5; $MgSO_4 \times 7H_2O$ 0.024; $CaCl_2$ 0.0001; glucose 10; Bacto-agar 2 and supplemented with L-serine at the concentration of 0.1 or 0.2 mM. The minimal medium of the same composition but without L-serine was used as a control.

M9 medium supplemented with L-serine was also used for isolation of *B. thuringiensis* strains from soil samples collected from woodland area near Zielona Góra (Poland). About 1 g (dry weight) of environmental sample was placed in test tubes containing 10 ml of 0.9% NaCl solution. The tubes were heat-shocked at 80°C for 15 min in a water bath. Next, 1 ml of tube content was poured into 50 ml of nutrient broth. The flasks were incubated at 37°C until germination was complete. The cells were sedimented by centrifugation and resuspended in 5 ml of 0.9% NaCl solution. The suspensions were serially diluted and spread on M9 medium and M9 medium supplemented with serine (0.2 mM). Plates were incubated at 37°C for 48 h. The same samples were simultaneously analysed using previously described nutrient agar method of Doroszkiewicz and Lonc (1999). The heated suspensions of soil were diluted and then plated on nutrient agar. Counts and *B. thuringiensis* colonies identification were made after incubation for 24 h at 37°C. All isolates with typical *B. thuringiensis* colony morphology (*i.e.* flat, cream, rough surface, irregular edges)

* Corresponding author: S. Andrzejczak, Department of Microorganisms Ecology and Environmental Protection, Institute of Genetics and Microbiology, University of Wrocław, Przybyszewskiego 63, 51-148 Wrocław, Poland; phone: (48) 71 3756366; **e-mail: sylwia@microb.uni.wroc.pl**

Table I
List of tested *Bacillus* strains

Strain	Origin
B. circulans PCM 2229 *B. firmus* PCM 1844 *B. sphaericus* PCM 485 *B. pumilus* 1852 PCM *B. megaterium* PCM 1855	Polish Collection of Microorganisms, Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences, Wrocław
B. megaterium MEG 001 *B. thuringiensis finitimus* KsAc1 *B. thuringiensis finitimus* KsS1 *B. thuringiensis finitimus* OpF3 *B. thuringiensis finitimus* OpS1 *B. thuringiensis japonensis* KpC1 *B. thuringiensis japonensis* KpF3 *B. thuringiensis japonensis* KsF1 *B. thuringiensis japonensis* OpAc1 *B. thuringiensis japonensis* OpPa1 *B. thuringiensis japonensis* OpPs1 *B. thuringiensis kurstaki* PO14 *B. thuringiensis tochigiensis* OpQ1	Collection of Institute of Genetics and Microbiology, University of Wrocław, Wrocław
B. thuringiensis aizawai BTNT0423 *B. thuringiensis kurstaki* CFTRI20 *B. thuringiensis thuringiensis* CFTRI18 *B. thuringiensis finitimus* XBIT-1966 *B. thuringiensis kurstaki* IwonaI *B. thuringiensis fukuokaensis* LBIT-499 *B. thuringiensis sumiyoshiensis* KNG6 *B. thuringiensis morrisoni* UNI101 *B. thuringiensis israelensis* LBE155 *B. thuringiensis alesti* LNG4-03	Kindly supplied by Dr. J.F. Charles, Pasteur Institute, Paris.

were determined to have the *B. thuringiensis* biochemical profile (Sneath, 1986) and were examined for the presence of parasporal bodies by phase-contrast microscopy.

All 28 tested strains grew in presence of 0.1 mM L-serine. However *B. circulans* PCM 2229, *B. firmus* PCM 1844, *B. sphaericus* PCM 485, *B. pumilus* 1852 PCM, *B. megaterium* PCM 1855, *B. megaterium* MEG 001 failed to grow in the presence of higher (0.2 mM) concentrations of L-serine. All *B. thuringiensis* strains appeared totally resistant to both L-serine concentrations applied.

Using different media to isolate *B. thuringiensis* strains, 524 colonies were obtained on M9 medium supplemented with L-serine and about 1000 on nutrient agar (approximate number because of specific *B. mycoides* growth). Basing on biochemical tests results and phase-contrast microscopy observations, 81 strains were identified as *B. thuringiensis*. Percentage of *B. thuringiensis* isolates on L-serine-medium was about 2.5 times higher than on nutrient agar (Fig. 1).

