

# Multivalent Repression and Genetic Derepression of Isoleucine-Valine Biosynthetic Enzymes in *Serratia marcescens*

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The regulation of the formation of isoleucine-valine biosynthetic enzymes was examined to elucidate the mechanism of isoleucine-valine accumulation by  $\alpha$ -aminobutyric acid-resistant (*abu-r*) mutants of *Serratia marcescens*. In the isoleucine-valine auxotroph, L-threonine dehydratase, acetohydroxy acid synthetase, and transaminase B were repressed when isoleucine, valine, and leucine were simultaneously added to minimal medium. These enzymes were derepressed at the limitation of any single branched-chain amino acid. Pantothenate, which stimulated growth of this auxotroph, had no effect on the enzyme levels. It became evident from these results that in *S. marcescens* isoleucine-valine biosynthetic enzymes are subject to multivalent repression by three branched-chain amino acids. The *abu-r* mutants had high enzyme levels in minimal medium, with or without three branched-chain amino acids. Therefore, in *abu-r* mutants, isoleucine-valine biosynthetic enzymes are genetically derepressed. This derepression was considered to be the primary cause for valine accumulation and increased isoleucine accumulation.

We described previously (7, 8) that  $\alpha$ -aminobutyric acid-resistant (*abu-r*) mutants of *Serratia marcescens* not only showed a marked valine accumulation but also had an increased activity of isoleucine accumulation from D-threonine. The mutants also had higher acetohydroxy acid synthetase levels than the wild-type strain. Moreover, D-threonine not only induced D-threonine dehydratase but also increased acetohydroxy acid synthetase level in the wild-type strain. In this case, the addition of valine or leucine overcame the effect of D-threonine on the formation of the latter enzyme. On the other hand, acetohydroxy acid synthetase levels in the *abu-r* mutants tested were not affected by the addition of these amino acids. Acetohydroxy acid synthetases of most of the *abu-r* mutants tested were feedback-inhibited by valine to the same extent as that of the wild-type strain, although two of the mutants had desensitized enzymes and increased enzyme levels. L-Threonine dehydratases of *abu-r* mutants were strongly feedback-inhibited by isoleucine, like that of the wild-type strain. On the basis of these data, it was considered that valine accumulation and increased isoleucine accumulation by *abu-r* mutants might be due to genetic derepression of isoleucine-valine biosynthetic enzymes.

The regulation of the formation of isoleucine-valine biosynthetic enzymes has been demonstrated primarily with *Escherichia coli* and *Salmonella typhimurium* (1, 4, 5, 13); little is known about this regulation in other bacteria. To elucidate the mechanism of isoleucine-valine accumulation by *abu-r* mutants of *S. marcescens*, it was considered important to know how the formation of isoleucine-valine biosynthetic enzymes is controlled, both in the wild-type strain and in the resistant mutants. This paper describes multivalent repression of isoleucine-valine biosynthetic enzymes in the wild-type strain of *S. marcescens* and genetic derepression in the *abu-r* mutants.

## MATERIALS AND METHODS

**Bacteria.** The bacterial strains used in this study were *S. marcescens* strain 1 (7); an isoleucine-valine auxotroph, strain 102 derived from strain 1; and several *abu-r* mutants described in preceding papers (8a).

**Media, cultural conditions, and enzyme assays.** The bacteria were cultured in 500-ml shaking flasks containing 150 ml of Davis-Mingioli minimal medium (3) modified by omitting the citrate and increasing the glucose concentration to 0.5%. Cultures of the wild-type strain and of *abu-r* mutants were grown in minimal medium. Strain 102 was grown in minimal medium supplemented with  $10^{-2}$  M isoleucine,  $10^{-2}$  M valine,

$10^{-2}$  M leucine,  $10^{-4}$  M glycyl-valine, and  $2 \times 10^{-5}$  M calcium pantothenate. The cells were harvested by centrifugation of cultures in early stationary phase and were washed with saline. The cells were resuspended to give  $160 \mu\text{g}$  of dry cells per ml in minimal medium with or without supplements. Supplements for strain 102 are indicated in Table 1. Incubation was at 30 C with reciprocal shaking (140 rev/min, 8-cm stroke). Growth was estimated by measuring the optical density at 660 nm with a Hitachi electric photometer (EPO-B type) and is expressed as dry cell weight calculated from a standard curve. The cells, cultured for 2 or 4 hr, were harvested by centrifugation. Cell-free extracts were prepared as described previously (8) and were used immediately for assays of L-threonine dehydratase, acetoxy acid synthetase, and transaminase B. The procedures for enzyme assays are described elsewhere (8a). Specific activities are expressed as micromoles of products formed per milligram of protein per minute.

