EFFECT OF AMINO ACIDS ON THE BIOSYNTHESIS OF β-AMINO ACIDS, CONSTITUENTS OF BACILLOMYCINS F

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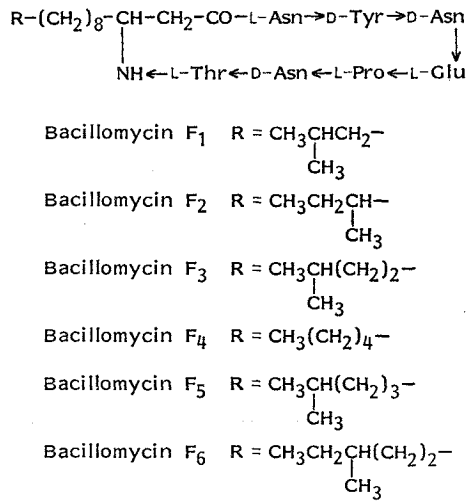
Bacillomycins F, antibiotics of the iturin group, are produced by *Bacillus subtilis* I164[1]. Their chemical structures have been determined[2]; they differ in the nature of the carbon chain of β-amino acids (Fig. 1). Such β-amino acids have been isolated only from antibiotics of the iturin group[3,4].

Fig. 1. Structure of bacillomycins F.



Factors affecting the production of bacillomycin L have been studied[5]. Several amino acids have been found to increase the production of this antibiotic but their effect on the biosynthesis of β-amino acids have not been determined.

In this paper, we describe the effect of leucine, isoleucine, valine and threonine on the production of bacillomycins F and on the nature of the carbon chain of the β-amino acid constituents of bacillomycins F.

B. subtilis I164 was grown in the medium of LANDY *et al.*[6] containing D,L-threonine, valine, leucine or isoleucine at various concentrations. Culture conditions were as described previously[1] and the production of bacillomycins F was determined by β-amino acids titration[5]. The effects of different amino acids on bacillomycin F production are summarized in Table 1. Amino acids which had the more positive effect on antibiotic synthesis were valine and isoleucine, which are not present in bacillomycins F. An unexpected result was the absence of increased bacillomycin F production in the presence of threonine, a constituent of the antibiotics.

Table 1. Effect of amino acids on the production of bacillomycin F.

Amino acid added	Concentration (g/liter)	Growth (OD at 600 nm)	Antibiotic	
			μM	μmol/g of dry cells
None		8.1	17	11
D,L-Valine	1	7.9	45	26
	2	7.6	65	38
	4	8.9	70	40
	8	10.2	20	9
D,L-Leucine	1	6.9	17	10
	2	6.6	15	10
	4	7.3	17	8
	8	7.1	15	7
D,L-Isoleucine	1	6.4	75	50
	2	6.5	69	46
	4	6.9	77	39
	8	6.2	72	40
D,L-Threonine	2	8.7	13	13
	4	11.4	9	12
	8	12.7	27	15

Table 2. Effect of amino acids on the nature of the carbon chain of the constitutive β-amino acids.

Amino acid added	Concentration (g/liter)	*iso*-C_{15}*	*anteiso*-C_{15}	*iso*-C_{16}	*n*-C_{16}	*iso*-C_{17}	*anteiso*-C_{17}
		%					
None		7	1	49	3	12	27
D,L-Valine	1			83		4	12
	2			91			9
	4			90			10
	8			90			9
D,L-Leucine	1	30		32		16	22
	2	24		28		29	19
	4	21		25		26	28
	8	35		21		21	22
D,L-Isoleucine	1			14			86
	2		7	8			85
	4		6	6			88
	8		6	4			90
D,L-Threonine	2	1	7				91
	4	5	52				43
	8	4	62				33

