



## Production of Probiotic *Streptomyces* Biomass from Starchy Wastewater

Suteera Srisamai, Phonlawat Srikhampa and Wasu Pathom-aree\*

Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

\*Author for correspondence; e-mail: wasu.p@cmu.ac.th

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### ABSTRACT

Ten probiotic *Streptomyces* strains isolated from soils and fish intestines were cultured in starchy wastewater collected from the potato chip factory in northern Thailand. Among these, 8 isolates were able to grow in starchy wastewater as sole source of nutrient and yielded equivalent growth as those cultivated in synthetic medium; Yeast extract protein starch soluble (YpSs) under the same condition (150 rpm at 30°C for 7 days). Two *Streptomyces*, F7-6 and F8-6 were chosen for time course experiment in starchy wastewater. The highest growth was observed on day 4 of cultivation with biomass yield of  $3.855 \pm 0.32$  mg/ml and  $2.766 \pm 0.63$  mg/ml, respectively.

**Keywords:** *Streptomyces*, probiotics, biomass production, starchy wastewater, amylase activity

### 1. INTRODUCTION

Members of the genus *Streptomyces* are filamentous actinobacteria, which are capable of producing secondary metabolites and extracellular enzymes such as amylase, protease and lipase. These enzymes are useful for the decomposition of organic and inorganic matters in natural environments [6, 7]. *Streptomyces* have been used as a probiotic to control bacterial diseases in livestock, poultry [9] and aquaculture [2, 3, 5]. Large scale production of *Streptomyces* biomass is needed for commercially probiotic products preferably at reasonable cost. Starchy wastewater is a by-product from the potato washing process in potato chip factories which

still contains high concentrations of starch. Since starch hydrolyzing activity is widely distributed among members of the genus *Streptomyces* [11, 12], starchy wastewater might be used as a low cost medium for its biomass production. In this study, starchy wastewater from a fried potato chip factory in the northern part of Thailand was used as substrate for cultivation of probiotics *Streptomyces*. The potential use of starchy wastewater as the sole nutrient source for growth of *Streptomyces* was evaluated. We anticipate that starchy wastewater can be used to produce *Streptomyces* biomass at cheaper cost than using conventional culture media.

## 2. MATERIALS AND METHODS

### 2.1 Microorganisms

Ten probiotic *Streptomyces* strains, were isolated from near shore soil samples and fish intestinal tracts using starch casein agar (SCA; soluble starch 10 g, casein 1 g,  $K_2HPO_4$  0.5 g, agar 15 g, distilled water 1000 ml, adjusted pH  $7.0 \pm 0.1$ ) supplemented with 25  $\mu$ g/ml nystatin and 10  $\mu$ g/ml nalidixic acid to minimize fungal and fast growing bacterial contamination [2]. These strains have previously been screened for their probiotic properties eg. inhibition of fish pathogens and non-haemolysis (data not shown). All isolates were stored on SCA slants at 4°C for further use.

### 2.2 Primary Screening of *Streptomyces* Strains for Amylase Activity

All *Streptomyces* isolates were screened for amylase activity on nutrient agar (NA) containing 2% soluble starch. Amylase activity was detected using iodine vapor. Starch hydrolysis was indicated by a clear zone against blue background of starch-iodine complex around amylase-producing colony after incubating at 30°C for 7 days. The hydrolysis zone (in mm) was calculated from clear zone diameter minus colony diameter. Amylase activity index (clear zone diameter (mm)/colony diameter (mm)) was also determined according to Knox et al. [8]

### 2.3 Biomass Production of *Streptomyces* Strains in Starchy Wastewater

Starchy wastewater was collected from Pepsico International Thai Trading company (Frito-Lay), Lamphun, Thailand. The nutritional value of this wastewater was determined using standard method [1] at Faculty of Agriculture, Chiang Mai University. This starchy wastewater was used as a

cultivation medium for *Streptomyces*. Ten agar plugs (5 mm in diameter) of *Streptomyces* culture were inoculated into 100 ml of sterile starchy wastewater (S). Yeast extract protein starch soluble (YpSs) broth was used as a control medium. Both cultures were incubated at 30°C on a shaker at 150 rpm. Cells were collected after 7 days by centrifugation (6000 xg for 10 min) and dried at 50°C for 2 days. Biomass yields of *Streptomyces* were expressed as dried cell weights.

