Full Length Article

Riceberry broken rice and soybean meal as substrates for exopolysaccharide production by *Bacillus tequilensis* PS21

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Abstract

This work aimed to study the effect of riceberry broken rice and soybean meal on exopolysaccharide (EPS) production by *Bacillus tequilensis* PS21. Various concentrations of riceberry broken rice (RBR) as carbon source at 4, 5, and 6%, soybean meal (SBM) as nitrogen source at 1, 2, and 3% were used whilst the fermentation conditions at 37 °C and media pH 7 were constant. The results showed that as fermentation time increased from 24, 48 to 72 h, the EPS content increased significantly along with bacterial growth. RBR (4, 5, and 6%) at 72 h resulted in no significant differences in EPS production. However, SBM at 1, 2, and 3% resulted in a statistically significant difference in EPS content at 72 h. Therefore, the optimized conditions for EPS production were RBR at 5% and SBM at 3% for an EPS content of 28.47 g/L. This work demonstrated a new way to add value to RBR and SBM to produce an EPS bioproduct for future applications including foods, cosmetics, and animal feeds.

Keywords: *Bacillus tequilensis*, exopolysaccharide, riceberry broken rice, soybean meal

Introduction

In recent years, waste has been increased daily in large quantities, adversely impacting ecosystems and human beings. The industry has a large amount of waste emitted in the production of products exported within and outside the country. By-products arising from the production process are difficult to eliminate and have a high cost. The need for methods of utilizing or recycling industrial waste is one of the current global environmental problems. Sugarcane molasses, broken rice, rice bran, and coconut water are agro- industrial wastes needed to be value added (Razack et al., 2013; Küçükaşik et al., 2011; Han and Watson, 1992, Seesuriyachan et al., 2011). These are agro-industrial wastes rich in sugars such as glucose, fructose, and sucrose. They also contain nitrogen and vitamins that are useful for multi-beneficial exopolysaccharide (EPS) biosynthesis. Several factors influence waste fermentation by microorganisms, including media composition, the fermentation conditions (pH,

temperature, concentration of oxygen), and the sources of carbon and nitrogen. (Pereira et al., 2006).

Riceberry rice well- known due to its distinct dark purple color, long-grained shape, and high antioxidant characteristics. It was developed by the Rice Science Center at Kasetsart University in Thailand and is a cross between Hom Nil rice and KhaoDok Mali 105. The shards of rice grains obtained by milling are referred to as broken rice. Broken rice is defined as rice with a length shortening of more than 20% (Soponronnarit et al., 1999). Riceberry broken rice (RBR) is a major byproduct of Thai rice processing plants. After the polishing phase, this product is separated and has the same chemical composition as white rice. The byproduct accounts for 20-30% of riceberry production (approximately 1,200-1,800 tons per harvesting season). Thus, we would like to find a new way to produce better value-added bioproducts using riceberry broken rice as a carbon substrate for EPS production.

Soybean meal (SBM) is a by-product of soybean extraction. It is a yellow or light brown color. Soybean meal consists of approximately 71.6% of soybean and approximately 7% of husks. Soybean meal and soy husks are produced approximately 1,923,000 tons/year. The most important protein source and contains large quantities of essential amino acids used to feed farm animals (Park et al., 2002). Soy waste can be used as a nitrogen source for the production of EPS.

EPSs are high molecular polymers consisting of sugar residues generated by EPS synthesizing microbes. EPS in the environment is found in the form of capsules or slime (Razack & Thangavelu, 2013; De Vuyst & Degeest, 1999). The microbial EPS offers a broad range of business applications, including food, cosmetics, pharmaceutics, milk, and medicines, as well as other industries, including medicine, wound dressing, food additives, cosmetic ingredients, medicine supplies, etc. As a result, EPS, as a value-added bioproduct, has a wide range of applications. The previous study reported that the concentration of carbon and nitrogen sources were able to affect EPS production (Lee et al., 2004; Smiderle et al., 2012).

This study aimed to identify the effects various concentrations of riceberry broken rice and soybean meal on EPS production from *Bacillus tequilensis* PS21.

Materials and Methods

Materials

Soybeans meal (SBM) was obtained from agricultural shop in Kantharawichai District of Mahasarakham Province as the nitrogen source of EPS production. Riceberries broken rice (RBR) was obtained from the Agrarian Network of E- San Enterprise in Roi Et Province as the carbon source. Prehandling, RBR, and SBM were ground in a fine 200 mm mesh powder and stored dry.

