Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal

Published online: http://jfat.mfu.ac.th

Original Research Article

Evaluation of different resistant starch types for stimulating growth of the dominant lactic acid bacteria inhabiting human colon

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ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form 9 March 2015
Accepted 18 March 2015

Keywords:
Intestinal microflora
Prebiotics
Resistant starch

ABSTRACT

Resistant starch (RS) refers to the portion of starch and starch products that resistant to digestion as it passes through the digestive system. The RS is subdivided into four types depending on its chemical structure: RS1 (physically inaccessible starch), RS2 (resistant granular starch), RS3 (retrograded starch), and RS4 (chemically modified starch), and considered as prebiotics that can be a supplement to the diet, leading to the growth stimulation of probiotics bacteria and the increase in intestinal concentrations of lactic acid and short-chain fatty acids (SCFA) assumed to promote host health. The aim of this study was to evaluate the activities of beneficial gut micro-flora isolated from human feces on three types of RS (RS2, RS3 and RS4). Two isolates of lactic acid bacteria were obtained from fecal sample of healthy people at 20-40 years old, and performed their very good growth in the medium containing 1% of either RS2, RS3, RS4, or fructo-oligosaccharides (FOS), a standard prebiotic, as carbon sources. All carbon substrates were treated through the digestive model system. The bacterial growth and pH changes were monitored, and fermentation products were determined. The growth of both bacterial isolates in the provided substrates at 20 hour cultivation reached to the maximum of 10^{10} CFU/mL. The highest content of lactic acid, 10,410 ppm, was determined in the fermentation of glucose by *Lactobacillus* sp. RCF10. RS3 was served as the best substrate for producing SCFA compared to RS4, RS2, and FOS, respectively. The highest content of SCFA was butyric acid, 11,575 ppm, which was higher than propionic and acetic acids. RS3 reveals the promising cheap prebiotic beneficial to human health.

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Published by School of Agro-Industry, Mae Fah Luang University

ISSN: 2408-1736

INTRODUCTION

The relationship between a nutritious diet and well-being has been one of the reasons for the increase in popularity of functional foods, which, in principle, apart from their basic nutritional functions, provide physiological benefits and/or reduce the risk of chronic diseases. Functional foods either contain or add a component with a positive health effect. (Gibson, 2004; Sanz *et al*., 2008). One of the added components could be resistant starch (RS) (Mikulíková *et al*., 2008), which is widely used as a functional ingredient, especially in foods containing high dietary fibre levels. The RS refers to the portion of starch and starch products that resistant to digestion as it passes through the digestive system. It is divided into four types: physically inaccessible starch (RS1), resistant granular starch (RS2), retrograded starch (RS3), and chemically modified starch (RS4), considered as prebiotics that can be a supplement to the diet. There is substantial research on the health benefits of RS, peer-reviewed studies that indicate benefits in intestinal health and also impact upon gastrointestinal diseases such as colon cancer, inflammatory bowel disease (IBD) and colitis (Asp, 1992; Englyst *et al*., 1992; Brouns *et al*., 2002; Nugent, 2005). The type of RS may play an important role in growth stimulation of probiotics bacteria and an increase in intestinal concentrations of lactic acid and short-chain fatty acids (SCFAs) assumed to be a health benefit for the host. However, there are still lacks of certain knowledge for the potential prebiotic properties of different types of RS for stimulating growth of lactic acid bacteria inhabiting human colon. The aim of this study was to isolate dominant lactic acid bacteria from human feces and evaluated some selected isolates for their capability to utilize certain types of RS (RS2, RS3 and RS4) that could reveal their potential prebiotic properties.

MATERIALS AND METHODS

Human fecal specimens for bacterial isolation

Fresh feces collected from two healthy adult volunteers (one man and one woman). The volunteers usually ingested a normal diet, presented no digestive disease and had not on antibiotics treatment for at least 3 months.

Isolation of lactic acid bacteria inhabiting human colon

Human feces were preserved in insulated bottles. For the isolation dominant lactic acid bacteria, five parts of carbonate-phosphate buffer solution were added to one part of feces (v/w) according to Barry *et al*. (1995). Further dilutions were made using PBS, and 100 microliters of each dilution were spread on the plates of MRS agar, Reinforced Clostridial Agar, *Streptococcus faecalis* Agar, and *Streptococcus thermophilus* Agar in order to isolate *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Streptococcus*, respectively. Plates were incubated at 37°C for 48 h under anaerobic conditions. Different majority colonies were collected and purified.

