Extraction of antibacterial peptides against *Helicobacter pylori* from bovine milk casein

Guo-Yue Wan, Ka-Man Lam, Ian-Ian Wong, Pedro Fong, Li-Rong Meng

School of Health Sciences, Macao Polytechnic Institute, Macao, China

Submitted: 21 April 2019; **Accepted:** 9 June 2019
Online publication: 24 March 2021

Arch Med Sci 2022; 18 (2): 376–381
DOI: https://doi.org/10.5114/aoms/109942

Corresponding authors:
Prof. Li-Rong Meng
School of Health Sciences
Macao Polytechnic Institute
Meng Tak Building
Room 730
Rua de Luís Gonzaga Gomes, Macao, China
Phone: +853-85993449
Fax: +853-28753159
E-mail: lrmeng@ipm.edu.mo

Dr Pedro Fong
School of Health Sciences
Macao Polytechnic Institute
Meng Tak Building
Room 705
Rua de Luís Gonzaga Gomes, Macao, China
Phone: +853-85993427
Fax: +853-28753159
E-mail: pedrofong@ipm.edu.mo

Abstract

Introduction: More than half of the world's population is infected with *Helicobacter pylori*, which may cause gastritis, peptic ulcer and even gastric cancer. The World Health Organization (WHO) has announced that *H. pylori* infection is a class I carcinogen and hence eradication of it is highly important. Bovine milk contains caseins, which can be digested by various enzymes in the human stomach to produce antibacterial peptides.
Material and methods: This study used *in vitro* methods to extract anti-*H. pylori* peptides from caseins by the gastric protease pepsin under environments with similar pH values to those found in the human stomach. The molecular weights and sequences of the peptides were identified by MALDI-TOF mass spectrometry and MS/MS Ion Search, respectively. Antibacterial activity tests were performed to calculate the minimum inhibitory concentration (MIC_{90}) of the extracts.
Results: The findings of this study revealed that the major products of bovine milk casein digestion by pepsin are casecidin 17 and β-casein 207–224. The extracts produced promising anti-*H. pylori* effects with the lowest MIC_{90} found at pH values of 1.5 and 2.0.
Conclusions: This study identified the anti-*H. pylori* effects of casecidin 17 and β-casein 207–224, which may help in developing therapeutic agents to modulate the effect of antibiotics on *H. pylori* infections.

Key words: antibacterial peptides, casein, casecidin 17, helicobacter pylori, minimum inhibitory concentration.

Introduction

Helicobacter pylori is a well-known causative agent of chronic dyspepsia, peptic ulcer, gastric mucosa-associated lymphoma and gastric cancer [1–3]. Currently, the global infection rate of *H. pylori* exceeds 50% of the population [4]. The treatment of *H. pylori* mainly comprises triple therapy such as omeprazole, clarithromycin and amoxicillin [5]. However, recent studies show that the curative rate of the triple therapy is gradually decreasing, mainly due to the emergence of resistant strains [6, 7]. Recently, the WHO has considered clarithromycin-resistant *H. pylori* to be one of the most urgently needed new antibiotic groups [8]. The mechanisms of resistance to clarithromycin mainly result from mutations of the peptidyl transferase region encoded in domain V of 23s rRNA [9]. The increase in clarithromycin resistance in the last decade has reduced the efficacy to eradicate *H. pylori* [10].

In the past decade, various antibacterial peptides were discovered through the studies of organisms' immune mechanisms [11]. These peptides showed antibacterial effects towards various kinds of bacteria, including *H. pylori*. For example, Chen *et al*. identified the first anti-*H. pylori* peptides from amphibian skins [12]. The peptide was composed of 23 amino acids with the sequence of GLLRASSVWGRKYYVDLAGCAKA. Another recent example is the venom peptide bicarinalin, which has produced similar anti-*H. pylori* activity as four antibiotics currently used in therapies against *H. pylori* [13]. Most of the discovered antibacterial peptides have a nonspecific innate immune response against exogenous pathogens. At present, the mechanisms of antibacterial activities can be divided into two groups. The first is the amphipathic α-helix structure of peptides that binds to the bacterial cell membrane to form pores and destroy the cell membrane by releasing the cell contents [14]. The second is the antibacterial peptides that directly enter the bacterial cells and act on the DNA, RNA, protein, mitochondria and cell membrane, inhibiting the synthesis, transcription and translation of the cells. The peptides may also interfere with cell metabolism and inhibit the formation of the cell membrane [14]. The study also demonstrated that peptides can kill bacteria at a faster rate than that of its multiplication [15]. The combination of the physical killing mechanisms and fast killing rate means resistant strains are potentially less likely to develop compared to most antibiotics [16]. Another advantage of antibacterial peptides is the highly degradable properties, which may lead to few drug residues and side effects [17].

