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(12) (B1)

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(24)

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2004 11 24

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(43)

10-2002-0049443
2002 06 26

(73) 375

(72) 104 603

1329-2 105 801

502 101 1410

244 506 1313

4 1 601

876-3

2 3 301

(74)

:

(54) L 1

L1) (amino-terminal)
(*Helicobacter pylori*)
RPL1
HP (2-20)
2

L1(ribosomal protein L1, RP
(amphipathic) 가

5

1 L1(ribosomal protein L1) HP (2-20)
 (diagram) ,
 2 (*Bacillus subtilis*) LB 가(agar)
 ,
 A : (no treatment)
 B : HP (2-20)
 C : 2
 3 (*Escherichia coli*) LB 가
 ,
 A : (no treatment)
 B : HP (2-20)
 C : 2
 4 (*Candida albicans*) PDB 가
 ,
 A : HP (2-20)
 B : (Melittin)
 C : 2
 5
 A : AML-2/WT
 B : SNU 638
 C : SNU 668
 : HP(2-20)
 : 2
 :

(*Helicobacter pylori*) L1(ribosomal protein L1,
 'RPL1') HP (2-20)
 , RPL1 가 2
 , 가
 (resistance) ,
 가 가
 (*Enterococcus faecalis*) , (*Mycobacterium tuberculosis*)
 (*Pseudomonas aeruginosa*) ()
 Stuart B. Levy, *Scientific American* , 46-53, 1998).
 (tolerance) (resistance) , 1970 (Tom
 (*Pneumococcus sp.*)
 asz et al., *Nature* , 227, 138-140, 1970).
 가 (autolytic) , (autolysin) 가
 (endogenous hydrolytic enzyme)

가 (Handwerger and Tomasz, *Rev. Infec. Dis.* , 7, 368-386, 1985).

가 (Liu and Tomasz, *J. Infect. Dis.* , 152, 365-372, 1985),

가 (phenotypic) 가 (Tuomanen E., *Revs. Infec. Dis.* , 3, S279-S291, 1986),

(down regulation)

가 , 가 (Tuomanen et al., *J. infect. Dis.* , 158, 36-43, 1988).

riocin) (nonlantibiotics) (signal peptide) (lantibiotics) (Cintas et al., *J. Bad.* , (Bevins et al., *Ann. Rev. Biochem.* , 59, 395-414, 1990),

(cysteine-rich) - (sheet) (helical) (proline-rich) (Mayasaki et al., *Int. J. Antimicrob. Agents* , 9, 269-280, 1998).

(Boman, H. G., *Cell* , 65:205, 1991; Boman, H. G., *Annu. Rev. Microbiol.* , 13:61, 1995).

(cecropin)

(cysteine) 가

(Blaser, MJ., *Trends Microbiol.* , 1, 255-260, 1991)

가

RPL1

230

8

가 (Putsep, K. et al., *Nature* , 398, 671-672, 1999),

RPL1 (Putsep K. et al., *Nature* , 398, 671-672, 1999).

가 가 (Putsep K. et al., *Nature* , 398, 671-672, 1999).

RPL1

가

()

가

(amino-terminal)

(*Helicobacter pylori*)

RPL1

RPL1

HP(2-20) RPL1 가

Fmoc (Merrifield, RB., *J. Am. Chem. Soc.* , 85, 2149, 1963) 1 (Merrifield) HP (2

-20) 2 17 19 (1).

95% , MALDI(Matrix-Assisted Laser Desorption Ionization)

가 2 가 1 HP (2-20)

17 19

가 가 HP (2-20) 8 10

20

가 가

(minimal inhibitory concentration, 'MIC')

s) , (*Bacillus subtilis*) (*Escherichia coli*) 가 (*Staphylococcus aureu*)

2 HP (2-20) 10 (1).

LB 가 (2 3)

HP (2-20)

(*Trichosporon beigelii*) (*Candida*)

MIC MTT (*Saccharomyces ce*)

HP (2-20) 4 2

(4) .

AML-2/WT SNU 638 SNU 668 , HP (2-20)

2 (5) .

가 가

, HP (2-20) 2 (3) .

