Int J Clin Exp Med 2017;10(1):795-801
www.ijcem.com /ISSN:1940-5901/IJCEM0039888

Original Article

Kinetin inhibits growth of *Helicobacter pylori* by down-regulation of replication genes

Min Ho Lee[1], Hye Jin Kwon[1], Do Hyun Kim[1], Ji Yeong Yang[1], Yoonjung Cho[1], Hyun Jun Woo[1], Min Ji Yeon[1], Min Park[1], Cheol Moon[2], Sa-Hyun Kim[2], Jong-Bae Kim[1]

[1]*Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju 26493, Republic of Korea;* [2]*Department of Clinical Laboratory Science, Semyung University, Jaecheon 27136, Republic of Korea*

Received July 11, 2016; Accepted October 20, 2016; Epub January 15, 2017; Published January 30, 2017

Abstract: *H. pylori* is a Gram-negative curved bacterium which primarily colonizes the epithelial layer of human stomach. Infection with this bacterium may lead to various gastric diseases from asymptomatic gastritis to gastric cancer. Because of its clinical importance, WHO designated *H. pylori* as a class I carcinogen. Kinetin is a type of cytokinins major role of which is to facilitate growth of the plants but it also plays a role to protect plants from infections. However, anti-bacterial effect of kinetin on *H. pylori* has not been elucidated. In this study, we performed agar dilution test and broth dilution test to determine MIC of kinetin against an *H. pylori* reference strain. In particular, we isolated *H. pylori* from gastric biopsies and confirmed inhibitory effect of kinetin against 60 clinical isolates of *H. pylori*. To elucidate the inhibitory mechanism of kinetin on *H. pylori* growth, we performed RT-PCR and found that kinetin down-regulated *dna*A and *hol*B expression both of which are necessary for bacterial replication. We also confirmed kinetin did not show cytotoxicity to gastric epithelial cells (AGS) with the dose completely inhibited *H. pylori* growth and kinetin did not inhibit the eukaryotic replication machinery. Moreover, kinetin reduced *H. pylori*-induced death of gastric cell line *in vitro*. Many recent reports are alarming the increased antibiotic resistance of *H. pylori* and limitation of current regimen for *H. pylori* eradication indicating necessity of new therapeutic agents. Although further studies seem to be necessary, our study collectively shows that kinetin inhibits growth of *H. pylori* by inhibiting replication of the bacteria and suggests kinetin as one of the potential candidates for *H. pylori* eradication.

Keywords: Kinetin, *H. pylori*, *dna*A, *hol*B

Introduction

Helicobacter pylori is a Gram-negative and spiral shaped bacterium. *H. pylori* colonizes the epithelial layer of human stomach for many years and it has been reported to infect more than half of the mankind [1]. Infection with this bacterium may lead to various gastric diseases from asymptomatic gastritis to gastric cancer [2]. Particularly, it has been proposed to act as a carcinogen causing gastric cancer [3]. Because of its clinical importance, WHO designated *H. pylori* as a class I carcinogen [3].

Kinetin is a type of cytokinins, a plant growth substance that plays a major role in cell division and cell differentiation [4]. Kinetin was isolated and identified by Miller and Skoog *et al.* as a compound from autoclaved herring sperm DNA that had cell division-promoting activity [5]. Although the major role of cytokinins is to facilitate growth of the plants, cytokinins such as kinetin also play a role to protect plants from infections. It was reported that kinetin has antifungal function by suppressing fungus spore germination in *Harpophoramaydis* [6]. In addition, cytokinins also reported to inhibit bacterial growth and virulence gene expression of *Agrobacterium tumefaciens* [7].

DNA replication is a biological step and occurs in all living organisms. In prokaryotes, DNA replication can be divided into stages: initiation, elongation, and termination [8]. At the initial phase, DnaA which activates initiation of DNA replication forms complex with *ori*C site, which has specific AT rich DNA unwinding element (DUE) regions [8]. Once bound to ssDUE, DnaA

provides docking platform for DnaB helicases to load on to the unwound region [8]. DnaB migrates along the single-stranded DNA in the 5' to 3' direction, unwinding the DNA as it travels [9]. The elongation phase begins with loading of DNA polymerase III at the 3' termini of the primer synthesized by DnaG [9]. DNA polymerase III consists of multicomponents including core polymerases (DnaE, DnaQ and HolE), sliding clamp (DnaN) and multiprotein clamploader (DnaX, HolA, HolB, HolC and HolD) all of which are necessary to appropriately function as a single machinery [9]. After polymerization of DNA, termination of the replication process involves the interaction with Ter protein which binds to a termination site sequence in the DNA to physically stop DNA replication, followed by chromosome segregation [9].

