

Screening High Efficiency Degradation Starch and Protein Strains from Potato Skins Slurry

Shaojuan Su

College of Life Science, Hebei University

Key Lab of Microbial Diversity Research and Application of Hebei Prov., Baoding 071002, China

E-mail: sushaojuan1002@163.com

Liping Zhang (Corresponding author)

College of Life Science, Hebei University

Key Lab of Microbial Diversity Research and Application of Hebei Prov., Baoding 071002, China

Tel: 86-312-5079696 E-mail: zhlping@hbu.edu.cn

Lige Guo

College of Life Science, Hebei University

Key Lab of Microbial Diversity Research and Application of Hebei Prov., Baoding 071002, China

Received: December 29, 2010

Accepted: January 18, 2011

doi:10.5539/mas.v5n2p235

This research was financially supported by Hebei Province Basic Research Project titled "Hebei microbial resource base information database" (Grant No. 10967146 D).

Abstract

[Objective] To isolate and screen strains that could yield both amylase and protease in the early stage of biogas slurry, determine the vitality of amylase and protease and improve the rates of biodegradation and gas yield. [Method] Use selective medium for early screening, and then apply the modified Yoo method and national professional standards concerning determination of protease activity to determine the vitalities of amylase and protease. [Result] Isolate and screen one strain that can yield both amylase and protease in the early stage of biogas slurry. The vitality of amylase and protease was 102.61U/mL and 92.93U/mL, respectively.

Keywords: Early stage of biogas slurry, Amylase and protease, Enzyme activity

Amylase and protease were the two important hydrolytic enzymes that have been most widely used with the largest output at home and abroad at present (Sangeeta, 2006, PP. 257-261). Microbial secretion of amylase and protease was relatively simple and cheap compared to the treatment of the two enzymes originated from plants and animals. Reports on amylase and protease originated from microorganism have been well documented and attracted researchers' attention all around the world (Wang, 2009, PP. 48-51). *Aspergillus* could produce a variety of extracellular enzymes, and secrete two enzymes, such as amylase and protease (Pandey, 2000, PP. 135-152). Activity in producing amylase and protease has been applied as an indicator of screening strains for water purification project. A strain with activities of 54.06U/mL for amylase and 35.6 U/mL for protease has been screened and applied as modifying agents for microbial water quality (Li, 2008, PP. 15-18). Strains isolated from the rumen contents with the enzyme activity of 54.06U/mL and 221.57U/mL respectively have been the solid basis for the application of feed production (Wang, 2009, PP. 48-51). However, there has been rarely documented about the systematic study on strains producing amylase and protease during the period of biogas fermentation. Therefore, it was profound necessary to investigate effects of enzyme activity of organic degrading bacteria, such as starch, protein, fiber, and fat etc, and substrate decomposition process in the early biogas fermentation on the yield of biogas fermentation.

Potatoes were the fourth cardinal crops, next to wheat, rice and maize currently, and its starch has been widely applied in commerce (Zhang, 2009, PP. 8-9). Subsequently, the resultant potato skin with large amount would cause environmental pollution and resource waste if unreasonable usage. They could be treated with anaerobic

fermentation, but there was a common issue that the conversion rate of raw materials was quite low in biogas fermentation. Raw materials were almost insoluble organic molecules, and could be taken into usage through decomposing them into small molecules by hydrolytic enzymes. Therefore, the hydrolysis process used to be the rate-limiting phase of anaerobic degradation (Meng, 2007, PP. 46-49). In the present paper, after isolating and screening strains that can yield both amylase and protease in the early stage of biogas slurry, determining the vitality of amylase and protease and improve the rates of biodegradation and gas yield, a strain with high activity of amylase and protease was obtained, which built a solid basis for the further improvement of biogas quality.

1. Materials and methods

1.1 Materials

1.1.1 Strains

Strains s1-s25 was isolated from biogas slurry.

1.1.2 Reagents

All reagents used in the present paper were of analytical grade.

1.1.3 Medium (Dong, 2001, PP. 352-374)

Slant medium: 10g peptone, 3g beef extract, 5g NaCl, 10g agar, and 1L distilled water.

Medium for primarily screening amylase: 10g peptone, 5g beef extract, 5g NaCl, 2g soluble starch, and 1L distilled water.

Medium for primarily screening protease: 10g skimmed milk powder, 10g peptone, 3g beef extract, 5g NaCl, 10g agar, and 1L distilled water.

Liquid seed medium: 10g peptone, 3g beef extract, 5g sodium chloride, and 1L distilled water.