2

2 가 가

가 가

(Propylene glycol),

ol), (tween) 61, (carrier) (ascorbic acid) (antioxidants), (chelating agents), (stabilizers) 0.1 2 mg/kg 0.5
 1 mg/kg 1 3 2 (infusion) (single dose) (bolus)
 (multiple dose) (fractionated treatment protocol)

< 1> RPL1 HP(2-20) HP (2-20) 17
 19 Fmoc(9-fluorenylmethoxycarbonyl) (Merrifield)
 (Merrifield, RB., *J. Am. Chem. Soc.*, 85, 2149, 1963)(1).
 -NH₂ Rink Amide MBHA-Resin
 -OH Fmoc- (chain) -Wang Resin
 Fmoc- (coupling) DCC(N-hydroxybenzo tri
 azole (HOBt)-dicyclo-hexycarbodiimide) Fmoc-
 omethane) , 20% NMP(piperidine /N-methyl pyrrolidone) Fmoc NMP DCM(dichor
 e-H₂O-triisopropylsilane(85: 5: 5: 2.5: 2.5, vol./vol.) 가 2 3
 (diethylether)
 (crude) 0.05% TFA가 (acetonitrle gradient) ()
 reverse phase, RP)-HPLC (Delta Pak, C₁₈ 300 , 15, 19.0mm x30 cm, Waters)
 6 N HCl 110 가 0.02 N HCl
 (Hitachi 8500 A)
 95% , MALDI (Hill
 , et al ., *Rapid Commun. Mass Spectrometry* , 5, 395, 1991)
 가 가

< 2>
 <2-1> 1 MIC 가
 (KCTC 1621) , (KCTC 1682) (KCTC 1918)
 가 (KCTC 2433)
 , 1%) - (mid-log phase) LB (1% , 0.5%
 1% 1x10⁴ /100 μℓ
 6 (Melittin) 25 μ M/ (well) 1/2 1 가 37
 MIC , 1 620 nm

[1]

	(μ M)		
	B .	S .	P . 가

HP (2-20)	3.12	12.5	12.5	6.25
2	0.39	0.78	1.56	0.39
	0.19	0.78	1.56	1.56

가 , HP (2-20) 가 HP (2-20) 10 P.
 <2-2> 가 가 가 B. LB (1
 % , 0.5% , 1%) 가 , 37 2 . 4x10⁵
 B. 4 μM 1 μM 가
 LB 가 (2 B.) (colony)
 가 (2 A) HP (2-20) 가 (2 B) (colony) (2 C).
 A) HP (2-20) 가 (3 B), 가 (3 C).
 가 HP (2-20)

< 3>
 <3-1> MTT

768), (KCTC 7707) , 96- (KCTC 7296) (TIMM 1
) 100 μℓ PDB (20% MTT
 가 , 5 mg/Mℓ MTT (3- [4,5- dimethyl- 2- thiazolyl] -2,5- diphenyl- 2H- tetrazoliu
 m bromide) 10 μℓ 가 5-6 (isopropanol) 100 μℓ ELISA
 (reader) (formazan) 0.04 N HCl- MIC 가
2

[2]

	(μ M)		
	C .	S .	T .
HP (2-20)	25.0	25.0	12.5 25.0
2	6.25	3.12 6.25	3.12
	3.12	3.12	3.12 6.25

가 , **2** HP (2-20) 4 HP (2-20) S. T. C
 . 4 8 가 ,
 <3-2> 가 가 , C. PDB
 2x10³ 20 μM 가 28 3 , PDB 가
 가 HP (2-20) 가 (4
 A) (4 B 4 C). 가 가

HP (2-20)

< 4>

SNU 638 5x10⁴ /Mℓ SNU 668 SNU 638 MTT SNU 668 90 μℓ 0.04 N HCl- 2x10⁵ 96- CO₂ /Mℓ AML-2/WT AML-2/WT 100 μℓ ELISA 540 nm 가

5

10 μM HP (2-20) 1 μM 가 가

< 5>

8% 가 (PBS, pH 7.0) 12.5 μM/ 1/2 37 1 , 1,000 g 414 nm 1% X-100 100% 1 % -X100 가 , 1% X-100 100% 1

1

$$\% \text{ 적혈구 파괴능 (hemolysis)} = \left(\frac{\text{흡광도 } A - \text{흡광도 } B}{\text{흡광도 } C - \text{흡광도 } B} \right) \times 100$$

A 414 nm 1% X-100 B 414 nm PBS C 414 nm 3

[3]

	% (μM)							
	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.097
HP (2-20)	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
	100	100	95	93	31	0	0	0