Only a few studies have been reported protective effect of cytokinins from infection, and especially anti-bacterial effect of cytokinins on clinically important bacteria is yet to be studied. In this study, therefore, we demonstrated anti-bacterial effect of kinetin on *H. pylori* and investigated its involved mechanism.

Materials and methods

Materials

H. pylori reference strain was purchased from ATCC (ATCC49503, Manassas, VA, USA). Mueller-Hinton broth, Mueller-Hinton agar and Brucella agar were purchased from Becton-Dickinson (Braintree, MA, USA). Bovine serum was purchased from Gibco (Long Island, NY, USA). Trizol reagent, random hexamer, and Moloneymurine leukemia virus reverse transcriptase (MMLV-RT) were purchased from Invitrogen (Carlsbad, CA, USA). Kinetin was obtained from Sigma-Aldrich (Saint Louis, MO, USA). EZ-Cytox cell viability assay kit was purchased from Daeil Lab Service (Seoul, Korea).

Bacterial culture

Brucella agar plate supplemented with 10% bovine serum was used to grow *H. pylori*. The bacteria were incubated at 37°C for 72 h in a humidified atmosphere with 10% CO_2. To cultivate bacteria in broth, bacterial colonies were collected and grown in Mueller-Hinton broth supplemented with 10% bovine serum. Turbidity of the bacterial suspension was set to MacFarland 0.33 (1×10^8 / mL) and incubated at 37°C for 72 h in a humidified atmosphere with 10% CO_2.

Agar dilution method to determine MIC

H. pylori were grown on the Brucella agar plate then the bacterial colonies were collected and suspended in saline. Turbidity of the bacterial suspension was set to MacFarland 4.0 (1.2×10^9/mL). Kinetin was 2-fold serially diluted in Mueller-Hinton agar supplemented with 10% bovine serum to prepare the media including kinetin in a range of concentrations (0, 31.2, 62.5, 125, 250, and 500 μM). After preparation of the media, 30 μl of the bacterial suspension was placed on the media. The bacteria were incubated for 72 h and growth of the bacterial colonies was observed. The lowest concentration that completely inhibited growth of the bacterial colony was determined as MIC (minimal inhibitory concentration).

Broth dilution method to determine MIC

H. pylori suspension turbidity of which was set to MacFarland scale 0.5 was prepared in Mueller-Hinton broth supplemented with 10% bovine serum. Various concentrations of kinetin were added and the bacteria were incubated at 37°C for 72 h in a humidified atmosphere with 10% CO_2. After 72 h, the final optical density (600 nm wavelength) of the bacterial suspension was measured by spectrophotometry.

Collection of H. pylori clinical isolates

Specimen collection was conducted at Yong-In Severance Hospital in Korea. *H. pylori* strains, isolated from 60 patients conducted gastroscopic examination to confirm the infection of *H. pylori*, were evaluated. Strains were identified as *H. pylori* by colony morphology analysis and urease test. The MICs were determined using a slightly modified agar dilution method (Mueller-Hinton agar base containing 10% bovine serum).

RT-PCR (reverse transcription-polymerase chain reaction)

Cultured *H. pylori* were collected and washed twice with PBS. After washing, total RNA was extracted by using Trizol reagent as described in the manufacturer's instructions. cDNA was synthesized by reverse transcription and subjected to PCR amplification as described in a

