Utilization of sweet potato starch wastewater for biofertilizer production by *Bacillus amyloliquefaciens*

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Abstract

Effluents from the sweet potato starch industry possess a high load of Chemical Oxygen Demand (COD) (10–35 g/l). The treatment of such wastewater by conventional activated sludge processes consumes a lot of energy, resulting in high treatment costs. A plant growth promoting bacterium (PGPB) *Bacillus amyloliquefaciens* strain EBL11 was isolated from soil. The isolate EBL11 performed a good capacity of promoting tea plant growth, which indicated that the strain can be used as a nonchemical alternative biofertilizer. In this study, we investigated the carbon and nutrient rich industrial processing effluents of sweet potato wastewater (SPW) as substrate for the cultivation of the biofertilizer strain EBL11. The isolate EBL11 can grow well using SPW as the culture media. The maximum yield of 8.7×10^9 CFU/ml of the isolate EBL11 was reached in 18 h at 32°C, pH 7.0 using the SPW as substrate. In conclusion, it is feasible to develop a hybrid biotechnological process, integrating the production of environmental friendly biofertilizer with treatment of intractable wastewater.
Keywords: Bacillus amyloliquefaciens, biofertilizer, plant growth promoting bacteria, sweet potato starch wastewater, tea.

1 Introduction

Wastewater from the sweet potato starch industry contains a high load of protein, pectin, and starchy materials with the COD of 10,000–35,000 mg/l [1]. Due to

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the high loading of COD, the treatment of such wastewater by conventional activated sludge processes can be complicated and costly consuming a lot of energy, resulting in high treatment costs. However, the wastewater with a high concentration of nutrients might be a good culture medium for microorganism cultivation.

Bacillus amyloliquefaciens was originally described as a potent producer of liquefying amylase and other industrial ectoenzymes [2]. Many strains of *B. amyloliquefaciens* as plant growth–promoting bacteria (PGPB) were isolated from soil, associated with plant roots and exert beneficial effects on plant development [3, 4]. In the present work, a strain of *B. amyloliquefaciens* EBL11 was isolated from soil, which could improve the growth of tea plant and tea yield. Therefore, the plant growth promoting effect of this strain suggests a potential application as biological fertilizer.

In this study, we investigated a feasibility of production of biofertilizer by *B. amyloliquefaciens* EBL11 using the sweet potato wastewater (SPW).

2 Materials and methods

2.1 Microorganism

An anti-fungi bacterial strain EBL11 was isolated on Potato-dextrose Agar (PDA) medium in Petri dish from soil, and maintained on the PDA slant tubes.

2.2 Optimization of culture conditions

SPW samples were collected from a sweet potato starch process waste stream in Changsha, China, mainly containing COD 17000 mg/l. The pH of SPW was adjusted to 7.0 by NaOH when the SPW was used as the culture medium. The starch, pectin and sugars in the SPW were used as the main carbon source throughout the investigation.

The isolate EBL11 was grown on the PDA plates at 30ºC for 2 days. A single clone was inoculated into 250-ml flask containing 100 ml of the PDA medium at 30ºC for 24 h. This seed culture was used to initiate the growth in fermentation medium used in this study. The basic medium (BM) is composed of as follows (g/l): $(NH_4)_2SO_4$ 5, $MgSO_4 \cdot 7H_2O$ 0.10, KH_2PO_4 0.50, NaC1 0.50, and K_2HPO_4 1.50. The fermentation was conducted in 250 ml Erlenmeyer flasks containing 100 ml medium inoculated with 5 ml of seed culture. Unless otherwise stated, the agitation rate and incubation temperature were 200 r/min and 30ºC, respectively.

All experiments were conducted in duplicate and the average values are reported. Key results were repeated three times to establish their validity.

2.3 Tea plant growth promotion

Tea plants (*C. sinensis*) from tea plantation (3 years old) of semi-tropical uplands, Hunan, China (113°19′E, 28°33′N) were used to study the efficacy of the PGPB strain EBL11 on the yield of tea. The plantation belongs to the typical

red soil. Tea plants have no clear differences owing to unified management conditions.

The treatments were conducted by irrigation of the biofertilizer (fermented SPW by strain EBL11) in four levels: without biofertilizer (CK), 100 ml/m^2, 200 ml/m^2 and 300 ml/m^2 for three times monthly. There were three replications with each replicate consisting of 50 bushes. The experiment was laid out in a randomized block design with a plot size of 10 m×20 m. Tea production was measured by one-hundred-bud weight and then fresh tea leaves was treated including fixation, rolling and baking, to prepare the green tea by the same procedure [5].

3 Results and discussion

3.1 Effect of carbon sources on the isolate EBL11 growth

The fermentation medium contained 100 ml BM, and 2% each carbon sources, including sugar, D-glucose, soluble starch, and SPW (90 ml) with BM (10 ml). After inoculation with 5 ml of seed culture, the medium was incubated at 30ºC for 20 h. The effect of carbon sources on the production of the isolate EBL11 are presented in Fig. 1. It was found that SPW was a suitable carbon source for the isolate EBL11 cultivation.

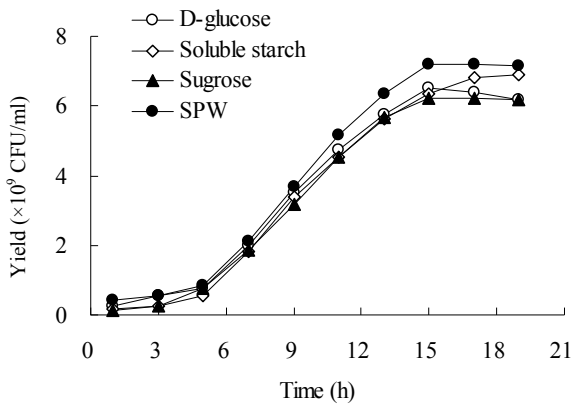

Figure 1: Effect of different carbon sources on the isolate EBL11 growth.