HP (2-20) 2

< 6>

6 (SPF) SD 2 0.5% 1 g/kg/15 Mℓ

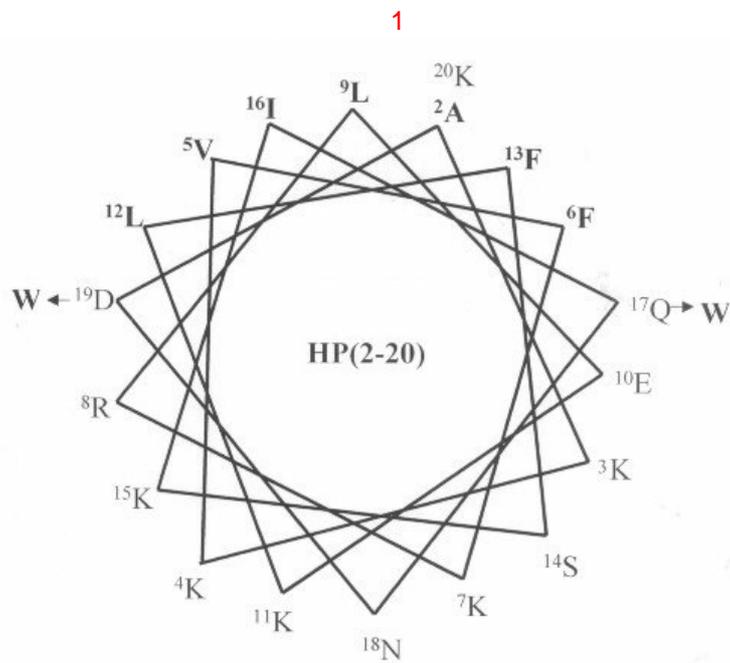
5 mg/kg

(LD₅₀) 10 mg/kg

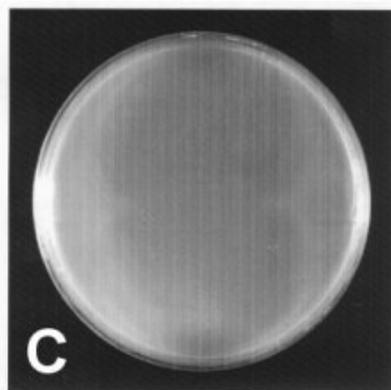
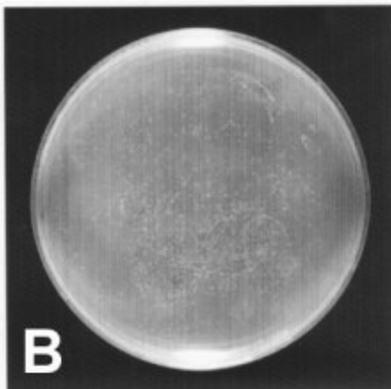
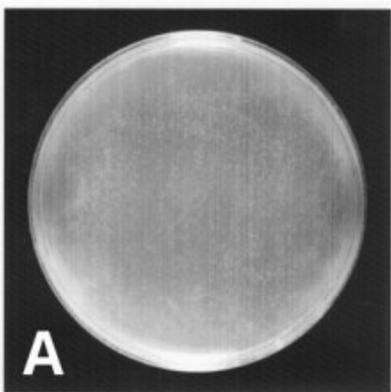
2

(57)

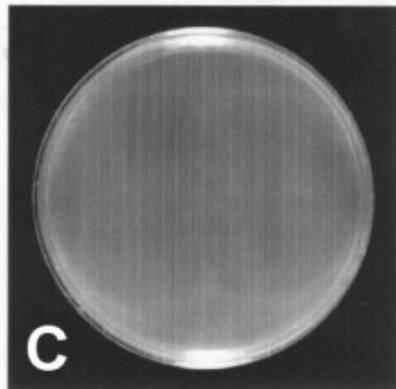
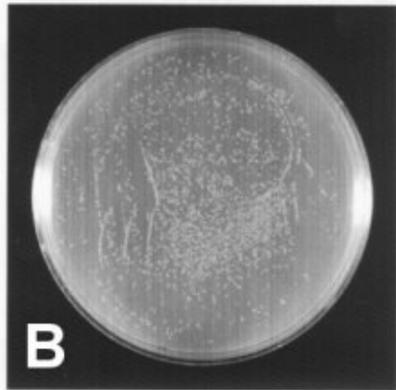
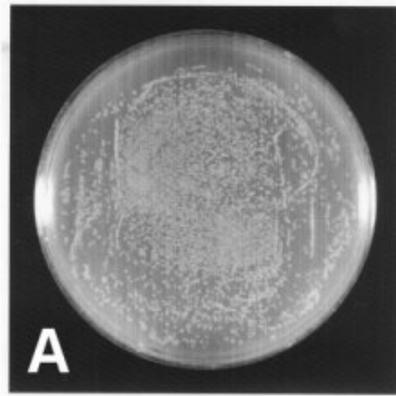
- 1. 1 가 HP (2-20) 17 가 가 19
- 가 2
- 2.
- 3.
- 4.
- 5.
- 6.
- 7.
- 1 8.
- 1 9.