Secondary screening medium: 10g peptone, 3g beef extract, 5g sodium chloride, 5g soluble starch, 0.3g potassium dihydrogen phosphate, 0.5g magnesium sulfate, 0.2g calcium chloride, and 1L distilled water.

1.2 Test methods

1.2.1 Isolation and screening of strains

The collected samples were coated in the plate medium for first screening by gradient dilution methods, and cultured for 2-4 days at a 37 °C incubator for further observation. Iodine solution was added into plates with amylase drops by drops, and whether there were starch hydrolyzed circles in plates was observed. The fact that there were transparent circles around strains proved that the strain could produce amylase; it was the same with protease. Strains that could both produce hydrolyzed circles in two kinds of plates were selected, inoculated into two kinds of solid medium, viz. amylase and protease, and cultured for 2-4 days at a 37 °C incubator for further observation. Strains that could produce both amylase and protease were selected. Its transparent circle diameter and strain diameter was measured by vernier caliper. According to the ratio between two data, enzyme producing vitalities of strains were primarily determined.

1.2.2 Secondary screening of strains and preparation of crude enzyme

Strains after primary screening were inoculated into the seed medium, and shaking cultured for 24 h at 37 °C at 120r/min. 10% feed fluid was inoculated into the liquid fermentation medium (100 mL/250mL flask), and shaking cultured for 72 h at 37 °C. Fermentation solution was centrifuged for 8 min at 4 °C at 12000 r/min, and the supernatant were kept in refrigerator at 4 °C for further investigations.

1.2.3 Determination of amylase activity

Using the modified Yoo (Shi, 1995, PP. 23-24) method, 5 ml 0.5% soluble starch solution was preheated at 40 °C water bath for 10 min, and 0.5 mL crude enzyme solution was added and mixed in the water bath for 5 min. After accurate reaction for 5 min, 5 mL 0.1mol/LH₂SO₄ was added to terminate the reaction. 5 mL dilute iodine solution was added to 0.5 mL reaction solution, and after coloring, the optical density was measured at 620 nm. Using 0.5 mL water instead of 0.5 mL reaction solution as blank, and tubes without enzyme solution (adding the same volume of blank medium supernatant) as control, enzyme activity was calculated according to the following formula:

$$\text{Enzyme activity (U)} = (\text{OD}_1 - \text{OD}_2) \times 100 / \text{OD}_1$$

Where OD₁ and OD₂ indicated the optical density of control and reaction solutions, respectively.

Activity definition: Hydrolyzing 1 mg starch in 5 min at pH 7.0 at 40 °C was 1 U.

1.2.4 Determination of protease activity

Protease activity was measured by the national professional standards with some modifications (SB/T 10317-1999). 1 mL crude enzyme solution was preheated at 40 °C water bath for 2 min, and 1 ml preheated casein solution was added. After 10 min precise insulation, 2 mL 0.4mol/L trichloroacetic acid was added to terminate the reaction. After 20 min water bath, the residual protein precipitation was discarded, and the resultant solution was centrifuged. 5mL 0.4mol / L sodium carbonate and 1mL Folin reagent were added to 1 ml supernatant, mixed till even, and heated and colored at 40 °C for 20 min. After cooling, the optical density of samples was measured at 660 nm by visible spectrophotometer. Blank experiment was the same with the previous method, and trichloroacetic acid was added to inactivate enzyme before adding casein. Enzyme activity was calculated according to the following formula:

$$\text{Protease activity (U/mL)} = A/10 \times 4$$

Where: A indicated the amount of produced tyrosine after reaction; 4 indicated 1 mL solution was taken from 4 mL reaction solution; 10 indicated reaction time (min).

2. Results and analysis

2.1 Isolating and screening of strains producing amylase and protease

Using selective culture method, 22 strains producing amylase and 12 strains producing protease were isolated, and the two kinds of strains were inoculated into two kinds of solid medium with both amylase and protease, and cultured for 2-4 days at 37 °C for further observation. Amylase plates were treated with iodine solution, and among them, 22 strains generated starch hydrolyzed circles. Circle diameter was marked as D_1 , strain diameter as D , and their ratio as D_1/D . After observation, there were 9 strains could generate visible transparent circles in the medium used for primarily screening protease, and their parameters were listed in Table 1. As seen from Table 1, strains s1, s5, s6, s7, s11, s17, s19, s21, s22 generated both amylase and protease, and could be applied as a primary screening strain for both enzymes.

