

Supplementary Materials: Inhibition of Aflatoxin Production in *Aspergillus flavus* by a *Klebsiella* sp. and Its Metabolite Cyclo(L-Ala-Gly)

Shohei Sakuda, Masaki Sunaoka, Maho Terada, Ayaka Sakoda, Natsumi Ishijima, Noriko Hakoshima, Kenichi Uchida, Hirofumi Enomoto and Tomohiro Furukawa

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Method

Analysis of the mycelial weight

Cyclo(L-Ala-Gly) water solution (100 µL), passed through a 0.25 µm filter, was added to potato dextrose liquid medium (1.9 mL) in a well of a microplate (24 wells). A spore suspension of *A. flavus* (5 µL, 1.1×10^5 CFU/µL) was inoculated into the medium and incubated statically for 4 days at 25 °C. The mycelia in each well were collected in a 1.5 mL microtube. After drying the mycelia at 50 °C for 4 days, the mycelial weights were calculated by subtracting the weight of a 1.5 mL microtube without mycelia from the total weight.

Query	1	ATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAR	60
Sbjct	1	ATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAG	60
Query	61	CTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGA	120
Sbjct	61	CTCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGA	120
Query	121	TAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTT	180
Sbjct	121	TAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTT	180
Query	181	CGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTC	240
Sbjct	181	CGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTC	240
Query	241	ACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACA	300
Sbjct	241	ACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACA	300
Query	301	CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA	360
Sbjct	301	CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA	360
Query	361	TGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTTCAGCGAGGAG	420
Sbjct	361	TGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTTCAGCGAGGAG	420
Query	421	GAAGGCR	480
Sbjct	421	GAAGGCG	480
Query	481	ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGC	540
Sbjct	481	ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGC	540
Query	541	GTAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGA	600
Sbjct	541	GTAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGA	600
Query	601	ACTGCATTGCAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCAGGTGTAGC	660
Sbjct	601	ACTGCATTGCAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCAGGTGTAGC	660
Query	661	GGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGA	720
Sbjct	661	GGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGA	720
Query	721	CTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG	780
Sbjct	721	CTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG	780

Figure S1. Alignment of the partial sequences of 16S rDNA from the strain KTTM (Query) and *Klebsiella aerogenes* NBRC 13534 (Sbjct)

Query	781	CCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCG	840
Sbjct	781	CCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCG	840
Query	841	TTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGG	900
Sbjct	841	TTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGG	900
Query	901	CCCGCACAAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACT	960
Sbjct	901	CCCGCACAAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACT	960
Query	961	CTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACCTCTGAGACAGGT	1020
Sbjct	961	CTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACCTCTGAGACAGGT	1020
Query	1021	GCTGCATGGCTGTCGTGAGCTCGTGTTGTGAAATGTTGGGTAAAGTCCCGCAACGAGCGC	1080
Sbjct	1021	GCTGCATGGCTGTCGTGAGCTCGTGTTGTGAAATGTTGGGTAAAGTCCCGCAACGAGCGC	1080
Query	1081	AACCCCTATCCTTTGTTGCCAGCGGT ^{YM} GGCCGGGAACCTCAAAGGAGACTGCCAGTGATA	1140
Sbjct	1081	AACCCCTATCCTTTGTTGCCAGCGGT ^{NC} GGCCGGGAACCTCAAAGGAGACTGCCAGTGATA	1140
Query	1141	AACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACA	1200
Sbjct	1141	AACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACA	1200
Query	1201	CGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAA	1260
Sbjct	1201	CGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAA	1260
Query	1261	GTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTA	1320
Sbjct	1261	GTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTA	1320
Query	1321	ATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGCCTTGTACACACCGCCCGTCAC	1380
Sbjct	1321	ATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGCCTTGTACACACCGCCCGTCAC	1380
Query	1381	ACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCAC	1440
Sbjct	1381	ACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCAC	1440
Query	1441	TTTGTGATTCATGACTGGGGTGAAG	1465
Sbjct	1441	TTTGTGATTCATGACTGGGGTGAAG	1465

Figure S1. (continued) Alignment of the partial sequences of 16S rDNA from the strain KTTM (Query) and *Klebsiella aerogenes* NBRC 13534 (Sbjct)