Antibacterial peptides have been found in mammals, plants, insects and even microorganisms. This inspired us to evaluate whether food also contains anti-*H. pylori* peptides. The aim of this study was to investigate whether the most common food source, milk, contains any peptides against *H. pylori*. Milk is rich in protein, of which caseins account for about 80% of the total protein [18]. Caseins, in addition to nutrition, produce numerous biologically active peptides through enzymolysis during food processing. Many of these peptides have demonstrated antimicrobial activities. For example, bovine αs1-casein f (99–109) has antibacterial activities against Gram-positive bacteria *Bacillus subtilis* and *Listeria innocua* [19] and αs2-casein f (181–207), f (175–207) and f (164–207) demonstrated antibacterial properties against both certain Gram-positive and Gram-negative bacteria [19].

This study aimed to extract antibacterial peptides from caseins and to determine their minimum inhibitory concentrations (MIC) against *H. pylori* under environments with different pH values.

Material and methods

Caseins (Sigma C5890) from bovine milk were purchased from Sigma (Guangzhou, China). Antibacterial peptide extraction was performed on the caseins, followed by concentration and identification of the peptides. A study had shown that casein has anti-*E. coli* activities, and hence it was used in this study to ensure the extracted antibacterial peptides were active. The antibacterial activities of the extracts were also tested on *H. pylori* (ATCC-43504). Eight concentrations of the extracts were used to calculate the MIC values at four environments with different pH values. The eight concentrations were 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, 1.5625 mg/ml, 0.78125 mg/ml, 0.390625 mg/ml and 0.1953125 mg/ml. They were all tested in environments with pH values of 1.5, 2.0, 2.5 and 3.0. The *H. pylori* were purchased from Tin Hang Technology, Hong Kong, China. The experiments were performed with blank solutions, negative and positive controls. The blank solutions did not contain antimicrobial peptides or *H. pylori*. They were used to ensure there was no contamination and also for measurement of the microplate spectrophotometer optical density values at 600 nm (Multiskan GO, Thermo Fisher Scientific). The negative controls contained *H. pylori* and no antibacterial peptides, whereas the positive controls contained the extracted antibacterial peptides and no *H. pylori*.

Recovery, storage, and identification of *H. pylori*

Strains of *H. pylori* (ATCC-43504) were recovered in accordance with the instructions provided by the supplier product information sheet (https://www.atcc.org/~/ps/43504.ashx). The cryopreserved strains were thawed in a 37°C water bath. The solutions (100 µl) were then inoculated with 6% sheep blood Columbia agar (Huankai Microbial, Guangdong, China) and incubated at 37°C for 3 days in a facultative anaerobic environment. In terms of storage, glycerin was added to brain heart infusion broth to prepare the cryopreservation medium, where the *H. pylori* were added and stored at –80°C. Identification of the *H. pylori* (ATCC-43504) was performed by assessment of colony morphology by microscopy, in which conformation was suggested to be needle-like with a glassy translucent appearance of colonies formed on the Columbia blood agar plate (Huankai Microbial, Guangdong, China). This indicates that the strains were Gram-negative spiral bacilli. Identification of *H. pylori* (ATCC-43504)

was also performed by biochemical assays, including oxidase, catalase and urease tests (Huankai Microbial, Guangdong, China). A combination of the above results served as the identification of the *H. pylori* strain.