Amino acids are essential for bacterial life, although their lack as well as surplus makes the growth of some species impossible. Our results show that some strains of the genus *Bacillus* are sensitive to action of L-serine, while all *B. thuringiensis* subspecies are resistant to growth inhibition caused by this amino acid. Our results do not correspond with previously published data of Singer and Rogoff (1986). The different results are probably consequence of application in our tests of minimal medium with different composition.

In our tests conditions L-serine-resistance seems to be a general phenomenon for all *B. thuringiensis* sub-

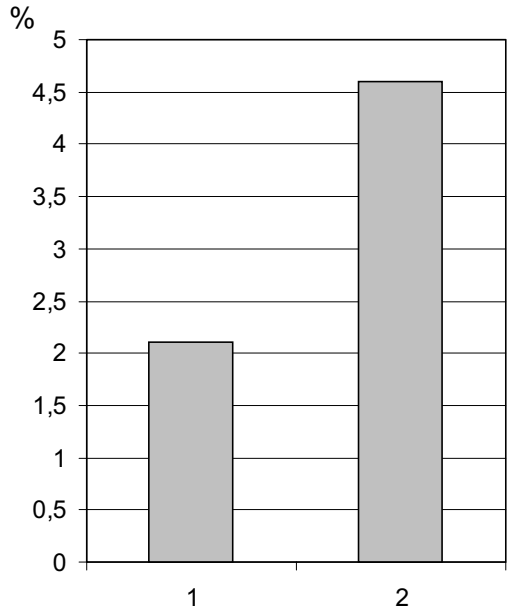

Fig. 1. Comparison of the rate of isolation of *B. thuringiensis* colonies using different media: 1 – nutrient agar, 2 – M9 medium supplemented with L-serine (0.2 mM)

species. However, L-serine-sensitive *Bacillus* strains are not able to grow in the presence of this amino acid, probably because of inhibition of homoserine dehydrogenase I (HDH I) activity, *i.e*. enzyme involved in L-threonine synthesis (S. Andrzejczak, unpublished data). On the other hand, it suggests that *B. thuringiensis* strains have L-serine-resistant HDH I and its activity is not inhibited even when serine concentration is relatively high. However the mechanism of this phenomenon still needs to be solved.

Application of L-serine as growth inhibitor increases effectiveness of *B. thuringiensis* strains isolation. It is clearly apparent that amino acid inhibits the growth of some species *i.e. B. mycoides*. Nevertheless, in natural environment L-serine-resistance seems to be a more frequent characteristic in genus *Bacillus* and other strains can be also isolated on minimal medium supplemented with serine.

Literature

Amos H. and G.N. **Cohen**. 1954. Amino acid utilization in bacterial growth. 2. A study of threonine-isoleucine relationships in mutants of *Escherichia coli*. *Biochem. J.* 57: 338–343.

Beerstecher Jr. E. and W. Shive. 1947. Prevention of phenylalanine synthesis by tyrosine. *J. Biol. Chem*. 167: 527–534.

Doroszkiewicz W. and E. **Lonc**. 1999. Biodiversity of *Bacillus thuringiensis* strains in the phylloplane and soil of Lower Silesia Region (Poland). *Acta Mirobiol. Pol.* 48: 355–361.

Gladstone G.P. 1939. Interrelationship between amino acids in the nutrition of *Bacillus anthracis. Br. J. Exp. Pathol.* 20: 189–200.

Lachowicz T.M., E. Morzejko, E. Panek and J. Piątkowski. 1996. Inhibitory action of serine on growth of bacteria of the genus *Bacillus* on mineral synthetic media. *Folia Microbiol.* 41: 21–25.

Leavitt R.I. and H.E. Umbarger. 1962. Isoleucine and valine metabolism in *Escherichia coli*. XI. Valine inhibition of the growth of *Escherichia coli* strain K-12. *J. Bacteriol*. 83: 624–630.

Saito K., K. Tanaka and K. Yamada. 2001. Growth inhibition by L-serine in *Bacillus subtilis* MF920. *Jpn. J. Food Chem.* 8: 117–120.

Singer S. and M.H. Rogoff. 1986. Inhibition of growth of *Bacillus thuringiensis* by amino acids in defined media. *J. Invertebr. Pathol.* 12: 98–104.

Sneath P.H.A. 1986. Endospore-forming Gram-positive rods and cocci, pp. 55–75. In: P.H.A. Sneath, N.S. Mair, M.E. Sharpe, and J.G. Holt (eds.), *Bergey's Manual of Systematic Bacteriology*, Williams & Wilkins, Baltimore.

Washburn M.R. and C. F. Niven. 1948. Amino acid interrelationships in the nutrition of *Streptococcus bovis. J Bacteriol*. 55: 769–76.