J. Microbiol. Biotechnol. (2015), 25(2), 178–184
http://dx.doi.org/10.4014/jmb.1404.04035

jmb

Utilization of Potato Starch Processing Wastes to Produce Animal Feed with High Lysine Content

Ying Li[1], Bingnan Liu[1], Jinzhu Song[1], Cheng Jiang[1], and Qian Yang[1,2*]

[1]*Bioengineering Center, School of Life Science and Technology, Harbin Institute of Technology, Harbin 150001, P.R. China*
[2]*State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, P.R. China*

Received: April 17, 2014
Revised: August 19, 2014
Accepted: September 4, 2014

First published online
September 4, 2014

**Corresponding author*
Phone: +86-451-86412952;
Fax: +86-451-86412952;
E-mail: yangq@hit.edu.cn

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

This work aims to utilize wastes from the potato starch industry to produce single-cell protein (SCP) with high lysine content as animal feed. In this work, *S*-(2-aminoethyl)-L-cysteine hydrochloride-resistant *Bacillus pumilus* E1 was used to produce SCP with high lysine content, whereas *Aspergillus niger* was used to degrade cellulose biomass and *Candida utilis* was used to improve the smell and palatability of the feed. An orthogonal design was used to optimize the process of fermentation for maximal lysine content. The optimum fermentation conditions were as follows: temperature of 40°C, substrate concentration of 3%, and natural pH of about 7.0. For unsterilized potato starch wastes, the microbial communities in the fermentation process were determined by terminal restriction fragment length polymorphism analysis of bacterial 16S rRNA genes. Results showed that the dominant population was *Bacillus* sp. The protein quality as well as the amino acid profile of the final product was found to be significantly higher compared with the untreated waste product at day 0. Additionally, acute toxicity test showed that the SCP product was non-toxic, indicating that it can be used for commercial processing.

Keywords: Lysine, optimization, microbial communities, single-cell protein

Introduction

Potato starch processing wastes of the potato starch industry, which include effluents and potato residues, cause serious environmental problems [7, 20]. A potato starch factory with annual starch production of 10 kt discharges 720 t of effluents and 192 t of potato residues per day for 100 days every year. Treatment of potato starch processing wastes has been described in previous works [15]. Effluents with a chemical oxygen demand of more than 30 g/l are rich with biodegradable components, such as starch, cellulose, and proteins, which can be used as nutrition for microorganisms. Potato residue refers to the thin solid by-product obtained after starch extraction, which contains 33 isolates (28 bacteria, 4 fungi, 1 yeast) [17] and is difficult to store because of its high water content of more than 80%. Effluent and potato residues from potato starch factories in China are discharged into rivers, causing serious pollution. Thus, the starch industry needs a more environment-friendly technique to meet emission standards.

Studies on potato processing wastes have mainly focused on wastewater treatment [3, 5, 14, 18] rather than waste utilization. Extraction of valuable products (*e.g.*, polygalacturonase, biogas) from wastes has been studied by several groups [7, 12, 19]. Among the various treatment methods for potato starch processing, conversion of the wastes into animal protein feed is an economical method that can be easily utilized in the potato starch industry. Co-fermentation of wastewater and potato residue to produce single-cell protein (SCP) can utilize all the waste from the potato starch industry without leaving effluents. However, in SCP production, digestible lysine is the first limiting amino acid that affects the quality of the animal feed [9]. The main nutrition parameters of animal feed are energy content, lysine content, and the ratio of other essential amino acids to lysine. Crude protein content, which is usually used as the standard for animal feed, cannot reflect the actual condition of these parameters. Alcaide *et al.* [1]

discussed the role of lysine in the final product.

The present work aims to improve the treatment method of wastes in the potato starch industry to produce SCP with high lysine content. *Bacillus pumilus* E1 was used, and an orthogonal design was adopted to optimize the process of fermentation to produce SCP with a high lysine content. The presence of microbial communities was analyzed using terminal restriction fragment length polymorphism (T-RFLP) for bacterial 16S rRNA genes.

Materials and Methods

Fermentation Process

A mutant strain of *B. pumilus* E1 that is resistant to 6.0 mg/ml of the lysine analog *S*-(2-aminoethyl)-L-cysteine hydrochloride was used in this study. The mutant strain was constructed by our laboratory and can produce 50.83 mg/ml lysine, which is 46.11% more than that produced by the wild strain (34.79 mg/ml).