Table 1. List of primer sequences and conditions used for RT-PCR

Primers	Sequences (5'-3')		Product length (bp)	Annealing temperature (°C)	Cycles
	Forward	Reverse			
dnaA	GGGCATGACTTTAGCGGTTA	TTAACGAATTGCACGCCAAC	128	55	25
dnaB	AATGGGCCGTTTATCGTCTC	CAAATCCGCTTGCAACTACG	231	55	24
dnaE	AATCCACCGGCTCCAAATAC	GCCAAACAAGTGTGGGAGTA	184	55	22
dnaN	GTTAGCGGTGGTTGAAAACG	CGGTTTCGCTATGCTCAGAA	233	55	22
dnaQ	CGCATGAAGCTTTGCAAGAA	GCATAGGCTCTATGGCTGAC	244	55	25
holB	TGCAAGCCTTTTTGAACACC	CGCGTTTTGGGCTTCTATAC	196	55	24
ORC1	CTGGTGCAGGTTAGAGGTG	GGTAGTGCAGTTTTCGATCC	189	66	29
PCNA	TTCCCTTACGCAAGTCTCAGCC	GGTTTACACCGCTGGAGCTAATA	238	52	31
GAPDH	CGGGAAGCTTGTCATCAATGG	GGCAGTGATGGCATGGACTG	349	55	21

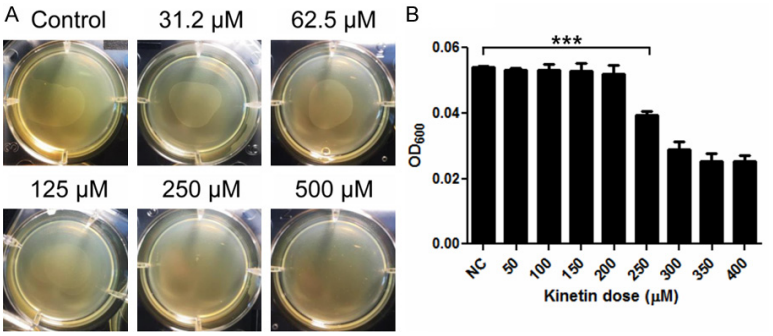

Figure 1. Determination of the MIC of kinetin on a *H. pylori* ATCC49503 strain by agar dilution and broth dilution method. A. *H. pylori* was grown on the Mueller-Hinton agar including indicated concentrations of kinetin. MIC of kinetin against *H. pylori* was determined after 72 h of incubation. B. *H. pylori* was grown on the Mueller-Hinton broth including indicated concentrations of kinetin. After 72 h of incubation, final optical density of the bacterial suspension was measured at 600 nm wavelength by spectrophotometry and MIC was determined. Data were from triplicate experiments and analyzed by Student's t-test (**P* < 0.05, ***P* < 0.01 and ****P* < 0.001).

Table 2. MIC of kinetin against the *H. pylori* clinical isolates

MIC (μM)	Number of bacteria (n = 60)
< 15.6	1 (1.7%)
31.3	1 (1.7%)
62.5	15 (25.0%)
125	26 (43.3%)
250	16 (26.7%)
500	1 (1.7%)

previous report [10]. The PCR primer sequences and PCR conditions used in this study are described in **Table 1**.

Western blotting

Cells were washed with PBS and then lysed at 4°C with lysis buffer containing 1% TritonX-100, protease inhibitor cocktail, and PBS. Lysates were centrifuged and the supernatants were subjected to Westernblot as described previously [11].

Statistical analysis

Data in the bar graphs are presented as mean ± standard error of mean (SEM). All the statistical analyses were performed using GraphPad Prism 5.02 software (GraphPad Software, San Diego, CA, USA). All the data were analyzed by unpaired Student's t-test and $P < 0.05$ was considered to be statistically significant (**P* < 0.05, ***P* < 0.01 and ****P* < 0.001).

Cell viability assay

AGS cells (1.5×10^4 per well) were plated in 96-well plates and incubated for 24 h before they were treated with various concentrations of kinetin. The cells were then further incubated for 24 h and subjected to WST (water soluble tetrazolium salts) assay by using EZ-Cytox cell viability assay kit according to manufacturer's instruction. Briefly, 10 μl of WST solution was added to the cultured media and incubated in the CO_2 incubator for 1 h. Absorbance of the supernatant at 450 nm wavelength was measured by spectrophotometer.