2.2 Secondary screening strains producing amylase and protease

9 strains producing both amylase and protease obtained from primary screening were inoculated into feed medium and shaking cultured for 24 h at 37 °C. 10% feed fluid was inoculated into the liquid fermentation medium and shaking cultured for 72h. After centrifugation, supernatant was selected to assay amylase and protease activities, and results were listed in Table 2. As seen from Table 2, amylase activity of strain s5 was relatively higher, and reached 113.01 U/mL, while protease activity was quite low, and only 18.16 U/mL; protease activity of strain s7 was 104.83 U/mL while amylase activity was quite low; two activities of strain s1 were 92.93U/mL and 102.61U/mL respectively, and failed to reach the highest level, but two enzymes resulted from the strain was of practical application values.

3. Discussion

During the early stages of biogas fermentation, bacteria producing non-methane organic substances provided nutrients, and meanwhile its strong aerobic respiration created suitable anaerobic environment for producing strains generating methane (Yu, 2008, PP. 15658-15660). Hydrolysis of organic matter was the beginning of the fermentation, and its speed determined the rate of the whole fermentation process. Treating potato skins with anaerobic fermentation could not only effectively reduce environmental pollution, but also improve resource utilization. In the present study, 1 strain that could effectively degrade starch and protein was isolated. The strain could efficaciously degrade raw potato skins, build a base for further improving the quality of biogas, and be of guidance significance for selectively adding biogas hydrolysis strains to fermentation system.

References

- Determination method of protease activity. Measurement of protease activity. Commercial industry standards, SB/T 10317-1999.
- Dong, X.Z., & Cai, M.Y. (2001). *Common bacteria identified manual system*. Beking: Science press, 352-374.
- Li, L., & Liu, D.M. (2008). Screening and identification of *Bacillus subtilis* strains with high-activity of amylase, protease. *Fishery Modernization*, 35(2):15-18.
- Meng, J., & Wang, D.Q. (2007). The research situation of microbe flora of biogas ferment. *Journal of Guangxi Agriculture*, 22(4):46-49.
- Pandey, A., Nigam, P., & Soccol, C.R., et al. (2000). Advances in microbial amylases. *Biotechnol. Appl. Biochem*, (31):135-152.

Sangeeta, N., & Rintu, B. (2006). Optimization of amylase and protease production from *Aspergillus awamori* in single bioreactor through EVOP factorial design technique. *Food Technol. Biotechnol*, 44(2):257-261.

Shi, Y.B., Jiang, Y.M., & Fan, Y., et al. (1995). Effects of protease on *Bacillus amyloliquefaciens* α -amylase activity. *Microbiology*, 22(1):23-24.

Wang, P.P., & Chang, J. (2009). Researches on screening fungus producing protease and amylase and studying their enzymatic characterization. *Chinese Journal of Animal Science*, 45(21):48-51.

Yu, F.B., Luo, X.P., & Guan, L.B. (2008). Research advances in biogas fermentation microorganism. *Journal of Anhui Agriculture Sciences*, 36(35): 15658-15660.

Zhang, T., & Li, W.Z. (2009). *Application study on potato starch*. Guangxi Journal of Light Industry, 5:8-9.

Table 1. Strain and hydrolyzed diameter and their ratio

Serial number	D/cm	D ₁ /cm	D ₂ /cm	D ₁ / D	D ₂ / D
s1	1.50	3.20	3.75	2.13	2.50
s2	1.20	2.30	-	1.91	-
s3	1.40	2.40	-	1.71	-
s4	1.00	2.30	-	2.30	-
s5	1.00	1.60	2.80	1.60	2.80
s6	1.80	3.60	3.60	2.00	2.00
s7	1.60	3.60	3.20	2.25	2.00
s8	1.00	1.60	-	1.60	-
s9	1.00	1.80	-	1.80	-
s10	0.90	2.30	-	2.55	-
s11	0.85	1.90	2.55	2.23	3.00
s12	1.00	1.50	-	1.50	-
s13	1.50	1.80	-	1.20	-
s14	0.90	1.80	-	2.00	-
s15	1.00	1.80	-	1.80	-
s16	0.75	1.50	-	2.00	-
s17	1.30	3.20	2.47	2.46	1.90
s18	0.50	0.80	-	1.6	-
s19	1.20	2.29	2.16	1.91	1.80
s20	1.00	1.80	-	1.80	-
s21	0.80	2.00	2.08	2.46	2.60
s22	1.00	1.90	2.28	1.90	2.28
s23	0.50	-	0.80	-	1.60
s24	1.40	-	2.80	-	2.00
s25	0.50	-	1.00	-	2.00

Table 2. Enzyme activity of strains after secondary screening

Serial number	Amylase activity	Protease activity
s1	102.61	92.93
s5	113.01	18.16
s6	60.65	24.22
s7	30.49	104.83
s11	46.80	25.18
s17	34.08	25.76
s19	18.25	17.11
s21	66.94	36.32
s22	68.18	26.26