Extraction and identification of antibacterial peptides

Caseins (20 g) were dissolved in 300 ml of deionized water at 37°C; the pH value of the solutions was adjusted to 1.5, 2.0, 2.5, or 3.0 with hydrochloric acid. Pepsin (0.6 g) (1 : 10000, P-5144, BoMei Biotechnology, HeFei, China) was added to the solutions for enzymolysis, which was carried out at 37°C for 5 h. During the experiments, hydrochloric acid was added drop-wise to maintain the pH value of the solution. After enzymolysis, the pepsins were inactivated by incubation in a water bath at 80°C for 20 min. The de-enzymatic solutions were then centrifuged at 3500 rpm and 9000 rpm for 30 min and 20 min, respectively. The supernatants were then collected and filtered through a 0.45 µm filter and re-filtered through a 0.22 µm filter. The mixtures were evaporated to dryness on a rotary evaporator and freeze-dried. The dry mixture (0.1 g) was sent to the National Center for Protein Sciences (Beijing, China) for molecular weight analysis and peptide sequencing analysis by MALDI-TOF mass spectrometry and MS/MS Ion Search, respectively.

Preparation of *H. pylori* and *E. coli* 0.5 McFarland Turbidity solution

Several *H. pylori* colonies from the 6% sheep blood Columbia broth were added to sterile tubes containing 0.9% normal saline, shaken for 15 s and adjusted to a turbidity of 0.5 in the microbial turbidimeter (DensiCHEK Plus, bioMerieux, USA). The same methods were performed for the preparation of the *E. coli* (ATCC-25922) solution.

Antibacterial activity test

Microdilution methods were used to evaluate the inhibitory effects of each group of antibacterial peptides against *E. coli*. Sterile Brucella broth (HopeBio, Qingdao, China) was used to dilute the antibacterial peptide extracts with pH = 1.5, 2.0, 2.5 and 3.0 through the following methods. The broth (600 µl) was added to a 48-well plate and 600 µl of 50 mg/ml antibacterial peptides was added to the first well, followed by seven half-dilutions to produce the 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, 1.5625 mg/ml, 0.78125 mg/ml, 0.390625 mg/ml and 0.1953125 mg/ml peptide solutions. The *E. coli* 0.5 (approximately 1.5 × 10^8 CFU/ml) McFarland Turbidity solution (2 µl) was then added to each of the peptide solutions and incubated at 37°C for 24 h in a facultative anaerobic environment. The mixtures were assigned to five categories by naked-eye observations. The categories were *clearly transparent*, *blurred* (80% suppressed), *turbidity significantly reduced* (50% inhibited), *turbidity mildly reduced* and *turbidity was not reduced*. Mixtures of blurred or turbidity significantly reduced categories were considered of having antibacterial activities against *E. coli*.

Minimum inhibitory concentration determination

The preparations of MIC assays of antibacterial peptides against *H. pylori* were the same as those for analysis of *E. coli*, with the difference being that each *H. pylori* assay was repeated 12 times for higher accuracy and consistency. Instead of performing naked-eye observation as in the *E. coli* assays, the *H. pylori* MIC assays were performed in sterile 96-well microliter plates (ChunBo Biologics, Haimen, China) and the absorbance of the spectrophotometer was set at 600 nm. The MIC_{90} values were defined as the lowest concentration of peptide that inhibits 90% of *H. pylori*. The inhibition percentage was calculated by the following equation: Inhibition (%) = (1 – (Optical density (OD) value of sample/Optical density (OD) value of negative control)) × 100%.

Statistical analysis

Data analysis was performed using the paired *t*-test comparative method embedded in the SPSS 20.0 software to calculate the *p*-values, which were considered to be statistically significant when *p* was less than 0.05. The sample groups were compared with the blank control, negative and positive control groups. Regression analysis was used to analyze the relationship between the concentration of each group of the antibacterial peptides and their OD values. Pearson correlation analysis was used to evaluate the relationship between pH changes and MIC values.

Results

Antibacterial peptide extraction rate

Casein (20 g) was hydrolyzed by pepsin at different pH values. It was then concentrated and freeze-dried to give the weight and extraction rate as shown in Table I.

Identification of antibacterial peptides

The MALDI-TOF mass spectrometry analyzed the molecular weight of the antimicrobial peptides at pH 2.0 and pH 3.0 (Figures 1 and 2). The peaks with the highest abundance at both pH values were located at 1881 m/z and 1994 m/z.

