International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:7, No:12, 2013

Study of the Antimicrobial Activity of Aminoreductone against Pathogenic Bacteria in Comparison with Other Antibiotics

Vu Thu Trang, Lam Xuan Thanh, Samira Sarter, Tomoko Shimamura, Hiroaki Takeuchi

***Abstract*—**Antimicrobial activities of aminoreductone (AR), a product formed in the initial stage of Maillard reaction, were screened against pathogenic bacteria. A significant growth inhibition of AR against all 7 isolates *(Staphylococcus aureus ATCC® 25923™, Salmonella typhimurium ATCC® 14028™, Bacillus cereus ATCC® 13061™, Bacillus subtilis ATCC® 11774™, Escherichia coli ATCC® 25922™, Enterococcus faecalis ATCC® 29212™, Listeria innocua ATCC® 33090™*) were observed by the standard disc diffusion methods. The inhibition zone for each isolate by AR (2.5 mg) ranged from 15±0mm to 28.3±0.4mm in diameter. The minimum inhibitory concentration (MIC) of AR ranging from 20mM to 26mM was proven in the 7 isolates tested. AR also showed the similar effect of growth inhibition in comparison with antibiotics frequently used for the treatment of infections bacteria, such as amikacin, ciprofloxacin, meropennem and levofloxacin. The results indicated that foods containing AR are valuable sources of bioactive compounds towards pathogenic bacteria.

***Keywords*—**Pathogenic bacteria, aminoreductone, Maillard reaction, antimicrobial activity.

I. Introduction

THE Maillard reaction, a heat-treatment-induced chemical reaction between amino and carbonyl groups, is significant for foods because it strongly affects the quality and acceptance [1]. The formation of aminoreductone (AR) in the initial stage of the Maillard reaction was first reported by Pischetsrieder et al. in a heating solution of lactose and N^{α}-acetyllysine [2]. Because AR can be detected after only short period of heating, it is an important indicator for estimating the extent of the Maillard reaction and heat treatment of food [3], [4]. Elucidation of the role and characteristics of AR are, therefore, of great interest to food scientists. So far, an antioxidant activity [5], a protective effect on photo-degradation of riboflavin, and an antimicrobial activity against *Helicobacter pylori*, a Gram-negative rod, of AR (1-(butylamino)-1,2-dehydro-1,4-dideoxy-3-hexulose) derived from the Maillard reaction of lactose and butylamine were reported [5]. Thus, we hypothesized that AR interferes with certain physiological behaviors such as viability and growth of other pathogenic bacteria.

Antibiotics provide the main basis for the therapy of bacterial infections. However, overuse and misuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms [6]. Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance [7].

In an attempt to seek alternative agents to antibiotics, and to further explicate the functional properties of AR, this study focused on investigating the effects of AR against food-spoilage and pathogenic bacteria: *Staphylococcus aureus* subsp. *aureus ATCC® 25923™, Salmonella enterica* subsp. *enterica serovar Typhimurium ATCC® 14028™, Bacillus cereus ATCC® 13061™, Bacillus subtilis ATCC® 11774™, Escherichia coli ATCC® 25922™, Enterococcus faecalis ATCC® 29212™* and *Listeria innocua ATCC® 33090™* and in the comparison with the effects of antibiotics (amikacin (AN), ciprofloxacin (CIP), imipennem (IPM) and levofloxacin (LVX)).