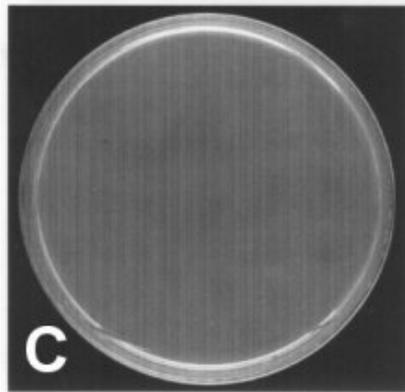
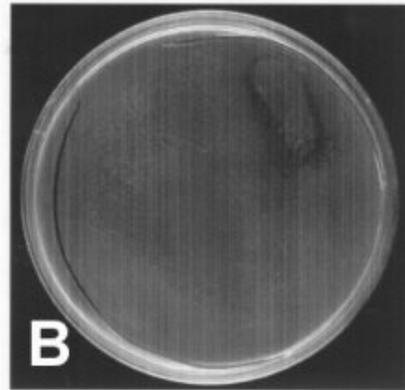
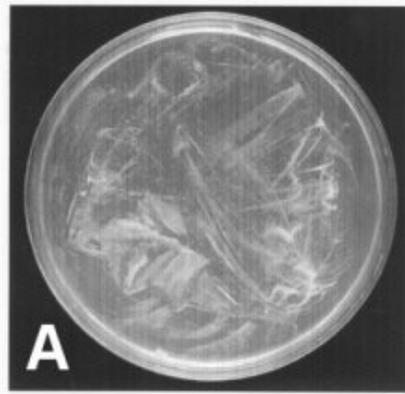
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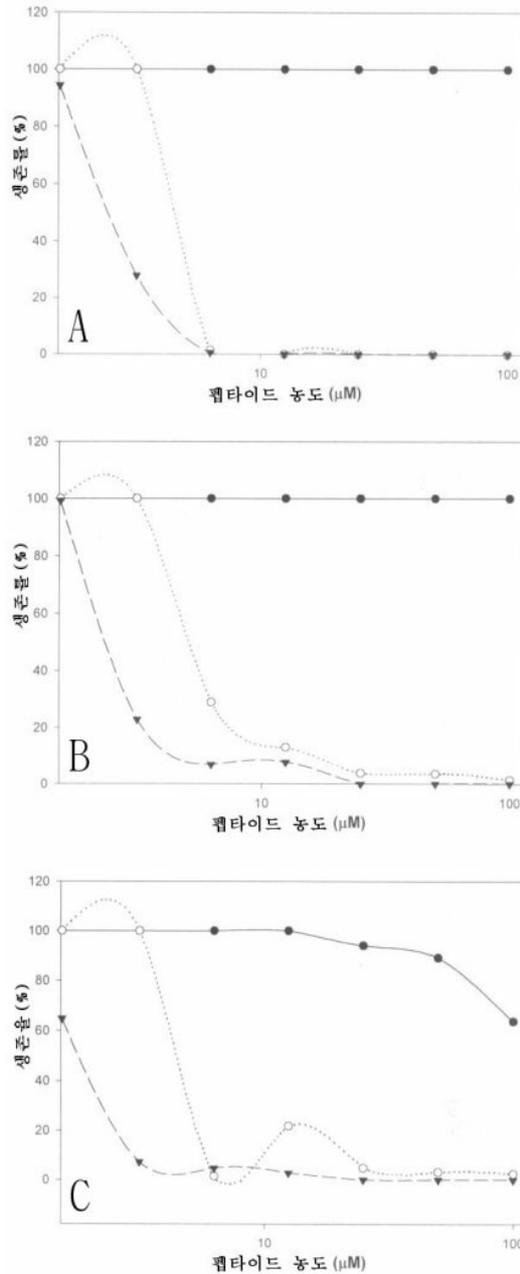
3



4



5



<110> HAHM, Kyung-Soo <120> Novel antibiotic peptide derived from ribosomal
 protein L1 of *Helicobacter pylori* and use thereof <160> 2 <170> KopatentIn 1.55 <210>
 1 <211> 19 <212> PRT <213> *Helicobacter pylori* <220> <221> PEPTIDE <222> (1)..(19) <223>
 2-20 amino acid sequence of RPL1 protein from amino-terminal end <400> 1 Ala Lys Lys Val Phe Lys
 Arg Leu Glu Lys Leu Phe Ser Lys Ile Gln 1 5 10 15 Asn
 Asp Lys <210> 2 <211> 19 <212> PRT <213> *Helicobacter pylori* <400> 2 Ala Lys Lys Val Phe
 Lys Arg Leu Glu Lys Leu Phe Ser Lys Ile Trp 1 5 10 15
 Asn Trp Lys