A mixed culture of *B. pumilus* E1, *Candida utilis*, and *Aspergillus niger* at a ratio of 7:2:1 was used. *A. niger* was used to degrade cellulose biomass, and *C. utilis* was used to improve the smell and palatability of the feed. *B. pumilus* E1 was cultured in LB medium at 37°C for 12 h. *C. utilis* was cultured in potato dextrose medium at 28°C for 48 h. *A. niger* was cultured on a solid medium of wheat bran and bean pulp (wheat bran: bean pulp: water = 2:1:3) at 28°C for 3 days. For inoculum preparation, each microorganism was cultured until the concentration (counted using a hemocytometer) reached 10^8 cell/ml (*A. niger* at 10^9 spores per gram). About 1% mixed inoculum was then added to the mixture of unsterilized waste water and potato residue (waste water: potato residue = 15:4 w/w) from a potato processing factory (Lixue Starch, Ltd., Heilongjiang, China). The processes were performed in a 10 L working volume stirred-tank bioreactor in the laboratory (Bioengineering NLF-22) for 6 days. The temperature, stirrer speed, and air flow rate were measured using sensors in the bioreactor. Silicone oil was used to control foaming. The standard fermentation conditions throughout the production period (unless stated otherwise) before optimization were as follows: temperature, 30°C; agitation, 300 rpm; natural pH, 7.0; and air-flow, 0.1 $vv^{-1}m^{-1}$.

Optimum Conditions for Lysine Production

$L_{16}(4^3)$ orthogonal design was used to optimize the processing conditions (Table 1). The temperatures (25°C, 30°C, 35°C, and 40°C), pH (6.5, 7.0, 7.5, and 8.0), and substrate concentrations (3%, 5%, 7%, and 9%) were compared during the process to demonstrate the influence of these parameters on potato waste media. A portion of the dried potato residue was added to the waste water to determine the substrate concentration.

Analysis of Microbial Community in the Potato Processing Waste Treatment System

Samples were collected from the reactors every day for microbial community analysis. For T-RFLP analyses, 1 ml of the sample was washed twice with sterilized pure water by centrifugation (10,000 ×*g* for 10 min, 4°C). The DNA in the sample was extracted and purified using a soil total DNA extract kit (Sangon Biotech Co., Ltd., Shanghai, China). Using the 8f and 1492r bacterial primer pair, PCR targeting of the bacterial 16S rRNA genes was performed. The 5′ end of the forward primer was labeled with 6-carboxyfluorescein (FAM) for T-RFLP analysis. PCR was then performed using one denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 3 min, with a final extension at 72°C for 10 min. The PCR products were digested for 3 h with *Hha*I, *Hae*III, or *Msp*I at 37°C, respectively. The digested products were then sent to a sequencing company (Life Technologies Corporation) for relative terminal restriction fragments (T-RFs) analysis. The results were analyzed using the database of Microbial Community Analysis (MiCA; http://mica.ibest.uidaho.edu/trflp.php) and a phylogenetic assignment tool (https://secure.limnology.wisc.edu/trflp/index.jsp).

Table 1. $L_{16}(4^3)$ orthogonal design.

Tests	pH	Temperature (°C)	Substrate concentration (%)
Test 1	6.5	25	3
Test 2	6.5	30	5
Test 3	6.5	35	7
Test 4	6.5	40	9
Test 5	7	25	5
Test 6	7	30	3
Test 7	7	35	9
Test 8	7	40	7
Test 9	7.5	25	7
Test 10	7.5	30	9
Test 11	7.5	35	3
Test 12	7.5	40	5
Test 13	8	25	9
Test 14	8	30	7
Test 15	8	35	5
Test 16	8	40	3

Acute Toxicity Test of the SCP Product

The sample was sent to Harbin Medical University for acute toxicity testing and for feeding of the SCP product to rats for 30 days. The test method followed the National Standards of China GB15193-2003.

Acute toxicity test. 20 Wistar rats (*Rattus norvegicus*), half male and half female, were used in this test. The male and female rats were fed with carboxy methyl cellulose (CMC) at 10 g/kg body weight. Signs of toxicity were detected after 14 days of treatment.

SCP product feeding test for 30 days. 80 pups (both male and

Table 2. The active lysine content of products in the orthogonal test.