II. Materials and Methods

A. Reagents

Mueller-Hinton broth (MHB) was obtained from Becton, Dickinson and Company. (Cockeysville, MD). Commercially available standard discs (φ = 6 mm) of amikacin (AN: 30 µg $disc^{-1}$), ciprofloxacin (CIP: 5µg $disc^{-1}$), imipenem (IPM: 10 µg $disc^{-1}$), and levofloxacin (LVX; 5 µg $disc^{-1}$) were also obtained from Becton, Dickinson and Company. Lactose monohydrate was purchased from Nacalai tesque, Inc. (Kyoto, Japan). *n*-Butylamine and agar were obtained from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were of the highest grade commercially available. Milli-Q water or sterilized water was used in all procedures

B. Preparation of AR and its Degradation Product

Purified AR was prepared according to our previous reports [2], [5]. Briefly, lactose monohydrate (262mM) and butylamine (1.16M) were dissolved in 1.28M phosphate buffer (pH 7.0). The sample solution (10ml) was heated at 100°C for 15min, cooled on ice and extracted three times with double volume of ethyl acetate and the ethyl acetate layer was evaporated to

V. T. Trang is with School of Biology and Food Technology, Hanoi University of Science and Technology, Hanoi, Vietnam (phone: 0084-438-680118; fax: 0084-438-682470; e-mail: trang.vuthu@hust.edu.vn; thutrangvu1981@ yahoo.com).
L. X. Thanh is with Hanoi University of Science and Technology, Hanoi, Vietnam. (e-mail: lamthanh555@yahoo.com).
S. Sarter is with Cirad UMR Qualisud, Hanoi, Vietnam; CIRAD, UMR Qualisud, F-34398 Montpellier, France (e-mail: sarter@cirad.fr).
T. Shimamura is with Faculty of Agriculture, Kochi University, Japan (email: tomokos@kochi-u.ac.jp)
H. Takeuchi is with Department of Clinical Laboratory Medicine, Kochi University School of Medicine , Japan (email: htake@kochi-u.ac.jp)

dryness under reduced pressure. The residue was dissolved in 10 ml of 20% methanol and filtered through a Sep-Pak Plus C18 cartridge (Waters Corporation, Milford, MA) (activated by 5 ml of ethanol and equilibrated using Milli-Q water) to remove brown components (melanoidin). The clear elute was evaporated again and freeze-dried under reduced pressure to collect the purified AR. The concentration of AR was calculated with the extinction coefficient of AR ($17.98M^{-1}$ cm^{-1}) at 320nm [2].

C. Bacterial Strains and Culture Conditions

The 7 food spoilage and pathogenic species: *Staphylococcus aureus* subsp. *aureus ATCC® 25923™, Salmonella enterica* subsp. *enterica serovar* Typhimurium *ATCC® 14028™, Bacillus cereus ATCC® 13061™, Bacillus subtilis ATCC® 11774™, Escherichia coli ATCC® 25922™, Enterococcus faecalis ATCC® 29212™* and *Listeria innocua ATCC® 33090™* were used in this study. All strains were grown on the MHB (Mueller-Hinton broth) plates supplemented with 1.4% agar and incubated at 37°C under aerobic conditions for 24h. Whenever appropriate, MHB liquid medium was used in this study [5].

D. Disc Diffusion Susceptibility Methods

Using the filter paper disc diffusion method on the MHB agar plate, the bacterial growth inhibition by AR was assessed [8]. Purified AR was diluted in Milli-Q water and dropped to the disc. Sterilized standard discs (φ = 6mm) containing 2.5mg of AR were placed on the MHB agar plates previously spread with 0.1ml of bacterial suspension (OD_{600} = 0.1) in MHB liquid medium. The plates were incubated for 24h at 37°C under aerobic conditions. Then the inhibition zones were measured and recorded in millimeter and an average diameter of at least two repetitions was calculated. Four standard commercially available antibiotic discs, such as AN (amikacin), CIP (ciprofloxacin)), IPM (imipenem) and LVX (levofloxacin), were also utilized in this assay.