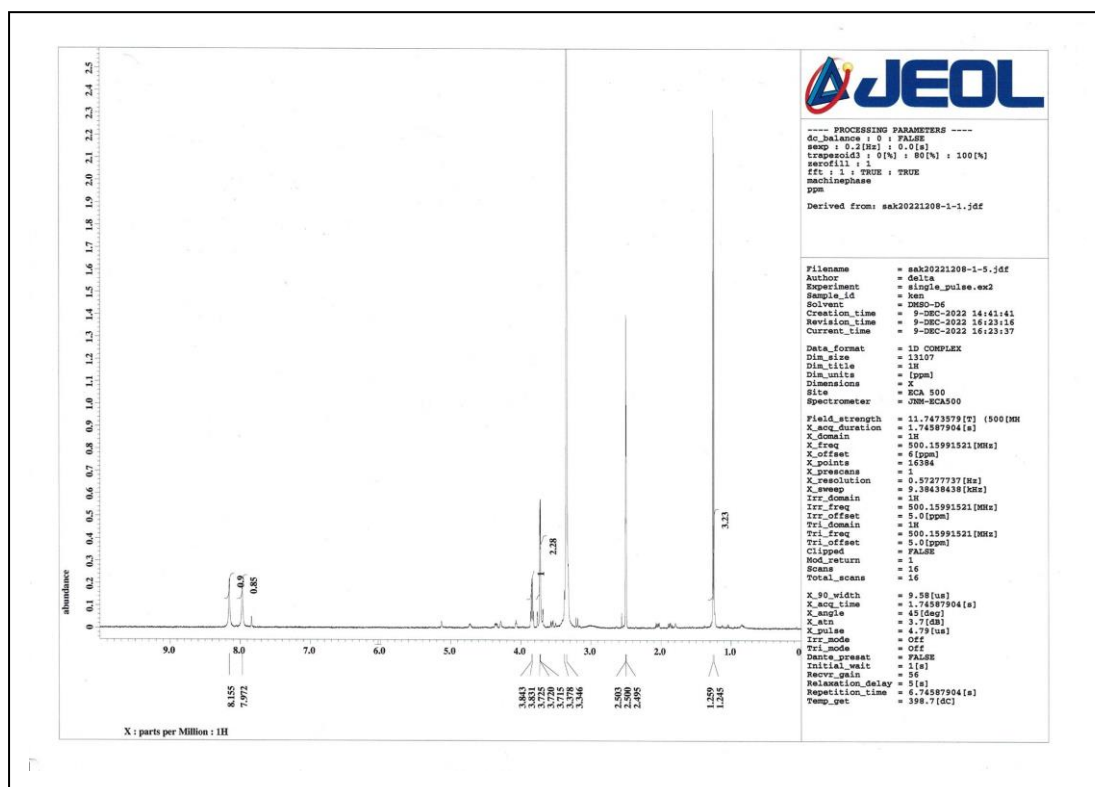


Figure S2. ^1H NMR spectrum of the active component in $\text{DMSO-}d_6$ (500 MHz)

