

Utilization of Cyclic Amides and Formation of ω -Amino Acids by Microorganisms¹

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In investigations on the utilization of cyclic amides by microorganisms, Noe and Nickerson (J. Bacteriol. 75:674, 1958) studied the metabolism of γ -butyrolactam by *Pseudomonas aeruginosa*, and Kato and Fukumura (Chem. Ind. [London], p. 1146, 1962) reported on the bacterial breakdown of ϵ -caprolactam. But the formation of ω -amino acids, hydrolytic products of these cyclic amides, was not detected in either case.

To determine the occurrence of "cyclic amide hydrolase," we screened a number of microorganisms utilizing the cyclic amides (γ -butyrolactam, δ -valerolactam, ϵ -caprolactam, and pyroglutamic acid) for the production of ω -amino acids. The media contained 0.1% glucose, 0.05% sodium citrate, 0.7% K_2HPO_4 , 0.2% KH_2PO_4 , 0.01% $MgSO_4 \cdot 7H_2O$, and the cyclic amide as the sole source of nitrogen. The utilization of cyclic amides was measured by growth of the microorganism, and the formation of ω -amino acids was detected by paper chromatography of the broth.

In the medium containing 0.2% L-pyroglutamic acid, 46 strains of bacteria (*Pseudomonas*, *Achromobacter*, *Serratia*, and *Proteus* species), 13 strains of streptomycetes, 27 strains of molds, and 4 strains of yeasts, such as *Hansenula*, *Pichia*, *Saccharomyces* and *Torula*, utilized the acid for their growth. However, accumulation of glutamic acid in the culture broth was not observed.

In the medium containing γ -butyrolactam, δ -valerolactam, and ϵ -caprolactam at a final concentration of 0.1%, 32 strains of bacteria, 3 strains of streptomycetes, 28 strains of molds, and 2 strains of yeast grew satisfactorily. Among these microorganisms, 7 strains of *Pseudomonas*, *Mycobacterium smegmatis*, and *Nocardia asteroides* accumulated ω -amino acids in the broth.

To determine which lactam was most readily utilized for growth, experiments were carried out with media S-5, S-6, and S-7, containing γ -butyrolactam, δ -valerolactam, and ϵ -caprolactam,

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TABLE 1. Utilization of lactams and formation of ω -amino acids*

Microorganisms	No. of strains					
	Tested	S-6 medium			S-7 medium	
		Growth	Formation of δ -aminovaleric acid	Formation of ϵ -aminocaproic acid	Growth	Formation of ϵ -aminocaproic acid
<i>Achromobacter delmarvae</i>	1	0	0	0	0	
<i>Pseudomonas aeruginosa</i>	9	3	0	0	0	
<i>P. fluorescens</i>	8	3	1	3	3	
<i>P. miyamizu</i>	1	0	0	0	0	
<i>P. myxogenes</i>	2	0	0	0	0	
<i>P. ovalis</i>	8	7	0	7	7	
<i>P. putida</i>	1	0	0	0	0	
<i>P. schuylkilliensis</i> ..	2	0	0	0	0	
<i>P. striafaciens</i>	1	0	0	0	0	
<i>P. taetrolens</i>	1	0	0	0	0	
<i>Mycobacterium smegmatis</i>	1	1	1	1	1	
<i>Nocardia asteroides</i> ..	1	0	0	0	0	
<i>Absidia orchidis</i>	1	1	0	1	0	
<i>Aspergillus nidulans</i>	1	1	0	1	0	
<i>A. parasiticus</i>	1	1	0	1	0	
<i>Penicillium corymbiferum</i>	1	1	0	1	0	
<i>P. chrysogenum</i>	2	2	0	2	0	
<i>Hansenula anomela</i> ..	1	1	0	0	0	
<i>Torula</i> sp. TH-1....	1	1	0	0	0	
Total.....	44	22	2	17	11	

* Shaking culture (110 cycle/min, 8-cm stroke, 45° angle) in test tubes was carried out for 6 days at 30 C. Growth of bacteria and yeasts was measured by the optical density at 660 m μ , and growth of molds and streptomycetes was visually observed. Routine identification and rough quantitative estimation of liberated ω -amino acids were carried out by paper chromatography of the broth and subsequent ninhydrin reaction.

respectively, at a final concentration of 0.2% (Table 1).

All of the tested microorganisms (except the two yeasts) utilized γ -butyrolactam for growth, but γ -aminobutyric acid was not detected in the culture broth. Therefore, γ -aminobutyric acid is assumed to be further metabolized without accumulation, as in the case of *Pseudomonas aeruginosa* (Noe et al., J. Bacteriol. **75**:674, 1958). δ -Valerolactam and ϵ -caprolactam were also utilized by many microorganisms for growth, and δ -aminovaleric acid and ϵ -aminocaproic acid were observed in the culture broth of strains of *P. fluorescens*, *P. ovalis*, and *M. smegmatis*.

The rate of formation of ω -amino acids from cyclic amides was compared by use of four strains of bacteria: *P. fluorescens* 174, *P. ovalis* 189, *P. ovalis* 193, and *M. smegmatis*. These bacteria were cultured for 72 hr in medium S-7, and the cells from 5 ml of culture broth were suspended in 0.5 ml of distilled water. A mixture of 0.5 ml of 0.1 M phosphate buffer (pH 5.6), 0.5 ml of 0.06 M δ -valerolactam or ϵ -caprolactam, and 0.5 ml of

cell suspension was incubated for 24 hr at 37 C. ω -Amino acids formed were identified by paper chromatography and measured by the colorimetric ninhydrin method. All four strains hydrolyzed δ -valerolactam and ϵ -caprolactam to produce δ -aminovaleric acid and ϵ -aminocaproic acid, respectively. Of the tested strains, *P. ovalis* 189 showed the highest activities; that is, the rates of formation of ω -amino acids were 4.38 and 4.08 μ moles per 24 hr per ml of the reaction mixture from δ -valerolactam and ϵ -caprolactam, respectively.

This is the first report of the formation of ω -amino acid from cyclic amides; it suggests the occurrence of "cyclic amide hydrolase" activity in microorganisms.

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