E. Determination of Minimum Inhibitory Concentrations (MIC)

MIC of AR against all 8 strains was determined using an agar dilution method described previously [5], [6]. Purified AR was diluted in Milli-Q water. 750µl of AR solution at given concentrations was separately added to each dish containing 14.25ml of yet-not-solidified MHB agar. The final concentrations of AR in the agar plates ranged from 0 to 30mM. Subsequently, each 10µl of bacterial suspension (OD_{600} = 0.1) was serially 10-fold diluted and inoculated onto the surface of the AR-supplemented agar plates, then incubated at 37°C for 48h under aerobic conditions. Sterilized water was used as a control for all experiments. The number of colony forming units (CFU) was determined as a measure of bacterial viability [6]. MIC was defined as the lowest AR concentration to inhibit 5×10^4 CFU compared to that of controls. In addition, the AR-derived Maillard reaction product (MRP) was also used and compared with AR in this study. All tests were performed in duplicate at least.

III. Results

A. Inhibition Effects of AR and Antibiotics against Pathogenic Bacteria

The inhibitory effects of AR to all test strains were examined by the standard disc diffusion method and agar dilution method which are widely used to study the bioactivity of chemical compounds. The inhibition zone for each isolate by AR (2.5 mg) was shown in Table I. The inhibition zone for each isolate by AR (2.5mg) ranged from 15±0mm *(Bacillus cereus ATCC® 13061™)* to 28.3±0.4mm *(Staphylococcus aureus ATCC® 25923™)* in diameter indicated that all isolates exhibited sensitivity to AR. Previously, Ukeda et al. and Trang et al. observed that the addition of Cu^{2+} could drastically decrease the AR concentration in the UHT-treated milk [9], [10]. Hence, to clarify the antimicrobial ability of AR on food spoilage and pathogenic bacteria, 5µg of Cu^{2+} was added into the disc containing 2.5mg of AR. In the present of Cu^{2+}, the drastically decrease of the inhibition zones were recognized (Table I). On the other hand, the disc containing 5µg of Cu^{2+} did not show the inhibition effect on tested strains. AR reportedly has a labile AR structure [4], so it can be subjected to the degradation process, followed by the advanced stages of the Maillard reaction, to form melanoidin, antimicrobial compounds, during storage [1], [2], [5]. However, in accordance with the decrease of AR concentration in the disc by the addition of Cu^{2+}, the growth inhibition activities were decreased respectively in all isolates. These results indicated that AR itself possessed the potential effectiveness to inhibit the growth of all isolates of food spoilage and pathogenic bacteria.

The fluoroquinolone antibacterial agents such as LVX and CIP [11], β-lactam antibiotic such as IPM [12] or aminoglycoside antibiotics such as AN [13] are widely used alone or in combination with other antimicrobial agents for the treatment of various serious infections caused by aerobic Gram-negative bacteria or aerobic Gram-positive cocci. Inhibition growth of those antibiotics against all tested bacteria was investigated by using the standard commercial discs and compare with the inhibition effect of AR (Table I). AR, a natural product formed during food processing, showed quite similar inhibition effects with 4 antibiotics used may provide insight to scientists searching for the alternative antibiotic reagents.

The inhibitory effects of AR to 7 tested strains were confirmed by an agar dilution method. Good agreement was found between the results obtained from the disc diffusion method and the agar dilution method. All the strains exhibited susceptibility to AR at concentrations lower than 26 mM (Table I). The MIC values ranging from 20mM (for *L. innocua*) to 26mM (for *S. typhymurium*, *B. subtilis,* and *E. coli*) in all isolates. The results suggest that AR can be used as an antimicrobial agent as an adjuvant therapy for the eradication of pathogenic bacteria infection.