Results

Inhibitory effect of kinetin on the growth of H. pylori

To determine the MIC of kinetin on *H. pylori*, we performed agar dilution test. Mueller-Hinton

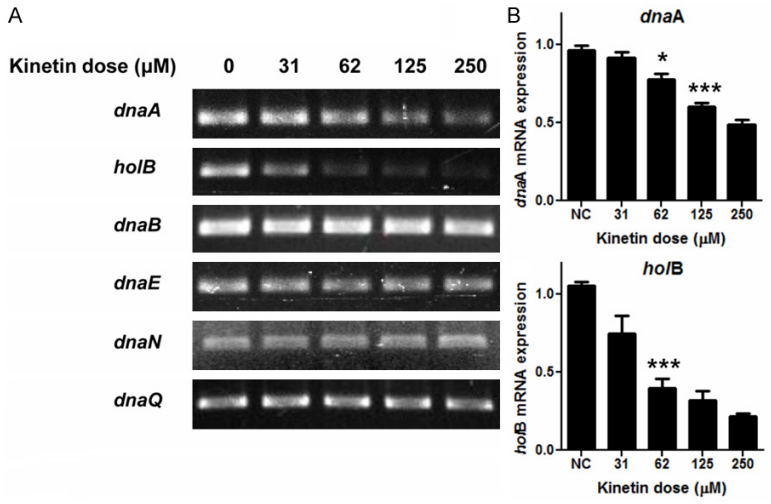

Figure 2. Effect of kinetin on the expression of replication machineries in *H. pylori*. A. *H. pylori* was treated with indicated concentrations of kinetin for 72 h and RNA was harvested. Collected samples were subjected to RT-PCR to detect mRNA expression level of replication machineries (*dna*A, *hol*B, *dna*B, *dna*E, *dna*N, *dna*Q and *pol*A). B. Density of *dna*A and *hol*B bands were measured and illustrated as graphs. Data were from triplicate experiments and analyzed by Student's t-test (**P* < 0.05, ***P* < 0.01 and ****P* < 0.001).

agar including various concentrations of kinetin was prepared. An *H. pylori* reference strain (ATCC49503) was suspended in PBS and 30 μl of the bacterial suspension set to McFarland scale 4.0 was placed on the agar, then MIC was defined after 72 h of incubation. In the agar dilution test result, MIC of kinetin against *H. pylori* was 250 μM (**Figure 1A**). To confirm whether kinetin can inhibit *H. pylori* growth not only in the reference strains but also in the clinical isolates, we collected clinical isolates of *H. pylori* from gastric biopsies and determined MIC of kinetin. Among the 60 clinical isolates, MIC of 26.7% (16/60) was 250 μM, 43.3% (26/60) was 125 μM and 25.0% (15/60) was 62.5 μM (**Table 2**). In addition, we also determined MIC of kinetin by broth dilution method because bacteria were grown in the broth condition in the next step of our experiment. We found that MIC in the broth dilution test was 250 μM (**Figure 1B**). Based on these results, we demonstrated kinetin has anti-bacterial effect on *H. pylori* and also determined MIC. In particular, we showed anti-bacterial effect of kinetin was equally applied on the clinical isolates of *H. pylori* as well as the reference strain.

Kinetin down-regulates dnaA and holB expression in H. pylori

To elucidate the mechanism by which kinetin inhibits growth of *H. pylori*, we investigated whether kinetin has an influence on the replication machinery of the bacteria. *H. pylori* was treated with kinetin and incubated in brucella broth supplemented with 10% bovine serum for 72 h. We collected RNA and performed RT-PCR to investigate the expression of the genes involved in the replication of *H. pylori*. In the result, we found that *dna*A and *hol*B mRNA expression levels decreased in a kinetin dose dependent manner, though expression of the other genes remained constant (**Figure 2A**). Kinetin induced reduction of *dna*A and *hol*B expression was statistically significant ($P = 0.0173$ and $P = 0.0006$) in the dose with 62 μM or above (**Figure 2B**). Both *dna*A and *hol*B, which are replication initiation protein and clamp-loader protein, are important proteins for bacterial replication, thus our result infers that kinetin inhibits *H. pylori* growth by down-regulating *dna*A and *hol*B expression.