Peptide sequencing analysis was performed by MS/MS Ion Search and the resulting amino acid sequences with 1881 m/z and 1994 m/z were YQEPVLGPVRGPFPIIV and LYQEPVLGPVRGPFPIIV, corresponding to residues 208–224 and 207–224, respectively (Table II). These sequences were matched with the corresponding peptides in the Antimicrobial Peptide Database [20], Milk Peptide Database [21] and MilkAMP Database [22]. The peptides with 1881 m/z were identified as casecidin 17 with APD (Antimicrobial Peptide Database) ID of AP01398, and there were no records in the above three databases for the peptide with 1994 m/z. Literature searches of this peptide were performed on the most common databases, including PubMed, Google Scholar, Cochrane Library Databases, and Science Direct, and only a single paper was found that mentioned its bioactivity, which is modulation of the bitter taste receptors and was named β-casein 207–224 [23].

Antibacterial activity and minimum inhibitory concentration determination

Antibacterial assays of the peptides against *E. coli* were performed using naked-eye observations. All the mixtures were assigned as blurred or turbidity significantly reduced, which indicate that *E. coli* were successfully inhibited by each group of antimicrobial peptides. This proved that the extracted antimicrobial peptides in each group were active.

Table I. Antibacterial peptide extraction rate

pH values	Weight of extraction [g]	Extraction rate (%)
1.5	5.84	29.2
2.0	5.15	25.8
2.5	4.35	21.8
3.0	7.36	36.8

With regards to the *H. pylori* MIC assays, all eight concentrations of the antibacterial peptides showed anti-*H. pylori* activity. The MIC_{90} of both pH 1.5 and pH 2.0 was 6.25 mg/ml, whereas for both pH 2.5 and pH 3.0, the MIC_{90} was 12.5 mg/ml.

Regression analysis showed that when the concentration of the peptide increased, the optical density (OD) values became smaller ($p < 0.001$) (Table III), indicating that the higher the concentration of the peptide is, the better is the anti-*H. pylori* effect. The Pearson correlation analysis between pH and the MICs of the antibacterial peptides in different concentrations showed a positive correlation between the pH and the MIC value with a correlation coefficient of 0.82. This indicates that the greater the pH value is, the larger is the MIC_{90} ($p < 0.001$). Hence, the anti-*H. pylori* effects of the peptides are higher in a lower pH environment, such as in the human stomach.