Cultivation of a bacterial strain

A purified culture of *B. tequilensis* PS21 was obtained from milk kefir in (Luang-In et al., 2016; Luang- In et al., 2018) in the Natural Antioxidant Innovation Research Unit, Biotechnology Laboratory of Mahasarakham University. The strain was aerobically cultured in sterilized Tryptic Soy Broth (TSB), pH 7.0 (17 g/L tryptone, 2.5 g/L K_2HPO_4, 5 g/L NaCl, and 2.5 g/L glucose) for 16-24 h shaking at 37°C at 150 rpm. The resulting bacterial suspension's optical density (OD_{600}nm) was adjusted to 0.1 before inoculation into the fresh TSB media.

Bacterial EPS production

RBR (4, 5, and 6%) and SBM (1, 2, and 3%) were mixed in 100 mL distilled water and adjusted to pH 7 and sterilized at 110 °C for 15 min. The resulting bacterial suspension's optical density (OD600nm) was adjusted to 0.1 before inoculation into the fresh RBR/SBM media and then seed inoculum (3% v/v) was transferred in culture media and incubation at 37 °C at 150 rpm agitation speed for 72 h. Bacterial counts on TSB agar using serial dilutions (10^{-2} - 10^{-8}) incubated at 37°C for 24 h were detected at several intervals at 72 h. A pH meter was used to measure the pH values, and the results were recorded in triplicate.

Extraction of crude EPS

The bacterial cultures were centrifuged at 4°C at 10,000 g for 15 min. Two volumes of cold ethanol (v/v) precipitated the supernatant and were kept at 4 ° C for 24 h. The was EPS centrifuged and washed twice with 95% (v/v) ethanol at 10,000 g at 4°C for 10 minutes. Fresh EPS was dried at 60°C in an oven and kept at -20°C.

Statistical analysis

All data were collected in triplicate, and the results were displayed as standard deviations (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) and Duncan's multiple range test by SPSS software (demo version).

Results and Discussion

Appearance of EPS

The EPS feature of *B. tequilensis* PS21 is the ropy or slimy appearance of the colony. Colonies show a slimy appearance on RBR agar media and incubated for 3 days at 37°C (Figure 1A). The formation of EPS sheets hanging on the culture surface of the supernatant appeared after 24 h of EPS ethanol precipitation (Figure 1B). The EPS was sticky and whit-ish (Figure 1C-1E).

Effects of various concentrations of riceberry broken rice and soybean meal on EPS production

The results showed that the EPS content increased significantly with bacterial growth as the fermentation time rose from 24, 48 to 72 h (Table 1). RBR (4, 5, and 6%) at 72 h resulted in no significant differences in EPS production. However, SBM at 1, 2, and 3% resulted in a statistically significant difference in EPS content at 72 h. Therefore, the optimized conditions for EPS production were RBR at 5% and SBM at 3% for the highest EPS content of 28.47 g/L. Significant increases in EPS production have come with increased concentrations of the substrates. EPS production was stimulated by the excess of carbohydrate in the medium and the limitation of carbon sources diminishes EPS synthesis (Van Geel-Scutten et al., 1998; De Vuyst & Degeest 1999). This is the first report of EPS production from RBR and SBM by *Bacillus* sp. with a higher yield than the previous reports by *Bacillus* sp (Moghannem et al., 2018; Ergene et al., 2018; Razack et al., 2013).