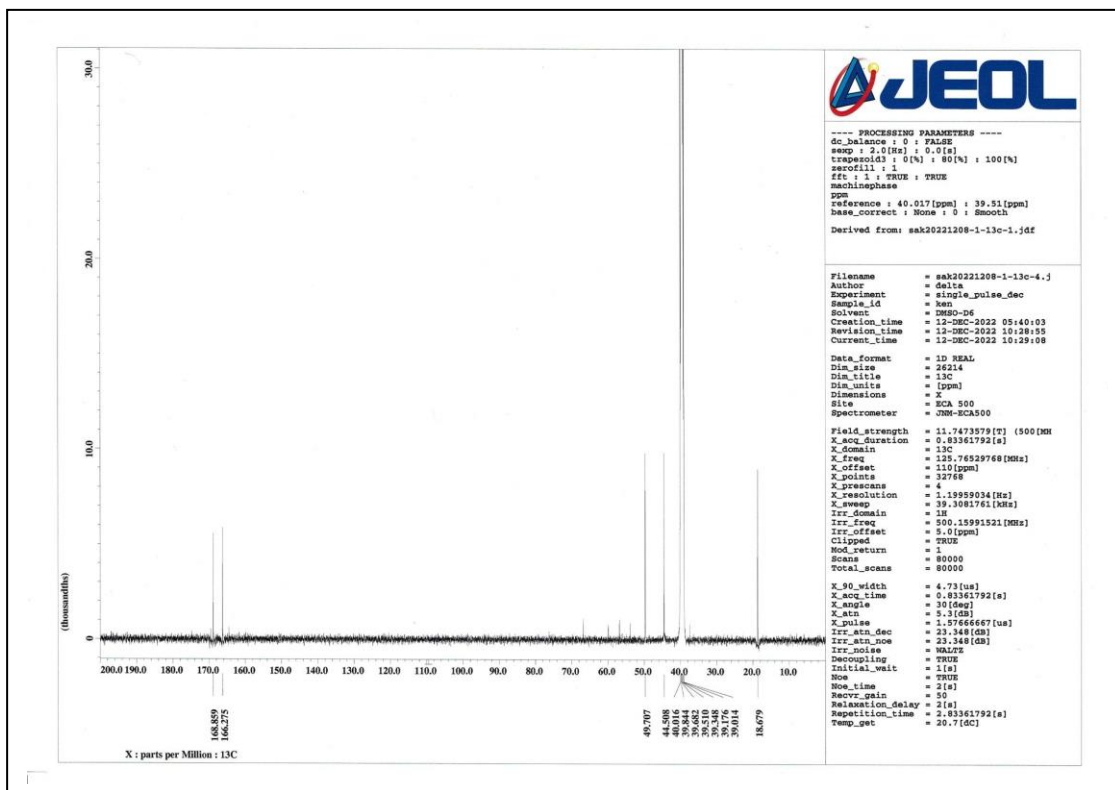


Figure S3. ^{13}C NMR spectrum of the active component in $\text{DMSO-}d_6$ (125 MHz)

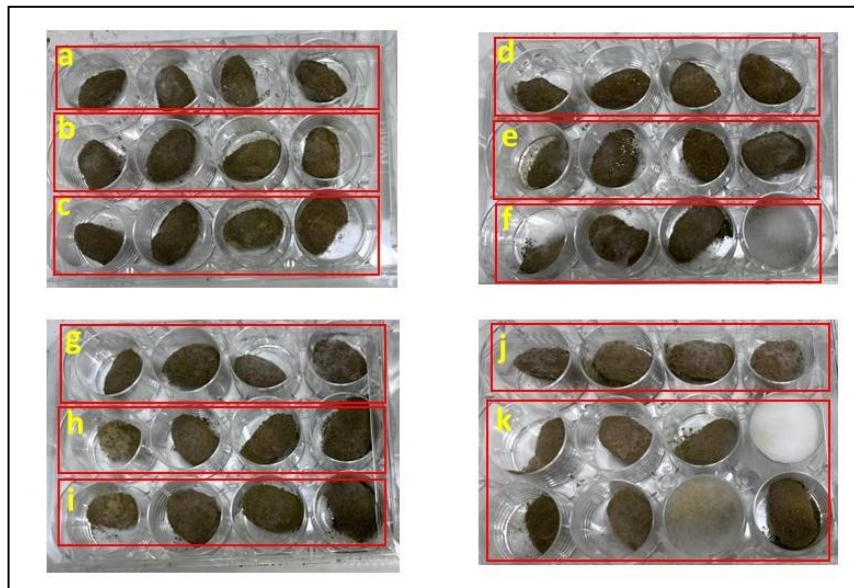


Figure S4. Effect of the strain KTTM on the growth of *A. flavus* on peanuts. Autoclaved law peanuts without shells and skins were dipped for a few seconds in the culture broth of the strain KTTM (a, 6.2×10^8 cells/mL) or a 10-time dilution series of dilutions of the culture broth (b, 6.2×10^7 cells/mL; c, 6.2×10^6 cells/mL; d, 6.2×10^5 cells/mL; e, 6.2×10^4 cells/mL; f, 6.2×10^3 cells/mL; g, 6.2×10^2 cells/mL; h, 6.2×10 cells/mL; i, 6.2 cells/mL; and j, <1 cell/mL), with a liquid Bennet medium used as the control (k), and incubated at 25 °C for 30 days.

