Note

Dihydrodipicolinate Synthase Deficiency of *Brevibacterium flavum* Strain BB69, a Threonine-producing Mutant with a Feedback-resistant Homoserine Dehydrogenase

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In the genus *Brevibacterium*, the following two types of threonine producers have been reported: mutants with HD^Rs^{1,2)} and those with a DPS⁻ or DPS^Ls^{3,4)} (Fig. 1). All of them were derived as threonine-producing mutants

resistant to AHV, a threonine analogue. The former are the conventional producers, while the latter are the novel producers which have been found by our recent studies^{3,4)} for the first time in the coryneform bacteria. So far, it has been shown that the threonine yields of simple HD^R or DPS^{-/L} type producers are almost the same, i.e., not better than around 10 g/l in the various kinds of production media containing 10% glucose.^{1,3)} However, the threonine productivity of a typical HD^R type threonine producer, *B. flavum* strain BB69, which was derived from a wild strain No. 2247 (ATCC14067) by two-step mutagenesis as a mutant with increased AHV resistance, was elevated to 13.5 g/l.¹⁾ Thus in this study, the strain BB69 was examined as to the mechanism of threonine production including its DPS activity.

Table I shows the activities of DPS and HD, and the threonine productivity of strain BB69. The DPS activity of strain BB69 was found to be reduced to about one-tenth that of the original strain No. 2247, and its HD was confirmed to be feedback-resistant. Thus, strain BB69 was found not to be a typical HD^R type producer, but had both an HD^R and a DPS^L. In general, the growth and threonine

Table I. PROPERTIES OF α -AMINO- β -HYDROXYVALERIC ACID-RESISTANT THREONINE-PRODUCING STRAINS, BB69 AND DK131, DERIVED FROM THE WILD STRAIN No. 2247 AND ASPARTATE-PRODUCING STRAIN No. 70, RESPECTIVELY

The threonine production of strain BB69 was examined by the same method as that used for strain DK131, i.e., method B³⁾ using Medium T2 (N30 M4) as the basal medium. The DPS activity was measured as described previously.³⁾ The HD activities of strains No. 2247 and BB69 were measured as described previously⁵⁾ with cells cultured by the same method as that used for the threonine production for strain BB69³⁾ except that the cultivation was done in Medium T2 (N20 M4) for 40 hr.

Properties	No. 2247	BB69	No. 70 ^d	DK131 ^e
L-Thr production ^a (Growth ^b) with DL-DAP at				
0 g/l	0.0 ^f (1.06) ^g	11.2 (1.09)	0.1 (0.84)	2.2 (0.72)
2		10.8 (1.16)		5.0 (0.95)
5		9.5 (1.12)	0.0 (0.87)	7.5 (1.10)
DPS activity ^c	57 ^h	6.2	87.1	0.0
HD				
Activity ^c	232	100	173	127
Inhibition (%) by L-Thr at				
0 mM	0	0	0	0
1	98		99	98
40		18		
80		44		

^a g/l.^b Absorbance at 562 nm of the culture broth after 26-fold dilution with 0.1 N HCl solution.^c nmol/min/mg protein.^{d,e,f,g,h} Data from refs. 4, 3, 1, 6, and 7, respectively.

Abbreviations: HD, homoserine dehydrogenase; HD^R, feedback-resistant HD; DPS, dihydrodipicolinate synthase; DPS⁻, DPS defect; DPS^L, low level DPS; DAP, diaminopimelic acid; AHV, α -amino- β -hydroxyvaleric acid.

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Fig. 1. Threonine and Lysine Biosyntheses and Their Regulation in *Brevibacterium flavum*.

PEP, phosphoenolpyruvate; ASA, aspartate- β -semialdehyde; DDP, dihydrodipicolinate; HSE, homoserine; Pyr, pyruvate; CIT, citrate; AK, aspartokinase; $\Rightarrow$, feedback inhibition.

productivity of DPS^- type threonine producers are enhanced by DAP, which is represented by a simple DPS^- mutant, strain DK131,³⁾ derived from strain No. 70, as shown in Table I. However, those of strain BB69 were not enhanced by DAP, and the productivity was rather decreased by the addition of 5 g/l of DL-DAP. They were considered to be attributed to its DPS^L character, i.e., strain

BB69 was not deficient in DAP, thus in the presence of the excess DAP, threonine and lysine that was formed from the DAP concertedly brought about the feedback inhibition of the aspartokinase. As reported in our preceding paper,⁴⁾ even among the simple DPS^- strains derived from strain No. 70, the growth enhancement by DAP was not remarkable in strains DK203 and DK330 compared with strain DK131. Thus, it is not irrational that the growth of strain BB69 with a DPS^L was not enhanced by DAP.

The above results suggest the usefulness of DPS mutations in combination with the HD^R character for the enhancement of threonine productivity, and also the possibility of obtaining excellent threonine producers having both HD^R s and a DPS^- or DPS^L s by deriving mutants resistant to higher concentrations of AHV.

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