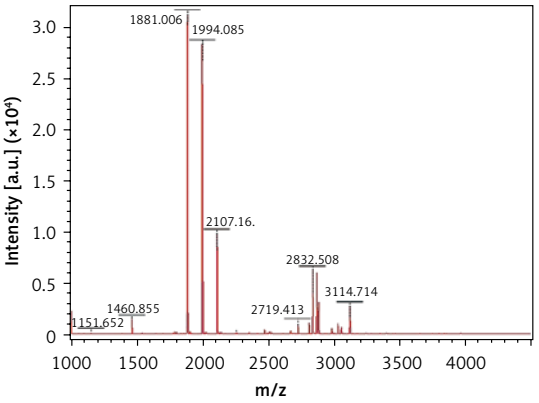

Figure 1. Mass spectrum of the antimicrobial peptide extraction with pH 2.0

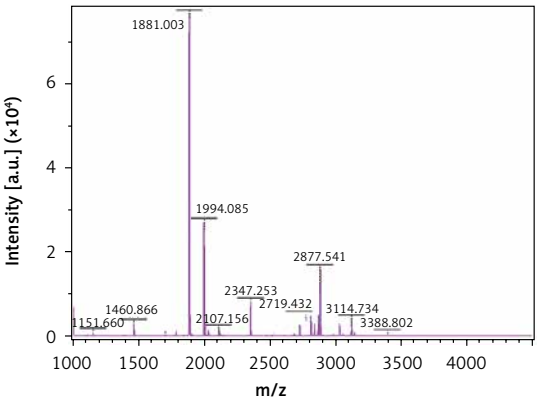

Figure 2. Mass spectrum of the antimicrobial peptide extraction with pH 3.0

Table II. Peptide profiles of the enzymolyzed casein at pH 2.0 and 3.0

pH	Observed	Calculated	Position	Sequence
2.0	2877.70	2876.56	179–204	SLSQSKVLPVPQKAVPYPQRDMPIQA
	2107.32	2106.22	206–224	LLYQEPVLGPVRGPFPIIV
	1994.24	1993.14	207–224	LYQEPVLGPVRGPFPIIV
	1881.14	1880.06	208224	YQEPVLGPVRGPFPIIV
3.0	2877.65	2876.56	179–204	SLSQSKVLPVPQKAVPYPQRDMPIQA
	1994.20	1993.14	207–224	LYQEPVLGPVRGPFPIIV
	1881.10	1880.06	208–224	YQEPVLGPVRGPFPIIV
	1460.94	1459.90	211–224	PVLGPVRGPFPIIV

Table III. Optical density values of the extract with different concentrations and at environments with different pHs

Extract concentration [mg/ml]	Average optical density (OD) values at			
	pH 1.5	pH 2.0	pH 2.5	pH 3.0
25	0.0505 ±0.1247	0.0503 ±0.0024	0.0552 ±0.0022	0.0456 ±0.0013
12.5	0.04993 ±0.0082	0.0453 ±0.0014	0.0462 ±0.0013	0.0536 ±0.0070
6.25	0.04933 ±0.0082	0.0687 ±0.0179	0.2530 ±0.1178	0.3168 ±0.0885
3.125	0.25273 ±0.0477	0.3324 ± 0.0530	0.3556 ±0.0621	0.3253 ±0.0230
1.5625	0.3202 ±0.0519	0.3116 ±0.0364	0.3204 ±0.0834	0.3267 ±0.0327
0.78125	0.34063 ±0.0332	0.3054 ±0.0384	0.3523 ±0.0502	0.03284 ±0.0413
0.390625	0.34503 ±0.0562	0.2983 ±0.0383	0.3595 ±0.0436	0.3364 ±0.0442
0.1953125	0.38013 ±0.0194	0.3431 ±0.0484	0.3650 ±0.0198	0.3759 ±0.0488

Values after ± are standard deviations.

Discussion

Due to an increase in *H. pylori* resistance to antibiotics, such as clarithromycin, the triple therapy treatment has become gradually less efficacious [8]. This study discovered that the major products of bovine milk casein digestion have promising anti-*H. pylori* effects. The two major peptides identified in this study were casecidin 17 and β-casein 207–224. The anti-*H. pylori* effects of bovine milk have been suggested by Wang *et al*. [24] and the non-*H. pylori* specific antibacterial activities against both certain Gram-positive and Gram-negative bacteria of bovine casein have also been demonstrated [19]. Casecidin 17 is well documented in the literature and several bioactivities have been suggested. Rojas-Ronquillo *et al*. found the angiotensin-converting enzyme inhibitory and antithrombotic properties of casecidin 17 with an inhibition efficiency ratio of 0.1%/peptide concentration (μg/ml) and 4.6%/peptide concentration (μg/ml), respectively [25]. Sandre *et al*. found that casecidin 17 has immunomodulatory activity in mice, probably by enhancing the antimicrobial activity of macrophages [26]. In terms of antibacterial properties, Birkemo *et al*. were the first to establish the inhibition effects of casecidin 17 of *E. coli*, indicated by *in vitro* MIC values of 0.4 mg/ml [27]. In this study, we found that the peptide extract mainly containing casecidin 17 and β-casein 207–224 had anti-*H. pylori* properties. Further studies will be focused on the anti-*H. pylori* activities of individual peptides.

As no antibacterial information on β-casein 207–224 can be found in various databases, we believe this study is the first to reveal its potential anti-*H. pylori* activities. β-casein 207–224 has only one more amino acid residue than does casecidin 17, and the rest of their sequences are in the same order. Hence, their structures and sequences are highly similar, which suggests that they may have similar biological functions. The reason for the small amount of information in the literature about β-casein 207–224 is that the hydrolysis of this peptide is not common in most experimental conditions. Here, the enzymolysis was performed in acidic environments, which could be an important factor for producing β-casein 207–224. As shown in Figures 1 and 2, the amount of β-casein 207–224 was much higher when the casein was enzymolyzed at pH 2.0, compared to at pH 3.0. Hence, β-casein 207–224 could be one of the major antibacterial peptides found in an empty human stomach, where the pH value is approximately 1.5 to 2.5. Furthermore, the MIC_{90} values at pH 2.0 are lower than those at pH 3.0, indicating that better anti-*H. pylori* activities were achieved in the pH 2.0 environment, where the concentration of β-casein 207–224 is higher (Figures 1 and 2).