Kinetin inhibits H. pylori-induced cell death of gastric cell line in an in vitro cell culture model

To further investigate whether kinetin can inhibit *H. pylori* growth and infection to gastric epithelial cells, we adopted *in vitro* model of *H. pylori* infection. We used AGS (gastric adenocarcinoma cell line) in this experiment because *H. pylori* colonizes on the human gastric epithelium. AGS cells were infected with *H. pylori* and treated with kinetin at the same time for 24 h. After incubation we observed cell viability and found that *H. pylori* infection (100 MOI) reduced cell viability of AGS cells (53.87%) but kinetin treatment (250 μM) partially recovered cell viability (76.12%) (**Figure 3A**). In the Western blotting result, PARP was cleaved by *H. pylori* infection but it was inhibited by kinetin treatment (**Figure 3B**). Based on our results, kinetin inhibited growth of *H. pylori* and the corresponding dose of kinetin rescued AGS cells from *H. pylori* induced cell death, thus our results collectively indicate that kinetin might inhibit infection of *H. pylori* by bacterial growth thereby reduced *H. pylori*-induced death of gas-

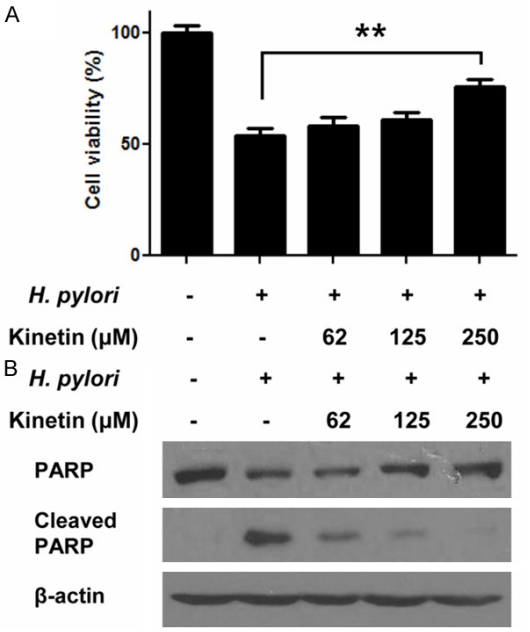

Figure 3. Inhibitory effect of kinetin on the *H. pylori* induced cell death in a gastric cell line. (A) AGS cells were infected with *H. pylori* (100 MOI) and treated with indicated concentrations of kinetin for 24 h. After incubation, cell viability was measured by WST assay. (B) AGS cells were treated as in (A) and the cell lysates were collected to conduct Westernblotting. Full-length PARP (116 KDa) and cleaved PARP (89 KDa) bands were detected by using specific antibody. β-actin was used as an internal control. Data were from triplicate experiments and analyzed by Student's t-test (**P* < 0.05, ***P* < 0.01 and ****P* < 0.001).

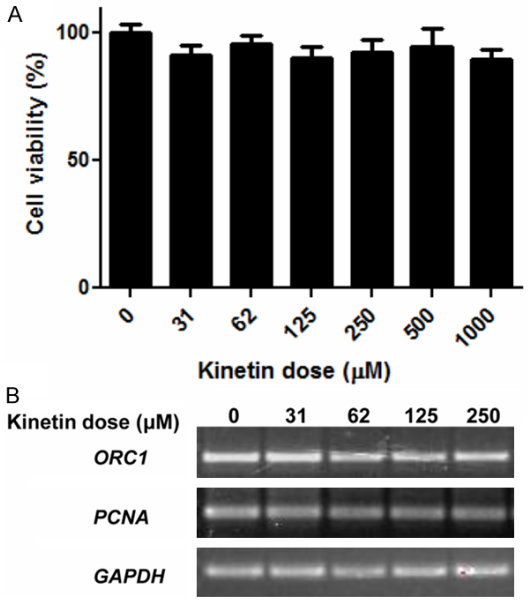

Figure 4. Evaluation of the cytotoxicity of kinetin on AGS cells. A. AGS cells were treated with indicated concentrations of kinetin and cell viability was measured by WST assay. B. AGS cells were treated with indicated concentrations of kinetin and RNA was harvested. Collected RNA samples were subjected to RT-PCR to detect mRNA expression level of eukaryotic replication machineries (*ORC1* and *PCNA*).

tric epithelial cells. In addition, we evaluated whether kinetin has cytotoxicity on human cells. AGS cells were treated with various concentrations of kinetin and cell viability was measured by WST based cell viability assay. Our result showed that 250 μM of kinetin decreased cell viability of AGS 7.4% and 1000 mM of kinetin decreased cell viability approximately 10.5%, nevertheless, the decrease of cell viability were statistically not significant (**Figure 4A**). We further investigated whether kinetin also inhibits the replication machinery of eukaryotic cells. Therefore, we performed RT-PCR for those genes in eukaryotic cells corresponding to prokaryotic replication machinery. In our result, mRNA expressions of *ORC1* (origin replication complex) and *PCNA* (proliferating cell nuclear antigen) genes were not changed by kinetin treatment in AGS cells (**Figure 4B**). These results indicate that kinetin does not show cytotoxicity on AGS cells in the dose completely inhibit the growth of *H. pylori*.