TABLE I
INHIBITORY EFFECTS OF AMINOREDUCTONE ON MICROORGANISMS

No.	Strain	*Inhibition zone (mm)						MIC (mM)
		AR (2.5 mg disc^{-1})	AR and Cu^{2+} (5 µg disc^{-1})	AN (30 µg disc^{-1})	LVX (5 µg disc^{-1})	IPM (10 µg disc^{-1})	CIP (5 µg disc^{-1})	
1	*S. aureus*	28,3±0,4	n.d.	29,8±0,4	31±0,7	32,8±0,4	31±0	24
2	*S. typhymurium*	23,7±0,2	n.d	27,7±1,2	35,8±0,4	33,7±1,2	35,7±1,6	26
3	*B. cereus*	15±0	n.d.	27,8±0,4	25,4±0,6	10,2±0,5	27,5±0,7	22
4	*B. subtilis*	17±0,7	n.d.	35,5±0,7	35,4±1,2	37,5±0,7	38,5±0,7	26
5	*E. coli*	20,9±0,07	n.d.	29±0,4	34,8±0,5	32,8±0,2	38,3± 0,2	26
6	*E. faecalis*	19,8±0,4	n.d.	18,5±0,7	26,9±0,1	17,9±1,6	27,5±0,7	24
7	*L. innocua*	23,3 ±0,4	n.d.	28±0	27,3±0,4	26,7±0,2	27,3±0,4	20

*Diameter of each disc was 6 mm and values are mean of duplicate; LVX, Levofloxacin; CIP, Ciprofloxacin; IPM, Imipenem; AN, Amikacin; n.d., not determined.

B. Screening for the Antimicrobial Activity of AR in a Combination with Antibiotics

Combined antibiotic therapy has been shown to delay the emergency of bacterial resistance and may also produce desirable synergistic effects in the treatment of bacterial infections [14]. Drug synergism could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome) [14]. The combination effects of AR and antibiotics were tested in all strains.

By observing the interaction of the zones of inhibition of bacterial growth produced by individuals (AR and antibiotics), no antibiotic antagonism occurred indicating that the uptake of food containing AR does not interfere with the activity of antibiotics in the treatment of pathogenic bacteria. In the case of *E. coli*, *S. typhymurium*, several resistant strains appeared in the inhibition zone of the discs containing IPM (Fig. 1). Despite that the standard disc diffusion method is widely used to study the bioactivity of chemical compounds, the ability of compounds in the diffusion through the nutrient agar medium is a major limitation in the evaluation of the antimicrobial effects using this method.

The antimicrobial effect showed in all tested strains arising to the possibility that foods containing AR could be valuable sources as antibacterial ingredients. However, the exact antimicrobial mechanism of AR is still unknown. Thus, in the near future, investigating the mechanisms by which AR, an early Maillard reaction product, possesses the antimicrobial activity is of interest to understand the application with these products for therapeutic efficacy. Over all, the present study can supply useful information for identifying the technological conditions which can favor the formation of the Maillard reaction products such as AR as functional ingredients in food.

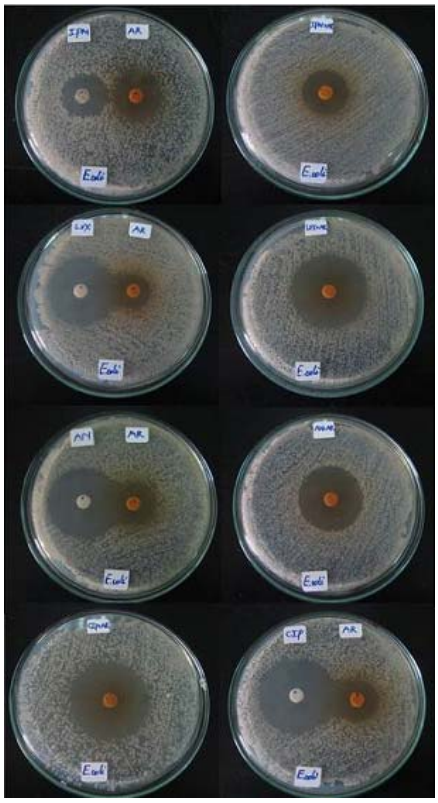

Fig. 1 The inhibition zones of 4 antibiotic discs (IPM (A), LVX (B), AN (C) or CIP (D)) in combination with 2.5 mg of AR and the mixtures of each antibiotic + AR in *E. coli*. Allows denote colonies appeared within the inhibition zones induced by IPM (A). The obscure border areas due to fusion at contact point of the inhibition zones induced by AR and antibiotics (LVX, AN and CIP), suggesting the synergistic effect to inhibit the growth. No colonies observed in the fused area in combinations as well as the mixtures indicate that AR has at least no antibiotic antagonism

ACKNOWLEDGMENTS

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.99-2011.22.

