# ANTIBACTERIAL ACTIVITY OF L-2-AMINO-4-CHLORO-4-PENTENOIC ACID ISOLATED FROM *AMANITA PSEUDOPORPHYRIA* HONGO

MITSUAKI MORIGUCHI, YOSHIFUMI HARA and SHIN-ICHI HATANAKA†

Department of Environmental Chemistry and Engineering, Faculty of Engineering, Oita University, Oita 870-11, Japan
†Department of Biology, College of Arts and Sciences, The University of Tokyo, Komaba, Meguro-ku, Tokyo 153, Japan

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A large number of non-protein amino acids are known from various plants and microorganisms. Most of them are analogs of protein amino acids and their biological effects have often been explained by this analogy[1,2].

In a previous paper, the isolation and identification of a chlorine-containing amino acid, L-2-amino-4-chloro-4-pentenoic acid (L-ACP) was reported from fruit bodies of *Amanita pseudoporphyria* Hongo[3]. This paper describes the antibacterial activities of L-ACP.

L-2-Amino-4-chloro-4-pentenoic acid was prepared by optical resolution of the synthetic racemate[3,4], using commercial acylase. The bacteria used in this study are listed in Table 1. They were grown in the test tubes (1.7×16.5 cm) containing 3 ml minimal medium of DAVIS and MINGIOLI[5] at 30°C on reciprocal shaking (140 rpm). Growth was determined by measuring the optical density at 610 nm.

Although no inhibitory effect by L-ACP was observed in nutrient medium by paper-disc agar diffusion methods, the growth of a considerable number of bacterial strains was inhibited in minimal medium even at a concentration as low as $1.34\times10^{-5}$ M. Almost none of the strains could grow at $6.70\times10^{-3}$ M. The results are shown in Table 1.

Effect of L-ACP concentration on the growth of *Pseudomonas aeruginosa* is shown in Fig. 1. Little inhibition occurred at $1.34\times10^{-7}$ M. As the concentration was raised from $1.34\times10^{-6}$ to $1.34\times10^{-3}$ M, the inhibition increased.

Table 2 shows the reversal of L-ACP inhibi-

Table 1. Antibacterial activity of L-ACP.

| Strain | Growth inhibition (%)[a] | |
|---|---|---|
| | L-ACP concentration | |
| | $1.34\times10^{-5}$ M | $6.70\times10^{-3}$ M |
| *Achromobacter aceris* IFO 3166 | 52 | 84 |
| *Aerobacter aerogenes* IFO 3319 | 32 | 74 |
| *Alcaligenes faecalis* IFO 3160 | 97 | 100 |
| *A. viscolactis* IAM 1517 | 85 | 100 |
| *Arthrobacter oxydans* IFO 12138 | 28 | 86 |
| *A. simplex* IFO 12069 | 98 | 100 |
| *Bacillus licheniformis* IAM 11054 | 92 | 100 |
| *B. natto* Sawamura IFO 3013 | 95 | 100 |
| *B. subtilis* IFO 3026 | 87 | 100 |
| *Escherichia coli* IFO 3806 | 92 | 100 |
| *E. coli* K-12 IFO 3208 | 0 | 100 |
| *E. intermedia* K-10 AKU 0011 | 58 | 78 |
| *Micrococcus luteus* IFO 3763 | 90 | 100 |
| *Pseudomonas aeruginosa* IFO 3447 | 100 | 100 |
| *P. desmolytica* IFO 12570 | 97 | 100 |
| *P. fluorescens* IFO 3461 | 98 | 100 |
| *P. polycolor* IFO 3918 | 27 | 97 |
| *P. putida* ICRB 3420 | 19 | 100 |
| *Serratia marcescens* IFO 3054 | 18 | 97 |

[a] Growth inhibition was calcd from absorbance at 610 nm in the absence and in the presence of L-ACP for 20 hours of cultivation.

Fig. 1. Effect of L-ACP concentration on the growth of *Pseudomonas aeruginosa*.

L-ACP concentration in the minimal medium: ○ None, ■ $1.34\times10^{-7}$ M, ◇ $1.34\times10^{-6}$ M, ● $1.34\times10^{-5}$ M, □ $1.34\times10^{-4}$ M, △ $1.34\times10^{-3}$ M.