## RESULTS

**Effect of branched-chain amino acids on isoleucine-valine enzyme levels in the isoleucine-valine auxotroph.** Although the enzyme levels in the wild-type strain slightly decreased in the presence of three branched-chain amino acids, it was difficult to know clearly the repression pattern of iso-

leucine-valine biosynthetic enzymes. Therefore, an isoleucine-valine auxotroph, strain 102, was used. This auxotroph showed slow growth when the mixture of isoleucine and valine was added to minimal medium. The slow growth was considered to be due to an intracellular lack of leucine and pantothenate because of blocking of the  $\alpha$ -ketoisovalerate supply in the cells. The addition of leucine and pantothenate restored growth. However, an increase in isoleucine concentration prevented growth. Since this seemed to be caused by competition between isoleucine and valine for entry into the cells, glycyl-valine was used as a partial substitute for valine, by the method of Umbarger and Brown (12).

The levels of L-threonine dehydratase, acetoxy acid synthetase, and transaminase B were low in cells from the complete medium (Fig. 1). These three enzymes were markedly derepressed in the isoleucine- or valine-limiting medium. Derepression also occurred in medium without leucine. When an excess of each amino acid was added to the cells derepressed by growing for 2 hr in the amino acid-limiting medium, immediate repression of three enzymes occurred (*not shown*). It was reported that, in *E. coli* K-12 and *S. typhimurium*, pantothenate is also necessary for the repression of isoleucine-valine biosynthetic enzymes (M. Freundlich, and H. E. Umbarger, *Bacteriol. Proc.*, p. 126, 1963). It was, therefore, investigated whether pantothenate might participate in multivalent repression in *S. marcescens*. In medium without pantothenate but with an excess of three branched-chain amino acids, three isoleucine-valine biosynthetic enzymes were not derepressed (Table 2). These results indicate that, in *S. marcescens*, isoleucine-valine biosynthetic enzymes are subject to multivalent repression by three branched-chain amino acids.

**Genetic derepression of isoleucine-valine biosynthetic enzymes in *abu-r* mutants.** It was pre-

TABLE 1. Supplements to the minimal medium for the isoleucine-valine auxotroph, strain 102

Supplement	Medium (concn) <sup>a</sup>			
	Complete (M)	Low isoleucine (M)	Low valine (M)	No leucine (M)
L-Isoleucine . . . . .	$10^{-3}$	$10^{-4}$	$10^{-3}$	$10^{-3}$
L-Valine . . . . .	$10^{-3}$	$10^{-3}$	$10^{-4}$	$10^{-3}$
L-Leucine . . . . .	$10^{-3}$	$10^{-3}$	$10^{-3}$	0

<sup>a</sup> Minimal medium containing  $10^{-4}$  M glycyl-L-valine and  $2 \times 10^{-5}$  M calcium pantothenate was supplemented with branched-chain amino acids as indicated.

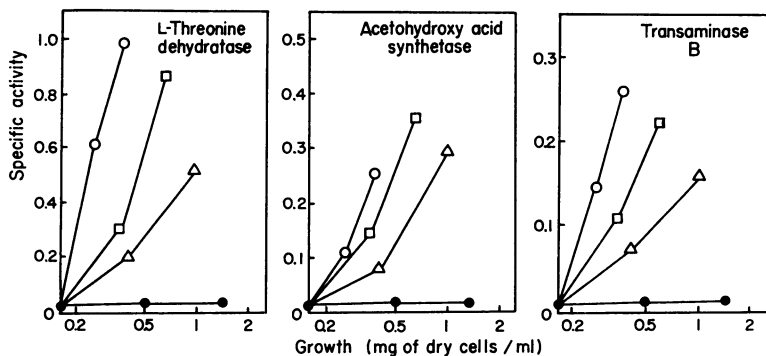


FIG. 1. Multivalent repression of isoleucine-valine biosynthetic enzymes in the isoleucine-valine auxotroph, strain 102. Supplements to minimal medium are indicated in Table 1. Symbols: ●, complete medium; ○, low isoleucine medium; □, low valine medium; Δ, no leucine medium.