Discussion

H. pylori is one of the clinically important bacteria because it causes various gastric disorders. We have demonstrated inhibitory effect of kinetin on *H. pylori* for the first time in this study. Here we have determined MIC of kinetin against an *H. pylori* reference strain by agar dilution test and broth dilution test. Furthermore, we isolated *H. pylori* from gastric biopsies and confirmed inhibitory effect of kinetin against 60 clinical isolates of *H. pylori*. Our study also has shown the putative inhibitory mechanism of kinetin on *H. pylori* growth. In our results, kinetin down-regulated *dna*A and *hol*B expression both of which are necessary for bacterial replication. In the *in vitro H. pylori* infection model, kinetin reduced gastric epithelial cell (AGS) death which was induced by *H. pylori* infection. In addition, kinetin did not inhibit the eukaryotic replication machinery corresponding to prokaryotic *dna*A and *hol*B and we also confirmed kinetin did not show cytotoxicity to gastric epithelial cells in the dose completely inhibited *H. pylori* growth.

As many virulence factors in *H. pylori* are associated with successful infection of the bacteria

or bacteria-induced pathogenesis, down-regulation of virulence factors may decrease chance for *H. pylori* to colonize on the host gastric epithelium as well as reducing pathogenesis by the bacteria. The most investigated virulence factors of *H. pylori* are CagA and VacA toxins. CagA and VacA toxins are closely associated with tumorigenesis by *H. pylori*. They disrupt intracellular signaling in host cells that lead to uncontrolled growth of the cells and inflammatory responses. However, kinetin did not inhibit expression of these toxins in our study (data not shown).

H. pylori is a gram-negative curved bacterium associated with the carcinogenesis on the stomach, thus World Health Organization classified *H. pylori* as a class I carcinogen [12]. Chronic infection of *H. pylori* on the stomach has been reported to induce various gastric diseases including gastritis, gastric ulcer and gastric cancer in severe case [12]. According to the global cancer statistics, 14.1 million new cancer cases have been occurred in 2012 and 8.2 million were died with cancer worldwide [13]. Among the cancer cases, gastric cancer was the fourth prevalent (8.5%) type worldwide and 35.4% of gastric cancer has occurred in Eastern Asia [13, 14]. Various reasons have been suggested to be responsible for gastric cancer rate such as dietary patterns, food storage, availability of fresh produces and especially prevalence of *H. pylori* infection [13, 14]. It is estimated that more than half of the adult population is infected with *H. pylori* worldwide and infection of which is responsible for 75% of all the gastric cancer cases [15]. Therefore, a lot of efforts for eradication of *H. pylori* infection still seem to be necessary for health promotion worldwide.

Triple therapy (clarithromycin, amoxicillin and proton pump inhibitor) is currently recommended for eradication of *H. pylori* as a first-line regimen [16]. However, numerous reports have informed the prevalence of clarithromycin resistance worldwide and decreased eradication rate of *H. pylori* by first-line therapyalarming the limitation of current empirical regimen being used for eradication of *H. pylori* [17-19]. Especially, clarithromycin resistance in Asia has been significantly increased to 32.46% in 2014 [19]. Moreover, levofloxacin resistance rate has been increased and resistance to metronidazole also showed to be high in *H. pylori* [19]. Collectively all these reports illuminate the seriousness of the antibiotic resistance and importance of appropriate selection for antibiotic regimen as well as discovery of a new therapeutic agent for eradication of *H. pylori*. Therefore, we believe that discovery of natural compounds inhibiting *H. pylori* growth such as kinetin is important and continuous researches are necessary for the development of supportive agents to increase eradication of *H. pylori*. Further studies seem to be necessary to completely understand the inhibitory mechanism of kinetin on *H. pylori*, and use of animal models also needs to evaluate the safety and effectiveness of kinetin *in vivo*.