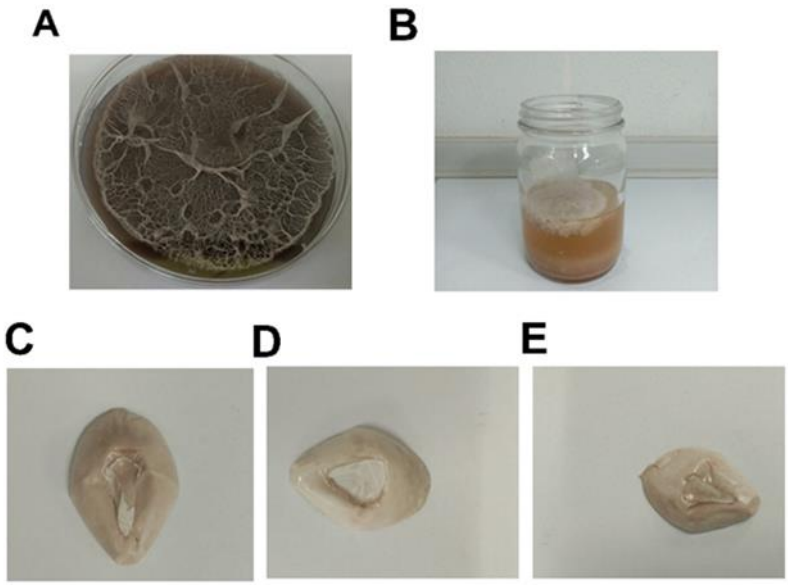

Figure 1. EPS slimes and EPS extracts. (A) EPS slime from BRB on agar. (B) EPS was precipitated with ethanol at 24 h. (C) EPS extract from RBR 4% SBM 3%. (D) EPS extract from RBR 5% SBM 3%. (E) EPS extract from RBR 6% SBM 3%.

Table 1: The effects of various concentrations of riceberry broken rice and soybean meal on EPS production by *B. tequilensis* PS21

Treatment	EPS content (g/L)		
	Time of fermentation (h)		
	24	48	72
RBR 4% SB 1%	$4.63\pm0.04^{C,f}$	$16.00 \pm 0.07^{B,f}$	$21.70\pm 0.04^{A,e}$
RBR 4% SB 2%	$7.96\pm0.01^{C,e}$	$20.83\pm0.04^{B,bc}$	$25.00\pm 0.00^{A,bc}$
RBR 4% SB 3%	$9.63\pm0.09^{B,de}$	$26.43\pm0.08^{A,a}$	$27.47\pm0.05^{A,a}$
RBR 5% SB 1%	$10.90\pm0.23^{C,de}$	$17.80\pm0.10^{B,e}$	$23.97\pm0.20^{A,cd}$
RBR 5% SB 2%	$10.86\pm0.56^{B,d}$	$18.26\pm0.57^{AB,d}$	$25.67\pm0.23^{A,b}$
RBR 5% SB 3%	$15.27 \pm0.30^{B,b}$	$19.93\pm0.50^{B,cd}$	$28.47\pm0.07^{A,a}$
RBR 6% SB 1%	$10.90 \pm0.31^{C,d}$	$18.29\pm0.32^{B,e}$	$22.90\pm0.13^{A,e}$
RBR 6% SB 2%	$13.37\pm0.17^{C,c}$	$19.90\pm0.17^{B,de}$	$25.39\pm0.26^{A,bc}$
RBR 6% SB 3%	$19.07\pm0.1^{C,a}$	$21.43\pm0.10^{B,b}$	$28.07\pm0.03^{A,a}$

* RBR: Riceberry broken rice; SBM: Soybean meal.
Data are mean ± S.D. of three replicates. Different capital letters within the same row and small letters within the same column indicate significant differences ($p < 0.05$) according to Duncan's multiple range test.