Several studies have produced antibacterial peptide mixtures with casecidin 17 through different methods [25–27]. Our study is the first to use a simple *in vitro* pepsin enzymolysis method to successfully establish casecidin 17 in an extract. According to the supplier product information sheet (https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/c5890pis.pdf), the caseins used in this study were obtained from bovine milk and contain four main types of casein: α-s1 casein, α-s2 casein, β-casein and κ-casein. Pepsin is one of the major enzymes in the human gastrointestinal tract required for the digestion of ingested proteins, including milk. Hence, this *in vitro* study simulated the digestion process of bovine milk casein by pepsin, and we found that the major products of such a process are casecidin 17 and β-casein 207–224. A study showed that bovine milk contains about 32 g of protein per liter [28]. Casein has been considered to be the main constituent in milk and it makes up approximately 80% of the total protein in bovine milk [18].

The MIC_{90} values in this study were obtained from *in vitro* experiments; hence, many other *in vivo* factors that may affect the activity of the peptides were excluded. For example, there are other enzymes that may digest casein and pro-

duce other types of antibacterial peptides, which may not be casecidin 17 and β-casein 207–224. Another excluded factor is that the human gastrointestinal tract is highly complex and contains food and many other bacteria; hence, casecidin 17 and β-casein 207–224 may bind to other bacteria or other substances and have less direct contact with *H. pylori*. Nevertheless, this study provides the basis for further investigation on casecidin 17 and β-casein 207–224 for novel anti-*H. pylori* peptide design. Further optimization of antibacterial peptide extraction could assist in developing therapeutic agents to modulate the effect of antibiotics on *H. pylori* infections.

Acknowledgments

The financial support of the Macao Polytechnic Institute Research Fund (Project No: RP/ESS-03/2018) is gratefully acknowledged. The authors thank the National Centre for Protein Sciences (Beijing, China) for providing the equipment for MALDI-TOF mass spectrometry and MS/MS Ion Search analysis.

Conflict of interest

The authors declare no conflict of interest.