3.2 Effect of nitrogen sources on the isolate EBL11 growth

The fermentation medium contained 90 ml SPW as carbon source, 10 ml BM, and 0.5% different nitrogen sources, including corn steep liquor, potassium nitrate, ammonium sulfate. A control experiment was conducted without addition of nitrogen source. After inoculation with 5 ml of inoculum, the medium was incubated at 30ºC for 20 h. The results of impact of nitrogen sources on the production of the isolate EBL11 are presented in Fig. 2. It was found that corn steep liquor was the most efficient nitrogen source for production of the strain.

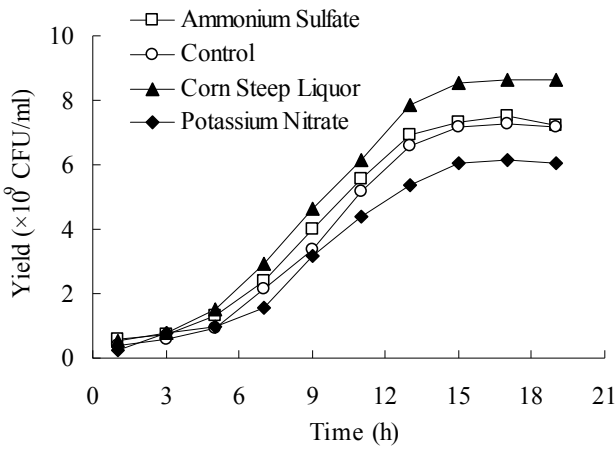

Figure 2: Effect of different nitrogen sources on the isolate EBL11 growth.

3.3 Effect of culture temperature on the isolate EBL11 production

The fermentation medium contained 100 ml SPW with 0.3% of corn steep liquor. After inoculation with 5 ml of inoculum, the medium was incubated at 24, 28, 32, 36 and 40ºC, respectively. The time courses of the isolate EBL11 production at different temperature are shown in Fig. 3. The maximum yield of 8.7×10^9 CFU/ml was reached at 32ºC after 18 h fermentation.

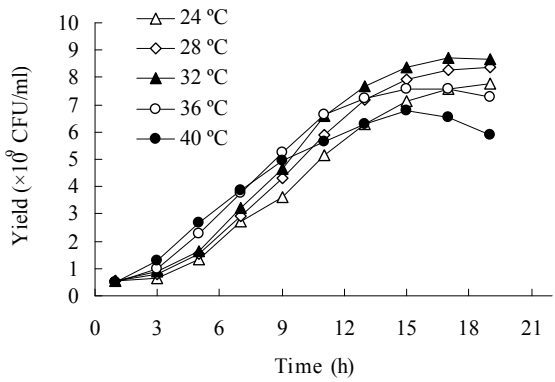

Figure 3: Effect of culture temperature on the isolate EBL11 growth.

3.4 Isolate EBL11 promoting the leaves growth of tea plants

B. amyloliquefaciens EBL11 was investigated for possible beneficial effects on the growth of tea plants. The results revealed that application of the biofertilizer was useful for the promotion of tea growth (Fig. 4). The tea yield was increased

significantly by irrigating 200 ml/m^2 and 300 ml/m^2 of fermented SPW by the isolate EBL11. In general, the results confirm those of previously published studies that have investigated the growth response of plants to inoculation with *B. amyloliquefaciens* [6, 7]. *B. amyloliquefaciens* was discovered to be soilborne and plant associated, i.e., to possess the abilities to stimulate plant growth and suppress plant pathogens by promoting seedling emergence, plant biomass, and disease control [2].

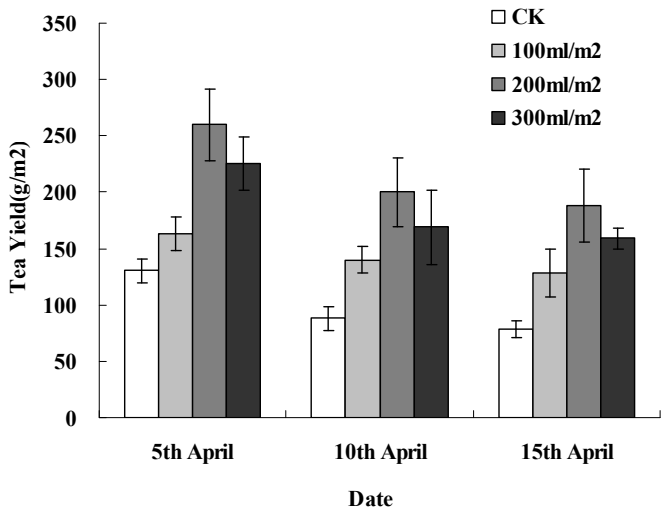

Figure 4: Effect of biofertilizer application on the yield of tea.

SPW is one of the most intractable wastewater because of its high strength of COD. A few studies have already focused on the utilization of such high organic loading wastewater as substrates or production media in fermentation processes [1, 8]. As far as the authors are aware, no research case was found in the literature for utilization of SPW for biofertilizer of *B. amyloliquefaciens* production.

4 Conclusions

A newly isolated *B. amyloliquefaciens* EBL11 from soil can be used as a nonchemical alternative biofertilizer for promoting tea plant growth and increase tea yield. It is feasible to develop a hybrid biotechnological process, integrating the production of environmental friendly biofertilizer with treatment of intractable SPW.

Acknowledgements

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