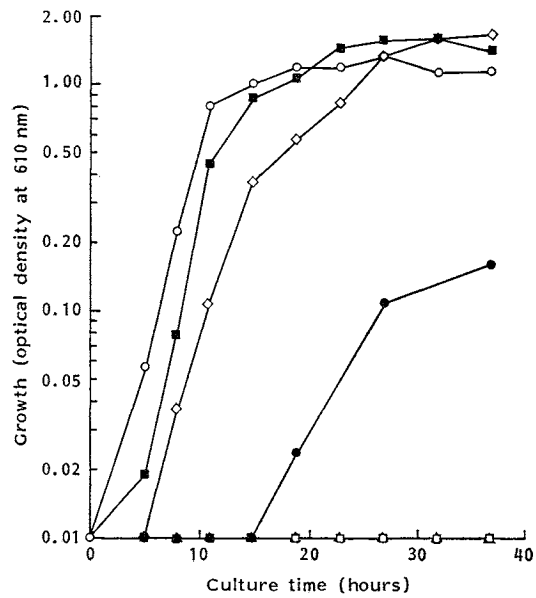


Table 2. The reversal of L-ACP growth inhibition by various amino acids.

| L-Amino acid[a] ($10^{-2}$ M) | Growth (610 nm)[b] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A.f.* | | *B.s.* | | *E.c.* K-12 | | *P.a.* | | *P.f.* | | *P.p.* | | *S.m.* | |
| | L-ACP concentration (M) | | | | | | | | | | | | | |
| | 0 | $1.34\times10^{-5}$ | 0 | $1.34\times10^{-4}$ | 0 | $1.34\times10^{-3}$ | 0 | $1.34\times10^{-4}$ | 0 | $1.34\times10^{-4}$ | 0 | $1.34\times10^{-4}$ | 0 | $1.34\times10^{-3}$ |
| None | 0.570 | 0.016 | 0.658 | 0 | 0.374 | 0.027 | 1.456 | 0 | 0.181 | 0.020 | 1.108 | 0.008 | 0.632 | 0.001 |
| L-Alanine | 0.781 | 0.732 | 0.125 | 0.033 | 0.571 | 0 | 0.514 | 0 | 0.281 | 0.027 | 1.221 | 0.096 | 0.319 | 0 |
| L-Valine | 0.222 | 0.186 | 0.390 | 0.572 | 0.020 | 0.011 | 0.687 | 0.263 | 0.087 | 0.039 | 1.026 | 1.048 | 0.621 | 0 |
| L-Leucine | 0.495 | 0.484 | 0.261 | 0.493 | 0.227 | 0.285 | 1.066 | 1.179 | 0.130 | 0.214 | 1.174 | 0.978 | 0.416 | 0.522 |
| L-Isoleucine | 0.563 | 0.046 | 0.379 | 0.262 | 0.169 | 0.008 | 1.082 | 0 | 0.057 | 0.023 | 1.209 | 0.028 | 0.381 | 0.011 |
| L-Methionine | 0.370 | 0.321 | 0.384 | 0.620 | 0.368 | 0.076 | 0.516 | 0 | 0.285 | 0.004 | 1.088 | 1.252 | 0.760 | 0.952 |
| L-Tryptophan | 0.197 | 0.015 | 0.285 | 0.016 | 0.437 | 0.001 | 0.681 | 0 | 0.112 | 0.025 | 1.000 | 0.029 | 0.468 | 0.017 |
| L-Proline | 0.735 | 0.028 | 0.212 | 0.025 | 1.018 | 0.019 | 0.790 | 0 | 0.130 | 0 | 1.241 | 0.030 | 0.291 | 0 |
| L-Serine | 0.041 | 0.028 | 0.006 | 0.030 | 0.439 | 0.002 | 0.480 | 0 | 0.147 | 0.015 | 1.190 | 0.061 | 0.231 | 0.054 |
| L-Threonine | 0.272 | 0.036 | 0.017 | 0.023 | 0.415 | 0.009 | 0.835 | 0 | 0.092 | 0 | 1.246 | 0.040 | 0.310 | 0 |
| L-Cysteine | 0.047 | 0.045 | 0 | 0 | 0.004 | 0 | 0.007 | 0 | 0.041 | 0 | 0.818 | 0.829 | 0 | 0 |
| L-Tyrosine | 0.584 | 0.027 | 0.433 | 0.002 | 0.141 | 0 | 1.322 | 0.433 | 0.422 | 0.049 | 1.119 | 0.210 | 0.147 | 0 |
| L-Lysine | 0.489 | 0.036 | 0.610 | 0 | 0.510 | 0.022 | 0.558 | 0 | 0.217 | 0.020 | 1.214 | 0.069 | 0.421 | 0.006 |
| L-Histidine | 0.206 | 0.019 | 0.239 | 0.034 | 0.100 | 0 | 0.683 | 0 | 0.317 | 0.017 | 1.112 | 0.039 | 0.358 | 0.002 |
| L-Arginine | 0.675 | 0.025 | 0.700 | 0.010 | 0.434 | 0.012 | 1.272 | 0 | 0.173 | 0 | 1.206 | 0.024 | 0.481 | 0.022 |
| L-Ornithine | 0.510 | 0.035 | 0.476 | 0.019 | 0.360 | 0.002 | 0.370 | 0 | 0.164 | 0 | 1.260 | 0.029 | 0.201 | 0 |
| L-Aspartic acid | 0.185 | 0.032 | 0.181 | 0.017 | 0.310 | 0 | 1.177 | 0 | 0.138 | 0 | 1.200 | 0.034 | 0.744 | 0 |
| L-Glutamic acid | 0.017 | 0.015 | 0.617 | 0.051 | 0.122 | 0.029 | 0.659 | 0 | 0.132 | 0 | 1.137 | 0.012 | 0.660 | 0 |