viously reported that *abu-r* mutants of *S. marcescens* had increased levels of L-threonine dehydratase and acetohydroxy acid synthetase (8). It was investigated whether these increased enzyme levels might be due to genetic lack of multivalent repression. When partially repressed wild-type cells were cultured in the minimal medium, the enzyme levels slightly increased; however, in medium containing excess branched-chain amino acids, the enzyme levels decreased (Fig. 2). On the other hand, in strain 130-1, the enzyme levels were very high in the presence or absence of three amino acids. The *abu-r* mutants other than strain 130-1 were tested for the enzyme levels. Generally, the higher resistance of the *abu-r* mutant resulted in higher enzyme levels. Plots of the levels of L-threonine dehydratase and acetohydroxy acid synthetase versus the level of transaminase B indicate that *abu-r* mutants almost

have coordinate derepression levels of isoleucine-valine biosynthetic enzymes (Fig. 3).

## DISCUSSION

Regulatory mechanisms of isoleucine-valine biosynthesis are very complicated owing to the following (11). Isoleucine-valine biosynthetic enzymes are bifunctional for both amino acids except L-threonine dehydratase; leucine and pantothenate are synthesized from  $\alpha$ -ketoisovalerate which is a precursor of valine; the last enzyme, transaminase B, participates in the formation of all three branched-chain amino acids. It has been known that, in *E. coli* and *S. typhimurium*, isoleucine-valine biosynthetic enzymes are multivalently repressed by isoleucine, valine, and leucine (13). However, the control of reductoisomerase, the third isoleucine-valine enzyme, was demonstrated to be regulated by induction (indirect multivalent repression; reference 1).

Regulatory mechanisms of isoleucine-valine biosynthesis in *S. marcescens* are illustrated in Fig. 4, on the basis of the experimental data. This report and previous papers (7, 8) have revealed that, in *S. marcescens*, isoleucine-valine biosynthetic enzymes are subject to almost the same pattern of feedback control as those in *E. coli* and *S. typhimurium*. In *S. marcescens*, as well as in *E. coli* W and *S. typhimurium*, three isoleucine-valine biosynthetic enzymes tested were multivalently repressed by three branched-chain amino acids. On the other hand, in *E. coli* K-12, repression of acetohydroxy acid synthetase is known not to require isoleucine. *S. marcescens* is, furthermore, similar to *E. coli* W and *S. typhimurium* with respect to less sensitivity of acetohydroxy acid synthetase activity to valine and to insensitivity of growth to valine (2, 9).

In *abu-r* mutants of *Serratia marcescens*, three isoleucine-valine enzymes tested were found

TABLE 2. Effect of pantothenate on the levels of isoleucine-valine biosynthetic enzymes in the isoleucine-valine auxotroph, strain 102

Medium <sup>a</sup>	Culture time (hr)	Growth (mg/ml) <sup>b</sup>	Specific activity		
			L-Threonine dehydratase	Acetohydroxy acid synthetase	Transaminase B
Complete	2	0.48	0.010	0.008	0.013
	4	1.30	0.023	0.012	0.010
Without pantothenate	2	0.38	0.013	0.010	0.015
	4	0.64	0.015	0.015	0.012

<sup>a</sup> Complete medium is indicated in Table 1; medium without pantothenate is prepared by omitting calcium pantothenate from complete medium.

<sup>b</sup> Cell dry weight.