Selection and identification of some dominant lactic acid bacteria inhabiting human colon

Collected bacterial isolates were screened for their capability to utilize starch using MRS medium containing 1% native rice starch as carbon sources, and incubating at 37°C under anaerobic conditions for 48 h. The isolates having their ability to produce lactic acid and short-chain fatty acids were identified by their cell morphology observed by Gram staining and under light microscope (Cappuccino and Sherman, 1999), and biochemical reactions conducted by following the standard determinative bacteriology procedure (Holt *et al*., 1994; Cappuccino and Sherman, 1999).

Evaluation of the prebiotic effect of RS

The selected isolates were grown in the medium containing 1% of either RS or FOS as carbon sources. All carbon substrates were treated through the digestive model system.

The digestion described by Goderska *et al*. (2008) was conducted in an insulated bottle placed in a shaking incubator maintained at 37°C and 150 rpm. Parameters of the process to stimulate the conditions of the following stages of the human gastrointestinal tract: the stomach, the small intestine and the large intestine were designed. The volume of the digested samples was 200 mL. The *in vitro* digestion process in the stomach was carried out by the addition of 60,000 U of pepsin (P 7000, Sigma) suspended in 2 ml of 0.1 M HCl. The pH value was reduced to 2.0 using 6 M of HCl. Solutions were incubated at 37°C for 2 h in a shaking incubator at 150 rpm. Then, small intestine stage the pH value was increased to 7.4 using 6 M NaOH and 10 mL of the pancreatic-intestinal extract (0.02 g of pancreatic extract, P 1750 Sigma; and 0.12 g bile salts, B 8381 dissolved in 10 mL 0.1 M $NaHCO_3$) were added. Solutions were incubated at 37°C for 4 h in a shaking incubator at 150 rpm.

The large intestine stage the pH value was increased to 8.0 using 6 M NaOH and two isolates of lactic acid bacteria (RCF10 and MRF4) were added. Solutions were incubated at 37°C for 20 h in a shaking incubator at 120 rpm. After 20 h bacterial fermentation, the bacterial growth and pH changes were monitored, the total acidity were determined by titration method in AOAC International (2000) and the concentration of lactic acid and short-chain fatty acids (acetic, propionic, and butyric acids) were determined with high performance liquid chromatography (HPLC).

Statistical evaluation

Data were analysed using the statistical analysis of SPSS version 15. The differences between the experimental groups and control containing glucose were evaluated using Duncan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The dominant lactic acid bacteria inhabiting human colon

The total of 45 isolates of Gram-positive were collected from MRS agar, Reinforced Clostridial Agar, *Streptococcus faecalis* Agar, and *Streptococcus thermophilus* Agar. Two isolates (RCF10 and MRF4) were selected and identified by morphological and biochemical characteristics. These selected isolates Gram-positive, non-spore forming, oxidase, and catalase negative, and identified as belonging to genera *Lactobacillus (Lactobacillus* sp. RCF10 and *Lactobacillus* sp. MRF4, respectively*).*

Evaluation of the prebiotic effect of RS

All types of RS substrates were treated through the digestive model system. The growth of two isolates (RCF10 and MRF4) were similar and reached the maximum of 10^{10} CFU/mL at cultivation for 20 hours, for the strain of *Lactobacillus* sp. RCF10 an increase in cell counts was observed from 8.81 to 10.20 Log CFU/mL, while for *Lactobacillus*