[a] The amino acid was added to the minimal medium with or without L-ACP.

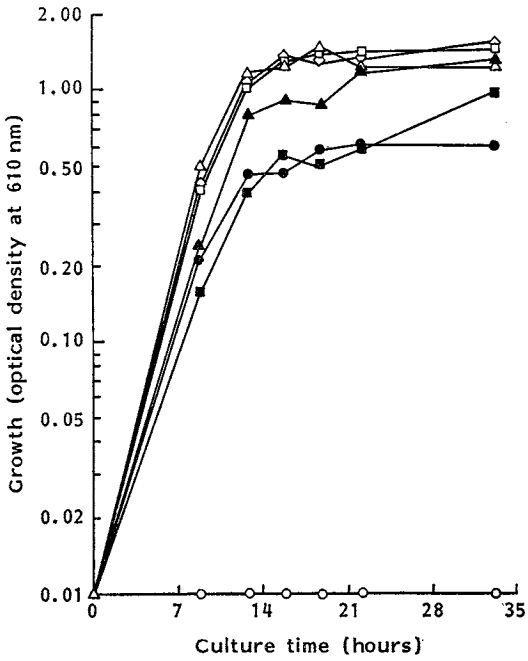
[b] The culture was carried out for 20 hours.

*A.f.*; *Alcaligenes faecalis*, *B.s.*; *Bacillus subtilis*, *E.c.*; *Escherichia coli*, *P.a.*; *Pseudomonas aeruginosa*, *P.f.*; *Pseudomonas fluorescens*, *P.p.*; *Pseudomonas putida*, *S.m.*; *Serratia marcescens*.

Fig. 2. Reversal of L-ACP inhibition on *Pseudomonas aeruginosa* by L-leucine.

L-Leucine concentration in the minimal medium with $1.34\times10^{-4}$ M L-ACP: ○ None, ● $1\times10^{-4}$ M, ■ $1\times10^{-3}$ M, □ $1\times10^{-2}$ M, ◇ $1\times10^{-1}$ M.

L-Leucine concentration in the minimal medium without L-ACP: △ None, ▲ $1\times10^{-1}$ M.




tion by various amino acids. L-Alanine, L-leucine, L-methionine and L-valine reversed the growth inhibition against *Alcaligenes faecalis*. L-Methionine, L-valine, L-leucine and L-isoleucine against *Bacillus subtilis*; L-leucine against *Escherichia coli* K-12; L-leucine, L-tyrosine and L-valine against *P. aeruginosa*; L-leucine against *Pseudomonas fluorescens*; L-methionine, L-valine, L-leucine, L-cysteine and L-tyrosine against *Pseudomonas putida*; L-methionine and L-leucine against *Serratia marcescens*.

Fig. 2 shows the effect of L-leucine on the growth inhibition of *P. aeruginosa* by L-ACP. Growth inhibition by $1.34\times10^{-4}$ M L-ACP was completely reversed by the addition of $1\times10^{-2}$ M L-leucine.

A saturated form of L-ACP, 4-chloronorvaline, was reported to be isolated from the culture broth of *Streptomyces griseosporeus*[6]. This amino acid also showed an antibacterial activity against *P. aeruginosa*, *S. marcescens*, *Klebsiella pneumoniae* and *B. subtilis*, and its anti-*Pseudomonas* activity was antagonized by L-leucine, L-isoleucine, L-valine and L-methionine[6].

In this study, we found that L-ACP can be regarded as an antimetabolite. L-2-Amino-4-chloro-4-pentenoic acid will be an interesting compound for the examination of mechanism of action of an antimetabolite and the production of amino acids by analog-resistant mutants.

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