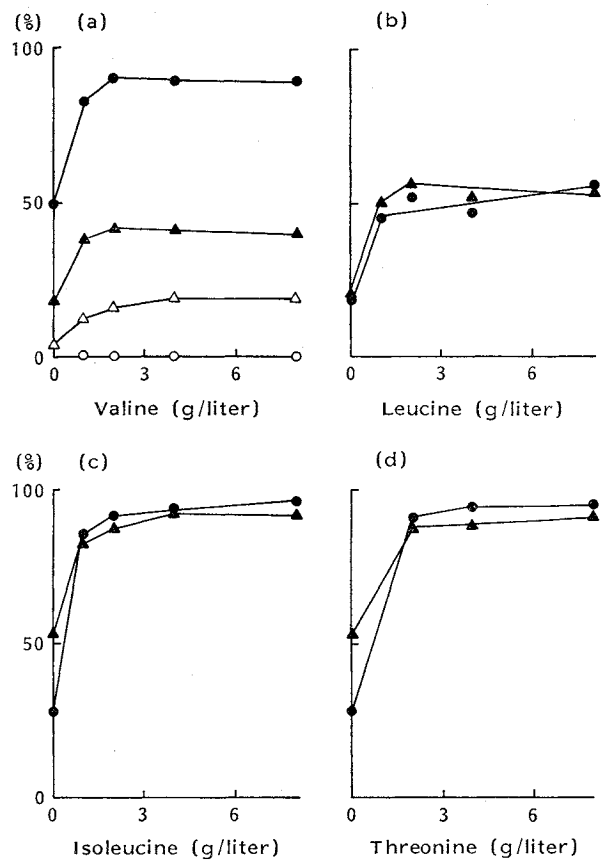
* *iso*-C_{15}: 3-Amino-13-methyltetradecanoic acid, *anteiso*-C_{15}: 3-amino-12-methyltetradecanoic acid, *iso*-C_{16}: 3-amino-14-methylpentadecanoic acid, *n*-C_{16}: 3-aminohexadecanoic acid, *iso*-C_{17}: 3-amino-15-methylhexadecanoic acid, *anteiso*-C_{17}: 3-amino-14-methylhexadecanoic acid.

Fig. 2. Comparison of the effect of various amino acids on the nature of β-amino acids and cellular fatty acids.

Effect of valine (a) on *iso*-C_{14} (○) and *iso*-C_{16} (●) β-amino acids, *iso*-C_{14} (△) and *iso*-C_{16} (▲) fatty acids.

Effect of leucine (b) on *iso*-(C_{15}+C_{17}) β-amino acids (●) and on *iso*-(C_{15}+C_{17}) fatty acids (▲).

Effect of isoleucine (c) and threonine (d) on *anteiso*-(C_{15}+C_{17}) β-amino acids (●) and *anteiso*-(C_{15}+C_{17}) fatty acids (▲).



As bacillomycins F differ only by the nature of the β-amino acids, the antibiotics obtained in various media were hydrolyzed and the β-amino acids were analyzed by gas chromatography as described previously[4]. Table 2 shows the results. Isoleucine and threonine increased odd *anteiso* β-amino acids corresponding to bacillomycins F_2 and F_6; leucine was less potent in increasing odd *iso* β-amino acids corresponding to bacillomycins F_1 and F_5; valine increased *iso*-C_{16} β-amino acid corresponding to bacillomycin F_3.

As branched amino acids are known to modify the composition of cellular fatty acids of *B. subtilis*[7], threonine, valine, leucine and isoleucine can be the precursors of β-amino acids *via* fatty acids by dehydrogenation and amination of the corresponding β-keto acid. Such a hypothesis was tested by analyzing the fatty acid composition of cells grown in various media. Cellular fatty acids were prepared according to the procedure of ITO *et al.*[8] and analyzed by gas chromatography as methyl esters. Fig. 2 compares the effects of the different amino acids on the increased β-amino acid and on the corresponding fatty acid. Isoleucine and threonine increased at the same level (about 95%) odd *anteiso* fatty acids and odd *anteiso* β-amino acids, leucine increased odd *iso* fatty acids and odd *iso* β-amino acids (about 55% for both); valine increased *iso*-C_{14} and C_{16} fatty acids but only C_{16} β-amino acid, valine did not induce synthesis of *iso*-C_{14} β-amino acid for which no bacillomycins F has previously been identified (Fig. 1).

Thus it appears that if fatty acids are the precursors of β-amino acids, the enzyme system responsible for β-amino acid synthesis selects, among the cellular fatty acids, those which correspond to the carbon chain of the β-amino acids.

Now we are trying to isolate a cell-free system able to synthesize β-amino acids and to determine whether β-amino acids are synthesized *via* fatty acids.

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