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Antibacterial and antiproliferative peptides in synbiotic yogurt—Release and stability during refrigerated storage

B. N. P. Sah,* T. Vasiljevic,* S. McKechnie,† and O. N. Donkor*[1]
*Advanced Food Systems Research Unit, College of Health and Biomedicine, and
†Advanced Food Systems Research Unit, College of Engineering and Science, Victoria University, Werribee Campus, PO Box 14428, Melbourne, Victoria 8001, Australia

ABSTRACT

The search for alternative therapeutics is on the rise due to the extensive increase in bacterial resistance to various conventional antibiotics and side effects of conventional cancer therapies. Bioactive peptides released from natural sources such as dairy foods by lactic acid bacteria have received attention as a potential source of biotherapeutic peptides. However, liberation of peptides in yogurt depends on proteolytic activities of the cultures used. Thus, this research was conducted to establish generation of inhibitory peptides in yogurt against pathogenic bacteria and cancer cells during storage at 4°C for 28 d. Water-soluble crude peptide extracts were prepared by high-speed centrifugation of plain and probiotic yogurts supplemented with or without pineapple peel powder (PPP). The inhibition zones against *Escherichia coli* and *Staphylococcus aureus* by PPP-fortified probiotic yogurt at 28 d of storage were, respectively, 25.89 and 11.72 mm in diameter, significantly higher than that of nonsupplemented control yogurts. Antiproliferative activity against HT29 colon cancer cells was also significantly higher in probiotic yogurt with PPP than in nonsupplemented probiotic yogurt. Overall, crude water-soluble peptide extracts of the probiotic yogurt with PPP possessed stronger inhibitory activities against bacteria and cancer cells than controls, and these activities were maintained during storage. However, activities were lowered substantially during in vitro gastrointestinal digestion. These findings support the possibility of utilizing dairy-derived bioactive peptides in the development of a superior alternative to the current generation of antibacterial and anticancer agents, as well as a functional ingredient in foods, nutraceuticals, and pharmaceuticals.

Key words: pineapple, probiotics, peptides, antibacterial activity, anticancer activity

INTRODUCTION

Rapid industrialization and urbanization has resulted in immense changes to lifestyle practices, leading to increased risks of various diseases and disorders, such as cancer. Cancer, an uncontrolled growth and spreading of abnormal cells, has become a major health burden in the United States and many other parts of the world (Siegel et al., 2012). Colorectal cancer is a widespread cancer, the fourth most common in men and third in women in Latin America (Goss et al., 2013). Side effects such as alopecia (hair loss), fatigue, nausea, and vomiting are associated with conventional cancer therapies, such as chemotherapy and radiotherapy, because they adversely affect healthy cells as they destroy malignant cells. In addition, there is increasing resistance against conventional chemotherapy. Consequently, there is an urgent demand for natural anticancer compounds, including bioactive peptides, as an alternative treatment to chemotherapy drugs, which could eliminate some drawbacks of chemotherapy.

Some bioactive peptides exhibit interesting cytotoxic activities against both malignant and microbial cells (Hoskin and Ramamoorthy, 2008). Positively charged antimicrobial peptides (**AMP**) can bind with negatively charged components of bacterial and cancer cells electrostatically, which may play a critical role for the disruption of bacterial and cancer cell membranes (Yeaman and Yount, 2003; Hoskin and Ramamoorthy, 2008). Most AMP are relatively small (6 to 100 AA), cationic, amphipathic, and α-helical peptides and demonstrate broad-spectrum antibacterial and antifungal activities, usually by lysing cell membranes (Giuliani et al., 2007; Yeung et al., 2011). The widespread increase in bacterial resistance to several common antibiotics has inspired scientists to focus on exploring new groups of antibiotics with new target sites and action modes.

Consequently, interest is growing in food-derived peptides as drug candidates, mainly due to several specific key merits over common chemotherapeutics. Notably, milk proteins emerge as a prolific source of biologically active peptides, which are encrypted in the

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[1]Corresponding author: Osaana.Donkor@vu.edu.au

primary structure of the proteins and could modulate the physiology of consumers following the proteolytic release of peptides with anticarcinogenic potential (Bhat and Bhat, 2011; Sah et al., 2015a). One way to obtain these bioactive peptides is by direct release from proteins by proteolytic actions of bacteria commonly used in manufacturing fermented foods (Choi et al., 2012). Therefore, yogurt appears to be an appropriate matrix for production of such functional ingredients.