Acknowledgements

This paper was supported by the Semyung University Research Grant of 2015.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jong-Bae Kim, Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju 26493, Republic of Korea. Tel: +82-33-760-2423; Fax: +82-33-760-2561; E-mail: kimjb70@yonsei.ac.kr; Dr. Sa-Hyun Kim, Department of Clinical Laboratory Science, Semyung University, Jaecheon 27136, Republic of Korea. Tel: +82-43-649-1624; Fax: +82-43-649-1624; E-mail: science4us@semyung.ac.kr

References

[1] Wiedemann T, Loell E, Mueller S, Stoeckelhuber M, Stolte M, Haas R and Rieder G. *Helicobacter pylori* cag-Pathogenicity island-dependent early immunological response triggers later precancerous gastric changes in Mongolian gerbils. PLoS One 2009; 4: e4754.

[2] Cover TL and Blaser MJ. *Helicobacter pylori* in health and disease. Gastroenterology 2009; 136: 1863-1873.

[3] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Monogr Eval Carcinog Risks Hum 1994; 61: 1-241.

[4] Barciszewski J, Massino F and Clark BF. Kinetin-a multiactive molecule. Int J Biol Macromol 2007; 40: 182-192.

[5] Amasino R. 1955: kinetin arrives: the 50th anniversary of a new plant hormone. Plant Physiol 2005; 138: 1177-1184.

[6] Degani O, Drori R and Goldblat Y. Plant growth hormones suppress the development of *Harpophora maydis*, the cause of late wilt in maize. Physiol Mol Biol Plants 2015; 21: 137-149.
[7] Hwang HH, Yang FJ, Cheng TF, Chen YC, Lee YL, Tsai YL and Lai EM. The Tzs protein and exogenous cytokinin affect virulence gene expression and bacterial growth of *Agrobacterium tumefaciens*. Phytopathology 2013; 103: 888-899.
[8] Terradot L and Zawilak-Pawlik A. Structural insight into *Helicobacter pylori* DNA replication initiation. Gut Microbes 2010; 1: 330-334.
[9] Nitharwal RG, Verma V, Dasgupta S and Dhar SK. *Helicobacter pylori* chromosomal DNA replication: current status and future perspectives. FEBS Lett 2011; 585: 7-17.
[10] Yoo TH, Lee MH, Park M, Lee J, Woo HJ, Kim HW, Yang JY, Kwon HJ, Yeon MJ and Kim JB. Detection of EBV-encoded small RNA from diffuse large B-cell lymphoma patients by RT-PCR method. Biomed Sci Lett 2015; 21: 227-232.
[11] Kim SH and Kim JB. Comparative proteome analysis of cyanidin 3-O-glucoside treated *Helicobacter pylori*. Biomed Sci Lett 2015; 21: 233-240.
[12] Romero-Gallo J, Harris EJ, Krishna U, Washington MK, Perez-Perez GI and Peek RM Jr. Effect of *Helicobacter pylori* eradication on gastric carcinogenesis. Lab Invest 2008; 88: 328-336.
[13] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
[14] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.
[15] de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D and Plummer M. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol 2012; 13: 607-615.
[16] Lee SW, Kim HJ and Kim JG. Treatment of *Helicobacter pylori* Infection in Korea: A Systematic Review and Meta-analysis. J Korean Med Sci 2015; 30: 1001-1009.
[17] Zhang YX, Zhou LY, Song ZQ, Zhang JZ, He LH and Ding Y. Primary antibiotic resistance of *Helicobacter pylori* strains isolated from patients with dyspeptic symptoms in Beijing: a prospective serial study. World J Gastroenterol 2015; 21: 2786-2792.
[18] Wu IT, Chuah SK, Lee CH, Liang CM, Lu LS, Kuo YH, Yen YH, Hu ML, Chou YP, Yang SC, Kuo CM, Kuo CH, Chien CC, Chiang YS, Chiou SS, Hu TH and Tai WC. Five-year sequential changes in secondary antibiotic resistance of *Helicobacter pylori* in Taiwan. World J Gastroenterol 2015; 21: 10669-10674.
[19] Ghotaslou R, Leylabadlo HE and Asl YM. Prevalence of antibiotic resistance in *Helicobacter pylori*: A recent literature review. World J Methodol 2015; 5: 164-174.