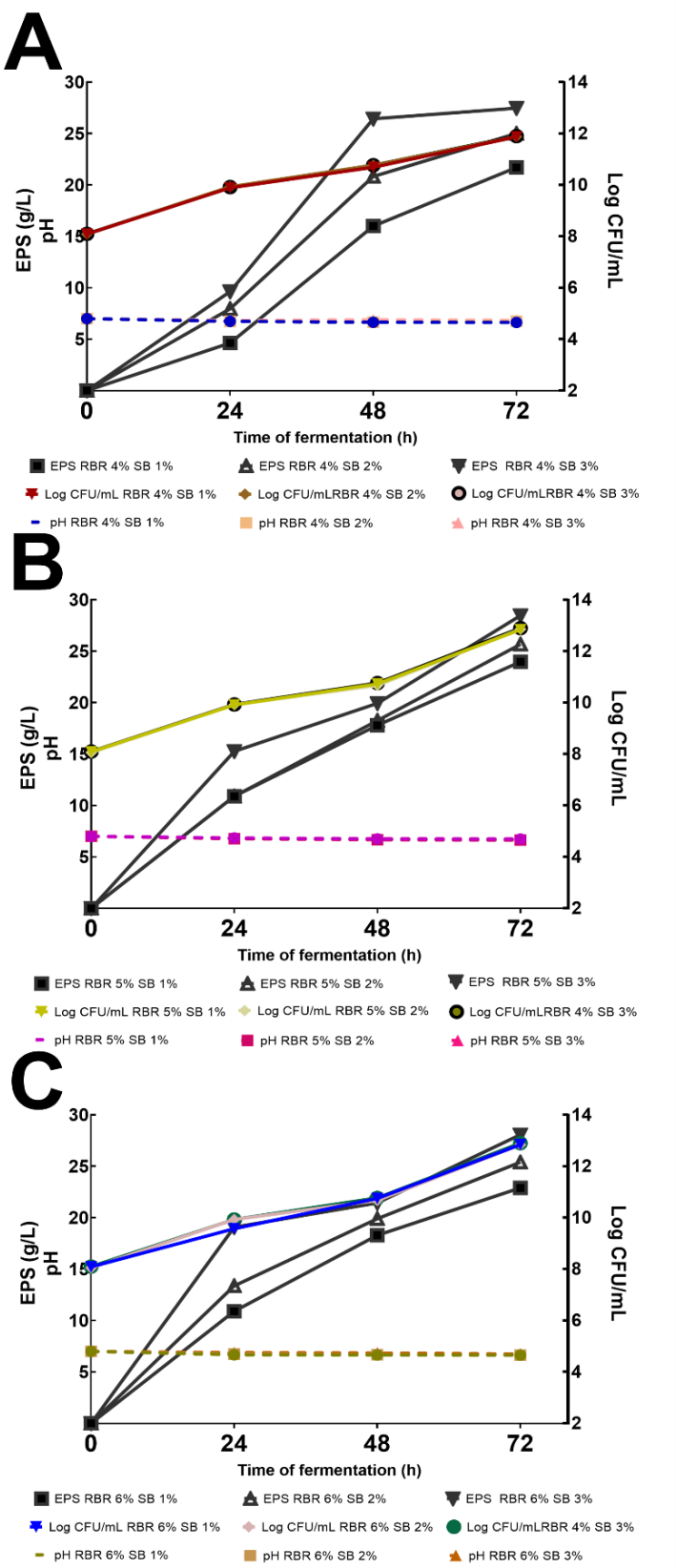

Figure 2. Number of microbial cells (dark lines), pH during fermentation (dotted lines) and EPS content (hyphen lines). (A) RBR 4% and SBM 1, 2 and 3%. (B) RBR 5% and SBM 1, 2 and 3%. (C) RBR 5% and SBM 1, 2 and 3%.

According to the previous works (Lee et al., 2004; Smiderle et al., 2012), EPS yields may be affected by the amount of agricultural waste and the types of agricultural waste used in production, as well as the microorganisms used. The EPS production of *P. pulmonarius* used peanut husks at 20 g/L, resulting in the highest EPS production at 5.60 g/L (Ogidi et al., 2020). Previous reports found that *Xanthomonas campestris* 1866 had xanthan gum production of 10.3 g/L using cocoa husks as a carbon source (Nery et al., 2013). The highest EPS production of 426 mg/L was detected using 60 g/L molasses concentration (Ergene et al., 2018). Various factors, different carbon sources and nitrogen sources were used. The results showed the best carbon source was molasses at 4% w/v has been EPS productivity was 4.2 g/L. The highest EPS productivity was 4.4 g/L from yeast extract at a concentration of 3 g/L. (Moghannem et al., 2018).

Number of microbial cells and pH of *B. tequilensis* PS21

The results showed that the initial pH value of the fermentation dropped from initially 7.00 to 6.67 at 72 h. The pH decline was caused by the production of organic acid as a result of the high consumption of carbon sources for cell growth. (Shih et al., 2006). Two other studies showed that growth in media with pH adjustment resulted in greater polysaccharides (De Vuyst et al., 2001). The pH of culture media was an important parameter affecting cell and EPS production (Liu et al., 2009). The difference in pH values in cultured *Bacillus* sp. ZBP4 affected EPS production because pH played a role in the synthesis of EPS at the gene level. Therefore, the pH value affected the enzyme in the synthesis of EPS (Ergene et al., 2018). The growth curve of *B. tequilensis* PS21 began at 10^8 CFU/mL at 0 h and reached 10^{12} CFU/mL at 72 h. (Figure 2). The *B. tequilensis* PS21 metabolite production followed a primary kinetics pattern, characterized by the metabolites' nearly simultaneous biosynthesis with growth and approaching a maximum rate near the end. EPS production decreased significantly after extended incubation which may probably be due to glycohydrolase degradation (Wang et al., 2014; Li et al., 2014). It can be seen that carbon and nitrogen sources are important for growth as well as EPS production because they provide the energy required for both processes.

Conclusion

The optimum conditions for EPS production from *B. tequilensis* PS21 showed that the maximum EPS was 28.47 g/L using 5% RBR and 3% SBM for 72 h of fermentation. *Bacillus* sp. yields higher yields than those from previously reported *Bacillus* sp. and is a new way to add value to broken rice and produce bio-products.

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