Time (day)	LT 1 (g/l)	LT 2 (g/l)	LT 3 (g/l)	LT 4 (g/l)	LT 5 (g/l)	LT 6 (g/l)	LT 7 (g/l)	LT 8 (g/l)	LT 9 (g/l)	LT 10 (g/l)	LT 11 (g/l)	LT 12 (g/l)	LT 13 (g/l)	LT 14 (g/l)	LT 15 (g/l)	LT 16 (g/l)
0	0.34 ± 0.09	0.31 ± 0.08	0.31 ± 0.01	0.37 ± 0.03	0.34 ± 0.09	0.31 ± 0.08	0.31 ± 0.01	0.37 ± 0.03	0.34 ± 0.10	0.31 ± 0.08	0.31 ± 0.01	0.37 ± 0.03	0.34 ± 0.09	0.31 ± 0.08	0.31 ± 0.01	0.37 ± 0.03
1	0.60 ± 0.04	0.59 ± 0.02	0.45 ± 0.04	0.37 ± 0.07	0.64 ± 0.02	0.73 ± 0.04	0.48 ± 0.09	0.57 ± 0.05	0.42 ± 0.06	0.38 ± 0.05	0.65 ± 0.02	0.60 ± 0.12	0.49 ± 0.07	0.55 ± 0.05	0.46 ± 0.04	0.48 ± 0.15
2	0.67 ± 0.04	0.67 ± 0.06	0.44 ± 0.01	0.41 ± 0.05	0.77 ± 0.06	0.80 ± 0.10	0.50 ± 0.04	0.65 ± 0.02	0.45 ± 0.05	0.50 ± 0.06	0.66 ± 0.07	0.60 ± 0.04	0.50 ± 0.04	0.63 ± 0.06	0.41 ± 0.05	0.60 ± 0.05
3	0.71 ± 0.03	0.68 ± 0.02	0.51 ± 0.02	0.51 ± 0.05	0.83 ± 0.05	0.83 ± 0.03	0.53 ± 0.05	0.69 ± 0.06	0.61 ± 0.07	0.63 ± 0.08	0.72 ± 0.10	0.67 ± 0.16	0.53 ± 0.10	0.75 ± 0.08	0.61 ± 0.04	0.68 ± 0.07
4	0.81 ± 0.08	0.72 ± 0.06	0.55 ± 0.02	0.66 ± 0.07	0.67 ± 0.03	0.92 ± 0.11	0.51 ± 0.07	0.57 ± 0.10	0.45 ± 0.04	0.54 ± 0.05	0.57 ± 0.03	0.96 ± 0.05	0.58 ± 0.15	0.55 ± 0.08	0.61 ± 0.06	0.75 ± 0.07
5	0.60 ± 0.02	0.67 ± 0.09	0.47 ± 0.03	0.61 ± 0.04	0.61 ± 0.02	0.75 ± 0.06	0.70 ± 0.11	0.50 ± 0.02	0.44 ± 0.06	0.50 ± 0.03	0.61 ± 0.05	0.53 ± 0.10	0.45 ± 0.01	0.51 ± 0.09	0.51 ± 0.08	0.75 ± 0.02
6	0.61 ± 0.04	0.53 ± 0.04	0.52 ± 0.01	0.67 ± 0.04	0.63 ± 0.04	0.62 ± 0.06	0.49 ± 0.04	0.56 ± 0.05	0.45 ± 0.05	0.44 ± 0.07	0.55 ± 0.02	0.55 ± 0.03	0.43 ± 0.03	0.58 ± 0.04	0.46 ± 0.08	0.79 ± 0.04

LT means Lysine content of products in the Test.

female) of Wistar rats were divided into four groups. In the negative control group, the rats were fed with CMC at 10 g/kg body weight. In the high, medium, and low dose groups, rats were given SCP at 3.3 g/kg, 1.65 g/kg, and 0.83 g/kg body weight, respectively, mixed in 10 g/kg of CMC for 30 days. All rats were fed a normal diet and water *ad libitum*.

Analytical Methods

Approximately 200 ml of samples was obtained at different time intervals to determine the crude protein, true protein, total sugar, reducing sugar, and active lysine contents. Culture samples (100 ml) were centrifuged (8,000 ×*g* for 10 min) to remove the substrate, and the supernatant was used to test total and reducing sugars. The remaining 100 ml sample was then centrifuged (10,000 ×*g*, 10 min). The pellet containing cell mass and unutilized substrate was dried, and then routinely analyzed for crude protein, true protein, and active lysine contents. The active lysine content was tested using the dye-binding lysine method [10]. Crude protein and true protein contents were determined by the Kjeldahl method [8] and the Lowry method [13], respectively. The 3,5-dinitrosalicylic acid method was used for reducing and total sugar testing [16]. Cellulose and hemicellulose contents were determined by the van Soest method [2].