Two *Streptomyces* strains that showed good biomass production in starchy wastewater were chosen for a time course experiment. Ten agar plugs (5 mm in diameter) of *Streptomyces* culture were inoculated into 100 ml of starchy wastewater, and incubated at 30°C on a shaker at 150 rpm. Cells were collected every day for 7 days by centrifugation (6000 xg for 10 min) and dried at 50°C for 2 days. The remaining supernatant was collected and kept at 4°C for determination of amylase activity.

### 2.4 Amylase Activity of Selected *Streptomyces* Strains

Amylase activity was determined using a dinitrosalicylic acid (DNS) method [4, 10]. A 0.3 ml of 1% soluble starch in 0.1 M citrate phosphate buffer (pH 6.5) was mixed with 0.05 ml supernatant. The mixture was incubated in a waterbath at 37°C for 30 min. DNS reagent (2 ml) was added to the mixture and then boiled at 100°C for 15 min. The heated mixture was cooled at once in an ice bath. The absorbance was measured by spectrophotometer at 540 nm. Sterile distilled water was used as a blank. One unit (U) of amylase activity was defined as the amount of enzyme necessary to release reducing sugars equivalent to 1  $\mu$ M of glucose per minute, at 37°C.

### 3. RESULTS AND DISCUSSION

#### 3.1 Primary Screening of *Streptomyces* Strains for Amylase Activity

All 10 probiotic *Streptomyces* strains were able to hydrolyze starch on starch agar. They produced clear zones with diameter ranging from 10.0-20.3 mm against a dark blue background after being

exposed to iodine vapor. An amylase activity index was used to select strains with high amylase activity for further study (Table 1). *Streptomyces* strains F7-6 and S9-8 exhibited the highest amylase hydrolysis indexes of 1.54 and 1.50, respectively, and were selected for a time course experiment.

**Table 1.** The diameter (mm) of hydrolysis zone and amylase activity index<sup>a</sup> of ten *Streptomyces* cultivation on starch agar plate after 7 days incubation at 30°C.

<i>Streptomyces</i> strains	Amylase activity index	Diameter of clear zone (mm)	Diameters of colony (mm)
F7-6	1.54	19.3	13.3
F8-6	1.14	19.0	16.6
S2F3	1.33	19.0	14.6
S9-4	1.42	20.3	14.3
S9-7	1.26	17.6	14.0
S9-8	1.50	18.0	12.0
S11-3	1.45	18.3	12.6
S13-2	1.25	10.0	8.0
S13-7	1.24	13.6	11.0
SAIA	1.42	18.3	13.0

<sup>a</sup> amylase activity index is calculated from clear zone diameter (mm)/colony diameter (mm).

#### 3.2 Biomass Production of Ten *Streptomyces* Strains in Starchy Wastewater

The starchy wastewater collected from Pepsico International Thai Trading company (Frito-Lay) was rich in starch ( $97.6 \pm 0.1\%$  w/v). Other nutrients were nitrogen ( $0.020\%$  w/v), lipid ( $0.014\%$  w/v), reducing sugars ( $0.003\%$  w/v) and suspension solids ( $0.17\%$  w/v). Ten *Streptomyces* strains that hydrolysed starch on starch agar plates were cultured in this starchy wastewater without any additional nutrient. Among these, 8 *Streptomyces* strains, namely F7-6, F8-6, S2F3, S9-4, S9-8, S11-3, S13-2 and SAIA were able to grow in starchy wastewater and yielded equivalent dried cell weights as cultures grown in control YpSs medium ( $p=0.884$ ), as shown in Figure 1.