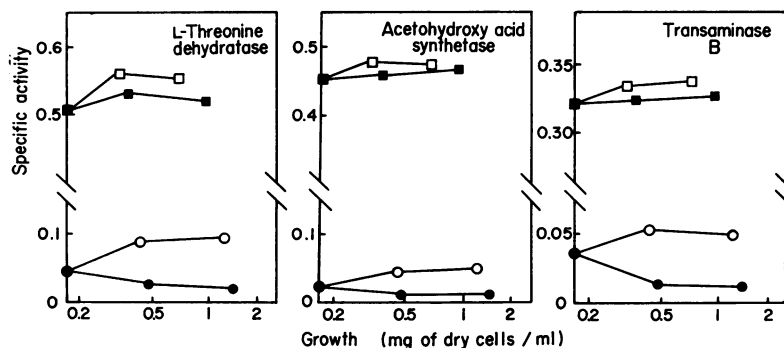


FIG. 2. Genetic derepression of isoleucine-valine biosynthetic enzymes in *abu-r* mutant, strain 130-1. Symbols: O, wild-type strain in minimal medium; ●, wild-type strain in minimal medium containing  $10^{-2}$  M L-isoleucine,  $10^{-2}$  M L-valine, and  $10^{-2}$  M L-leucine; □, strain 130-1 in minimal medium; ■, strain 130-1 in minimal medium containing  $10^{-2}$  M L-isoleucine,  $10^{-2}$  M L-valine, and  $10^{-2}$  M L-leucine.

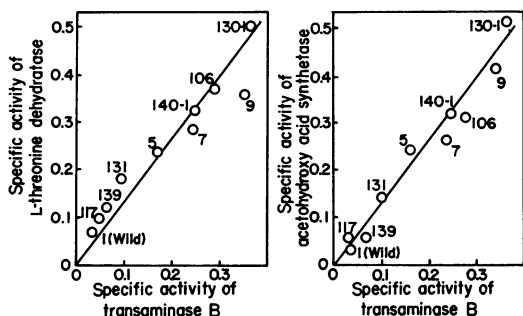


FIG. 3. Coordinate derepression levels of isoleucine-biosynthetic enzymes in *abu-r* mutants. The cells were cultured in minimal medium and were in early stationary phase. Numbers refer to the strains of *abu-r* mutants.

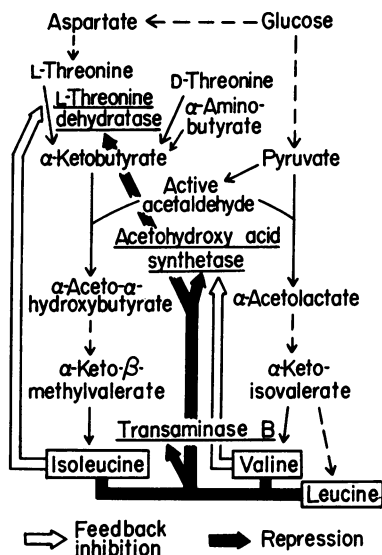


FIG. 4. Regulatory mechanisms of isoleucine-valine biosynthesis in *Serratia marcescens*.

to be genetically derepressed, since the enzyme levels were hardly affected by the simultaneous addition of three branched-chain amino acids. These *abu-r* mutants were derepressed not only for acetohydroxy acid synthetase but also for L-threonine dehydratase and transaminase B, although an *abu-r* strain of *E. coli* K-12 was reported to be derepressed only for acetohydroxy acid synthetase (11). Also, D-threonine, L- $\alpha$ -aminobutyric acid, and  $\alpha$ -ketobutyric acid caused phenotypic derepression of these three enzymes in *S. marcescens* (unpublished data). These facts indicate that genetic basis for the regulation of isoleucine-valine biosynthesis in this strain slightly differs from that in *E. coli* K-12. Moreover, it was found that leaky revertants of isoleucine

auxotrophs derived from an *abu-r* mutant accumulated large amounts of leucine in the culture medium and that some *abu-r* mutants had cross-resistance to azaleucine, which was specifically antagonized by leucine (unpublished data). These facts suggest that the *abu-r* mutation might be accompanied by the lack of feedback control of leucine biosynthesis. The investigation of leucine enzymes will clarify the genetic basis of *abu-r* mutants.

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