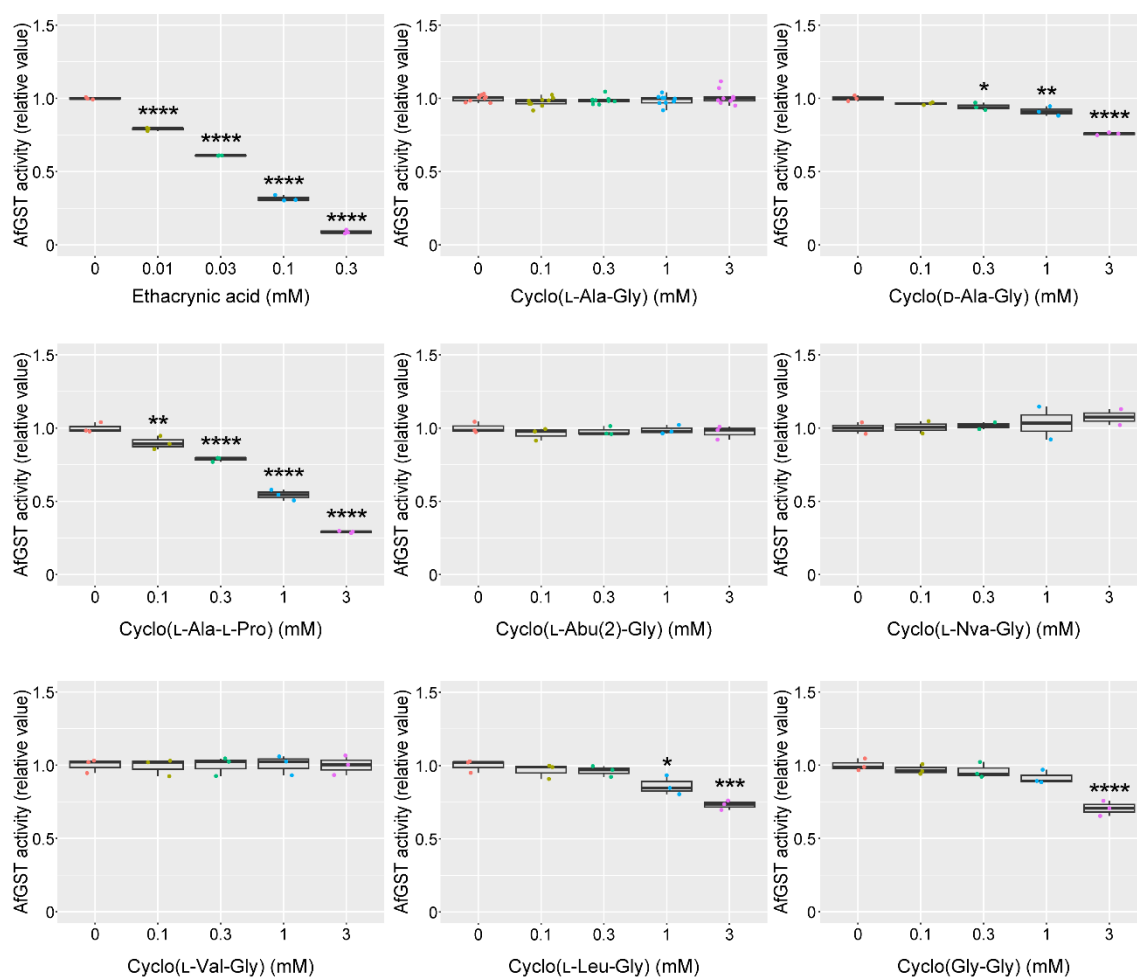


Figure S5. Effects of cyclo(L-Ala-Gly) and related compounds on *A. flavus* glutathione-S-transferase activity
 Boxplots of the relative activity of *A. flavus* glutathione-S-transferase (AfGST) when treated with ethacrynic acid and diketopiperazines. The colored dots indicate individual values. $n = 4$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ versus no added control, ordinary one-way ANOVA followed by Dunnett's test.