Several investigations have been conducted to increase the functionality of yogurt such as probiotic inclusion in culture and prebiotic supplementation (Donkor et al., 2007a; Al-Sheraji et al., 2012; Sah et al., 2015b, 2016). A prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Gibson et al., 2004). Common prebiotics are inulin, fructooligosaccharides, galactooligosaccharides, and other oligosaccharides, such as resistant starch and lactulose (Thammarutwasik et al., 2009). Inulin represents a group of plant polysaccharides having linear fructans with β-(2←1) fructosyl-fructose glycosidic linkages, and "inulin HP" is a long-chain inulin with a degree of polymerization of 10 to 60, the average being 25 (Roberfroid, 2007). Besides inulin, pineapple peel powder (**PPP**) appears to be a good source of dietary fiber, protein, and minerals, with apparent prebiotic potential (Sah et al., 2015c).

Although prebiotic supplementations may result in several functional benefits for probiotic organisms and ultimately consumers, the approach may influence the bioactivity of yogurt because bacterial proteolytic enzymes further hydrolyze milk proteins and peptides during storage (Donkor et al., 2007b). However, studies are still largely limited regarding the effects of prebiotic addition on inhibitory activities against bacteria and HT29 human colon cancer cells of the released peptides in yogurt during storage. This work thus aimed to assess the effect of PPP addition on performance of *Lactobacillus acidophilus* (ATCC 4356), *Lactobacillus casei* (ATCC 393), and *Lactobacillus paracasei* ssp. *paracasei* (ATCC BAA52) in regard to the liberation of bioactive peptides with antibacterial and anticancer potential in yogurts during 28 d of refrigerated storage at 4°C.

MATERIALS AND METHODS

Substrates and Chemicals

McCoy's 5A (Modified) medium and trypsin-EDTA (0.25%) were procured from Life Technologies (Carlsbad, CA). Bovogen Biologicals Pty Ltd. (Melbourne, Australia) supplied fetal bovine serum (**FBS**). CellTiter 96 AQ_{ueous} One Solution reagent containing a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; **MTS**] and an electron-coupling reagent (phenazine ethosulfate) was purchased from Promega Corp. (Madison, WI) for the cell proliferation assay. Antibiotic/antimycotic solution (100×) and staurosporine solution (from *Streptomyces* sp.) were obtained from Sigma Chemical Co. (St. Louis, MO). Pepsin (cat. no. P7000; pepsin A; EC 3.4.23.1, 570 U/mg solid, from porcine gastric mucosa), pancreatin [cat. no. 1494057; pancreatin, amylase, and protease United States Pharmacopeia reference standard; each mg contains 344 USP units of amylase activity and 358 USP units of protease activity], and bile (catalog number B3883; bile bovine) were also purchased from Sigma Chemical Co. Cellstar T25 and T75 flasks, 96-well flat-bottomed microplate (Cellstar, Greiner Bio-One GmbH, Frickenhausen, Germany) were obtained from Interpath Services Pty. Ltd. (Heidelberg West, VIC, Australia). Ampicillin sodium salt was purchased from Progen Industries Ltd. (Darra, QLD, Australia). Thermo Fisher Scientific Australia Pty Ltd. (Scoresby, VIC, Australia) supplied nutrient agar no. 1 (CM0003; Oxoid, Basingstoke, UK). Aqueous solutions were prepared in Milli-Q water (18.2 MΩ·cm) obtained from a Millipore water purification system (Millipore Australia Pty Ltd., North Ryde, NSW, Australia). Skim milk powder and whole pineapples were bought from a local supermarket (Woolworths Limited, Werribee, Australia). Pineapple peel powder was prepared from the peel of pineapple (*Ananas comosus* [L.] Merrill) as described by Sah et al. (2015b).

Propagation of Cultures and Preparation of Yogurts Supplemented with Prebiotics

Pure cultures of *Streptococcus thermophilus* ASCC 1275 and *Lactobacillus delbrueckii* ssp. *bulgaricus* Lb1466 (*L. bulgaricus*) were obtained from the Victoria University Culture Collection (Werribee, Australia). *Lactobacillus acidophilus* ATCC 4356, *L. casei* ATCC 393, and *L. paracasei* ssp. *paracasei* ATCC BAA52 (*L. paracasei*) were procured from Cell Biosciences Pty Ltd. (Heidelberg, VIC, Australia). All organisms were stored at −80°C in de Man, Rogosa, and Sharpe broth containing 40% (vol/vol) glycerol. The strains resuscitated after 3 successive transfers were used to prepare starters as described by Sah et al. (2014).

Set-type plain and probiotic yogurts with inulin or PPP supplementation or without supplementation (control) were prepared as described by Sah et al.