Results and Discussion

Optimum Fermentation Conditions for Lysine Production

As the first limiting amino acid in SCP production, digestible lysine is the main parameter affecting the quality of animal feed. Previous studies have shown that the limiting amino acid in the diet should be considered to meet animal protein requirements and to increase the average daily gain of weight [23].

An $L_{16}(4^3)$ orthogonal design was used to optimize the conditions of the treatment process. The active lysine content was tested every day and the results are shown in Table 2. The active lysine content increased by 159.46% on day 0 and reached a maximum of 0.96% on days 3 to 4. Results of range analysis and variance analysis of the orthogonal test are shown in Table 3. The range of pH, temperature, and substrate concentration was 0.0079, 0.0211, and 0.0276, respectively, indicating that the effect of the parameters on active lysine content followed the order substrate concentration > temperature > pH. The optimal level of the three parameters was pH 6.5, 40°C, and substrate concentration of 3%. In the variance analysis of the repeat tests, substrate concentration and temperature had significant effects on active lysine content, whereas the influence of pH was not significant. Thus, considering economic factors, the optimum fermentation conditions can be considered to be 40°C, substrate concentration of 3%, and natural pH (about 7.0). In most fermentation processes, pH is a very important parameter that affects

Table 3. The range analysis and variance analysis of the orthogonal test.

Parameters	Range	F	Significance
pH	0.0079	0.6637	Not significant
Temperature	0.0211	4.0199	Significant
Substrate concentration	0.0276	9.5490	Significant

Table 4. Nutrients in potato residue and fermentation products.

Nutrients	After optimization (g/100 g)	Before optimization (g/100 g)	Potato residue (g/100 g)
Lysine	1.15 ± 0.09[a]	0.88 ± 0.09[b]	0.37 ± 0.12[c]
Crude protein	30.29 ± 0.71[a]	27.62 ± 0.47[b]	20.69 ± 0.58[c]
True protein	4.32 ± 0.12[a]	4.59 ± 0.02[a]	2.83 ± 0.28[b]
Crude fat	2.71 ± 0.21[a]	2.52 ± 0.43[a]	2.43 ± 0.56[a]
ADF	14.35 ± 2.32[a]	10.98 ± 3.11[a]	14.95 ± 3.27[a]
NDF	20.12 ± 3.67[a]	17.34 ± 2.98[a]	19.23 ± 3.24[a]
Moisture content	9.88 ± 0.63[a]	10.07 ± 2.01[a]	10.35 ± 0.67[a]
Ash	11.38 ± 2.73[a]	12.42 ± 2.11[a]	11.45 ± 1.96[a]

Values followed by different letters in each row differ significantly at $p \leq 0.05$.
ADF, acid detergent fiber.
NDF, neutral detergent fiber.

biomass synthesis and product yields. However, our results show that waste water is a buffered system that cannot be regulated easily, which may be why pH had no significant effect on the active lysine content.

Kinetics of the Fermentation Process

A batch fermentation process was performed to treat the wastes of potato processing plants. Nutrients in the potato residue and fermentation products (before and after optimizing) were analyzed (Table 4). The active lysine content after optimization was 1.15%, which increased to 30.41% for the control (not optimized) and 223.59% for the potato residues. The crude protein content was 30.29% after optimization, which increased to 46.40% for the potato residues, but exhibited almost no change in the control. Neutral detergent fiber and acid detergent fiber were also tested. Changes in the fibers were not noticeably different between the potato residues and the SCP products, both before and after optimization. The effective treatment of potato processing wastes by mixed microorganisms suggests that the obtained microbial consortium was suitable for biomass degradation and nutrient conversion. In addition, since no chemicals were supplemented during the treatment process, the costs were kept at a low level.

The kinetics of active lysine content, crude protein, true protein, and reducing sugar during the process are shown in Fig. 1. The results showed that the active lysine content increased during the first 4 days after optimization, and then decreased to 0.74% on the fifth day, indicating that bioconversion may have been limited by the growth of *B. pumilus*, as suggested by results of our microbial community analysis. Changes in the active lysine content can reveal key growth events during fermentation [6]. The tests of total/reducing sugar and crude/true protein can show consumption of the substrate and product yields. Reducing sugars were consumed in 1 day, but the total sugar changed only minimally, except on the last day. This finding suggests that reducing sugars were consumed quickly at the start of the fermentation process, possibly due to the relatively low levels of reducing sugars in potato processing wastes and the rapid growth rate of microorganisms. After the reducing sugars were consumed, the microorganisms then utilized the starch and cellulose in the waste. Crude protein and true protein increased constantly, with a rapid increase on the fourth day.