References

1. Lee Y, Chiang T, Chou C, et al. Association between Helicobacter pylori eradication and gastric cancer incidence: a systematic review and meta-analysis. Gastroenterology 2016; 150: 1113-24.e5.
2. Chojnacki C, Poplawski T, Blonska A, Blasiak J, Romanowski M, Chojnacki J. Expression of tryptophan hydroxylase in gastric mucosa in symptomatic and asymptomatic Helicobacter pylori infection. Arch Med Sci 2019; 15: 416-23.
3. Agin M, Batun I, Ozdemir S, Dora F, Tumgor G. Prevalence of Helicobacter pylori in Turkish children with celiac disease and its effect on clinical, histopathological, and laboratory parameters. Arch Med Sci 2019; 15: 1475-81.
4. Abadi ATB, Kusters JG. Management of Helicobacter pylori infections. BMC Gastroenterology 2016; 16: 94.
5. Zagari RM, Rabitti S, Eusebi LH, Bazzoli F. Treatment of Helicobacter pylori infection: a clinical practice update. Eur J Clin Invest 2018; 48: 10.1111/eci.12857. Epub 2017 Dec 4.
6. Shao Y, Lu R, Yang Y, Xu Q, Wang B, Ye G. Antibiotic resistance of Helicobacter pylori to 16 antibiotics in clinical patients. J Clin Lab Anal 2017; 32: e22339.
7. Akpinar MY, Aksoy EK, Sapmaz F, Goktas Z, Uzman M, Nazligul Y. Comparison of moxifloxacin-based therapies and standard bismuth-based quadruple therapy for first-line treatment of Helicobacter pylori infection. Arch Med Sci Civil Dis 2018; 3: 81-6.
8. Flores-Treviño S, Mendoza-Olazarán S, Bocanegra-Ibarias P, Maldonado-Garza HJ, Garza-González E. Helicobacter pylori drug resistance: therapy changes and challenges. Expert Rev Gastroenterol Hepatol 2018; 12: 819-27.
9. Abadi ATB. Resistance to clarithromycin and gastroenterologist's persistence roles in nomination for Helicobacter pylori as high priority pathogen by World Health Organization. World J Gastroenterol 2017; 23: 6379-84.
10. Abadi ATB. Strategies used by helicobacter pylori to establish persistent infection. World J Gastroenterol 2017; 23: 2870-82.
11. Li W, Tailhades J, O'Brien-Simpson NM, et al. Proline-rich antimicrobial peptides: potential therapeutics against antibiotic-resistant bacteria. Amino Acids 2014; 46: 2287-94.
12. Chen L, Li Y, Li J, Xu X, Lai R, Zou Q. An antimicrobial peptide with antimicrobial activity against Helicobacter pylori. Peptides 2007; 28: 1527-31.
13. Guzman J, Tene N, Touchard A, et al. Anti-Helicobacter pylori properties of the ant-venom peptide bicarinalin. Toxins 2017; 10: 21.
14. Sibel Akalın A. Dairy-derived antimicrobial peptides: action mechanisms, pharmaceutical uses and production proposals. Trends Food Sci Technol 2014; 36: 79-95.
15. Agyei D, Danquah MK. Rethinking food-derived bioactive peptides for antimicrobial and immunomodulatory activities. Trends Food Sci Technol 2012; 23: 62-9.
16. Kim S, Wijesekara I. Development and biological activities of marine-derived bioactive peptides: a review. J Funct Foods 2010; 2: 1-9.
17. Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 2006; 24: 1551-7.
18. Kunz C, Lonnerdal B. Human-milk proteins: analysis of casein and casein subunits by anion-exchange chromatography, gel electrophoresis, and specific staining methods. Am J Clin Nutr 1990; 51: 37-46.
19. McCann KB, Shiell BJ, Michalski WP, et al. Isolation and characterisation of a novel antibacterial peptide from bovine αS1-casein. Int Dairy J 2006; 16: 316-23.
20. Wang G, Li X, Wang Z. APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Res 2016; 44: D1087-93.
21. Nielsen SD, Beverly RL, Qu Y, Dallas DC. Milk bioactive peptide database: a comprehensive database of milk protein-derived bioactive peptides and novel visualization. Food Chem 2017; 232: 673-82.
22. Theolier J, Fliss I, Jean J, Hammami R. MilkAMP: a comprehensive database of antimicrobial peptides of dairy origin. Dairy Sci Technol 2014; 94: 181-93.
23. Georgalaki M, Papadelli M, Chassioti E, et al. Milk protein fragments induce the biosynthesis of macedocin, the lantibiotic produced by Streptococcus macedonicus ACA-DC 198. Appl Environ Microbiol 2010; 76: 1143-51.
24. Wang X, Hirmo S, Willen R, Wadstrom T. Inhibition of Helicobacter pylori infection by bovine milk glycoconjugates in a BAlb/cA mouse model. J Med Microbiol 2001; 50: 430-5.
25. Rojas-Ronquillo R, Cruz-Guerrero A, Flores-Nájera A, et al. Antithrombotic and angiotensin-converting enzyme inhibitory properties of peptides released from bovine casein by Lactobacillus casei Shirota. Int Dairy J 2012; 26: 147-54.
26. Sandre C, Gleizes A, Forestier F, et al. A peptide derived from bovine beta-casein modulates functional properties of bone marrow-derived macrophages from germ-free and human flora-associated mice. J Nutr 2001; 131: 2936-42.
27. Birkemo GA, O'Sullivan O, Ross RP, Hill C. Antimicrobial activity of two peptides casecidin 15 and 17, found naturally in bovine colostrum. J Appl Microbiol 2009; 106: 233-40.
28. Haug A, Hostmark AT, Harstad OM. Bovine milk in human nutrition: a review. Lipids Health Dis 2007; 6: 25-511X-6-25.