REFERENCES

[1] M. A. J. S. van Boekel, "Effect of heating on Maillard reactions in milk,"

Food Chem., vol. 62, May 1998, pp. 403-414.
[2] M. Pischetsrieder, C. Schoetter, and T. Severin, "Formation of an aminoreductone during the Maillard reaction of lactose with N^{α}-acetyllysine or proteins," *J. Agric. Food Chem.,* vol. 46, Feb. 1998, pp. 928-931.
[3] T. Shimamura, H. Ukeda, and M. Sawamura, "Relationship between the XTT reducibility and aminoreductone formed during the Maillard reaction of lactose: the detection of aminoreductone by HPLC," *Food Sci. Technol. Res.,* vol. 10, 2004, pp. 6-9.
[4] M. Pischetsrieder, F. Rinaldi, U. Gross, and T. Severin, "Assessment of the antioxidantive and prooxidative activities of two aminoreductones formed during Maillard reaction: Effects on the oxidation of *β*-Carotene, N^{α}-Acetylhistidine, and *cis*-Alkenes," *J. Agric. Food Chem.,* vol. 46, Feb. 1998, pp. 2945-2950.
[5] V. T. Trang, H. Takeuchi, H. Kudo, A. Aoki, S. Katsuno, T. Shimamura, T. Sugiura, and H. Ukeda. "Antimicrobial activity of aminoreductone against *Helicobacter pylori*." *J. Agric. Food Chem.,* vol. 57, Sep. 2009, pp. 11343–11348.
[6] H. Harbottle, S. Thakur, S. Zhao, and D. G. White, "Genetics of Antimicrobial Resistance," *Anim. Biotechnol.* vol. 17, Sep. 2006, pp. 111-124.
[7] R. Khan, B. Islam, M. Akram, S. Shakil, A. Ahmad, S. M. Ali, M. Siddiqui, and A. U.Khan, " Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical origin," *Molecules*, vol. 14, Jan. 2009, pp. 586-597.
[8] H. Takeuchi, T. Nakazawa, T. Okamoto, M. Shirai, M. Kimoto, M. Nishioka, S. Con, N. Morimoti, and T. Sugiura, "Cell elongation and cell death of *Helicobacter pylori* is modulated by the disruption of *cdr*A (Cell division-related gene A)," *Microbiol. Immunol.* vol. 50, Jul. 2006, pp. 487-497.
[9] H. Ukeda, T. Shimamura, T. Hosakawa, Y. Goto, and M. Sawamura, "Monitoring of the Maillard reaction based on the reduction of tetrazolium salt XTT," *Food Sci. Technol Int*, vol. 4, 1998, pp. 258-263.
[10] V. Trang, Y. Kurogi, S. Katsuno, T. Shimamura, and H. Ukeda, "Protective effect of aminoreductone on photo-degradation of riboflavin," *Int. Dairy J.* vol. 18, Dec. 2008, pp. 344-348.
[11] K. Mori, C. Maru, K. Takasuna, K. Furuhama, "Mechanism of histamine release induced by levofloxacin, a fluoroquinolone antibacterial agent," *Eur. J. Pharmacol*., vol. 394, Mar. 2000, 51-55.
[12] T. Darville, "Imipenem and Meropenem," *Seminars Ped. Infect. Diseases*, vol. 10, 1999, pp. 38-44.
[13] D. Carrier, N. Chartrand, and W. Matar, "Comparison of the effects of amikacin and kanamycins A and B on dimyristoylphosphatidylglycerol bilayers," *Biochem. Pharmacol*., vol. 53, Jun.1997, pp. 401-408.
[14] G. Adwan, and M. Mhanna, "Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens," *Middle-East J. Sci. Res*., vol. 3, 2008, pp. 134-139.