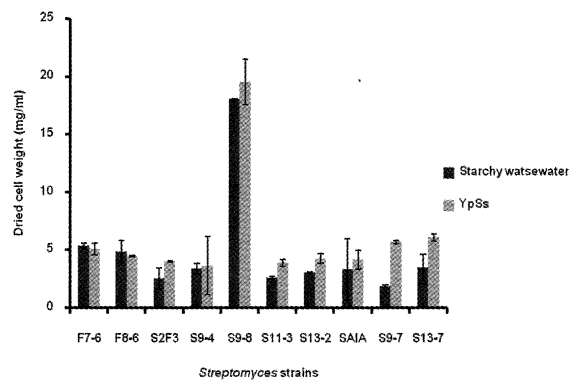
Strains F7-6 and F8-6 were selected for a time course experiment of biomass production, as they gave higher yields in starchy wastewater than in YpSs (Figure 1). It is evident from Figure 2A that strains F7-6 and F8-6 produced the maximum dried cell weight of  $3.855 \pm 0.32$  and  $2.766 \pm 0.63$  mg/ml on the 4<sup>th</sup> day of growth, respectively.

#### 3.3 Amylase Activity of Selected *Streptomyces* Strains

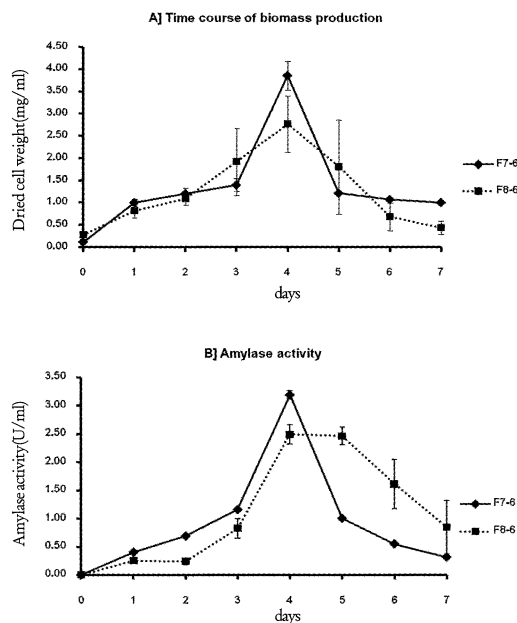
From the experiments described above, *Streptomyces* strains F7-6 and F8-6 can produce amylase enzymes on starch agar plates, grow rapidly and produce good cultures in starchy wastewater after four days of incubation. Amylase production was

growth associated. In time course experiments, amylase production started in day 1 and reached the highest activity on day 4 corresponding to their growth as clearly seen in Figure 2B. Strains F7-6 and F8-6 showed maximum amylase activities of  $3.191\pm0.07$  and  $2.492\pm0.16$  U/ml, respectively. Similar observations were reported in *Streptomyces griseoflavus* [13].

However, the maximum amylase activities of strains F7-6 and F8-6 were higher than those previously reported from *S. griseoflavus* ( $1.66 + 0.07$  U/ml) [13]. This observation showed that these two *Streptomyces* strains could use their amylase to hydrolyze starch in the culture medium to produce biomass.



**Figure 1.** Dried cell weight of ten *Streptomyces* strains cultivated in starchy wastewater and YpSs. After 7 days growth, eight strains grown on starch yielded equivalent growth as those cultured in YpSs ( $p=0.884$ ).



**Figure 2.** Dried cell weight (mg/ml) (A) and amylase activity (U/ml) (B) of *Streptomyces* strains F7-6 and F8-6 during 7 days growth.

#### 4. CONCLUSION

In this study, starchy wastewater from a potato chip factory was investigated as a nutrient source for cultivation of probiotic *Streptomyces*. The detection of amylase activity clearly indicated the ability of these *Streptomyces* to use starch as their nutrient for growth. The biomass yields obtained were equivalent to those from conventional medium based on dried weight. Our results indicated the feasibility of biomass production from starchy wastewater, which could be used as a cheap alternative to expensive commercial synthetic media to culture microorganisms with biotechnological potential such as *Streptomyces*.

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