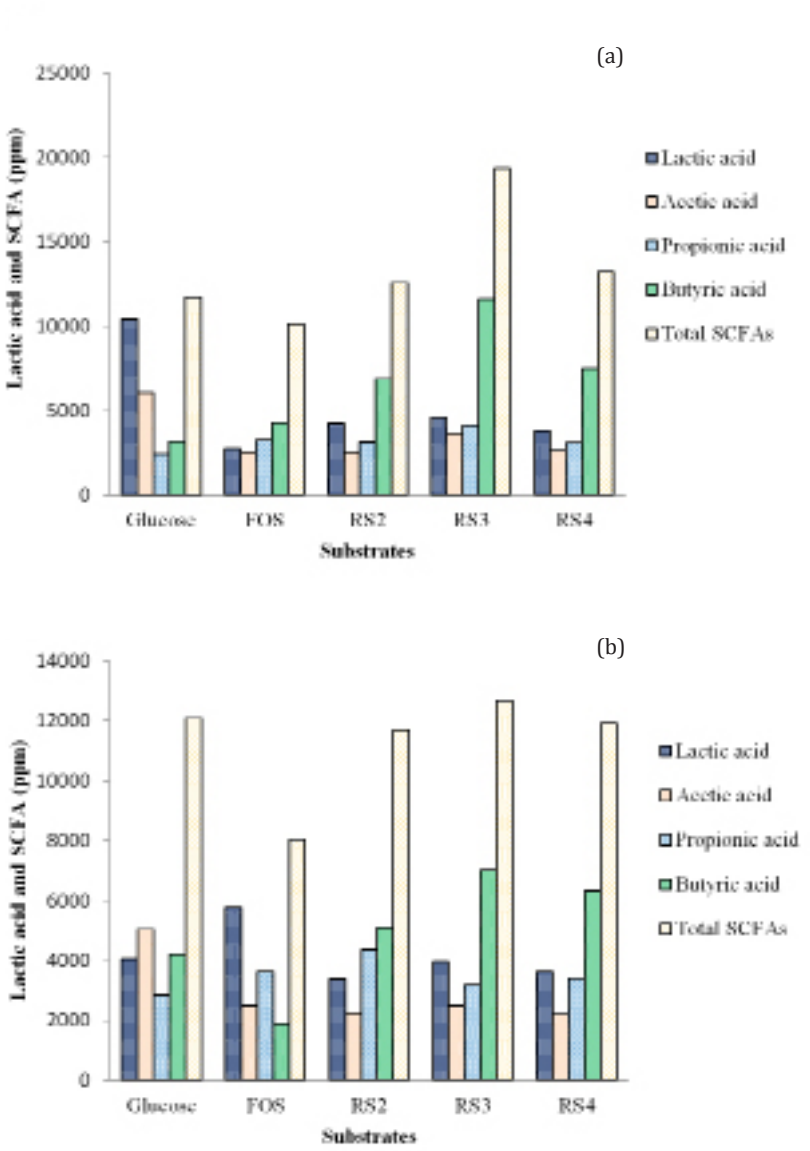

Figure 1. Contents of the short chain fatty acids (SCFAs) produced by *Lactobacillus* sp. RCF10 (A) and *Lactobacillus* sp. MRF4 (B) on the medium containing 1% of either RS or Fructo-oligosaccharide incubated at 37°C for 20 h in a shaking incubator at 150 rpm.

sp. MRF4 an increase in cell counts was from 8.76 to 9.92 Log CFU/mL. The pH and the total acidity of both species were ranged from 4.23-5.46 and 0.255-0.687%, respectively. The HPLC studies of cultured media indicated that major metabolites produced during fermentation of RS by the dominant lactic acid bacteria inhabiting human colon were lactic, acetic, propionic, and butyric acids (Figure 1). The highest content of lactic acid, 10,410 ppm, was determined in the fermentation of glucose by *Lactobacillus* sp. RCF10. However, the content of lactic acid produced by *Lactobacillus* sp. MRF4 was higher in the fermentation of FOS than glucose and all types of RS. The results of SCFA analyses indicated that RS3 had more potential for producing SCFA than RS2 and RS4. The highest content of SCFA was butyric acid, 11,575 ppm, which was detected in the medium containing RS3 after cultivating *Lactobacillus* sp. RCF10. These results indicated that RS played an important role of the potential prebiotic properties under the digestive model system.

CONCLUSION

Retrograded starch (RS3) was best served as the substrate for producing SCFA by the dominant lactic acid bacteria inhabiting human colon. Butyric acid was found as the highest content of SCFA, 11,575 ppm, which was higher than propionic and acetic acids. Results revealed the promising beneficial effects of RS3 as a prebiotic.

ACKNOWLEDGEMENT

This research was supported by Suranaree University of Technology, Nakhon Ratchasima, Thailand.

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