The active lysine content peaked on the fourth day. Crude and true protein levels also exhibited a rapid increase near

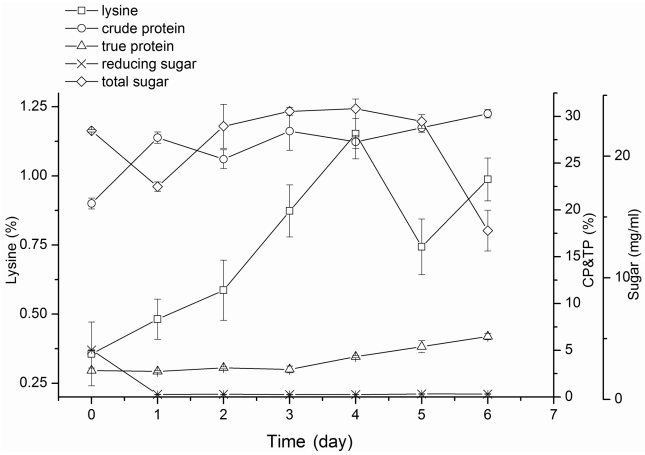

Fig. 1. Kinetics of the fermentation process.
The changes of lysine, crude protein (CP), true protein (TP), reducing sugar, and total sugar during the fermentation under the optimized condition. The symbols are shown in the figure.

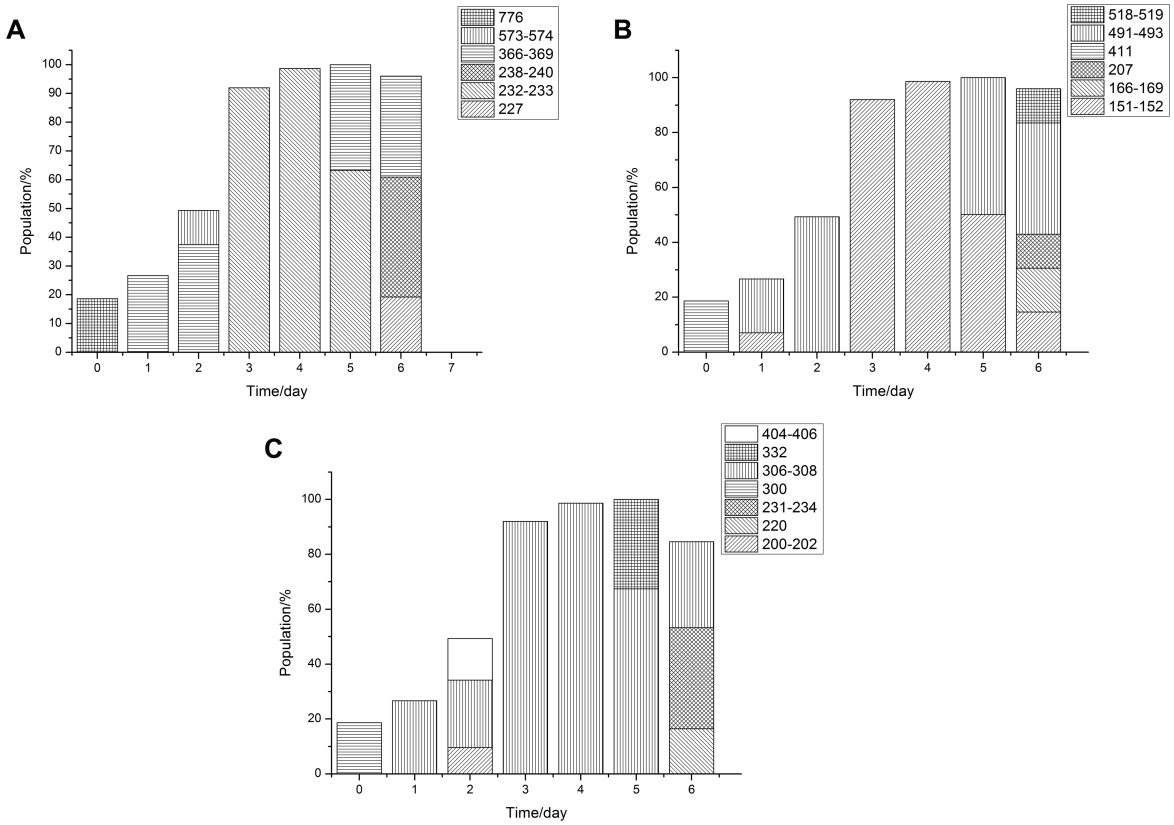

Fig. 2. Relative abundance of T-RFs of bacterial 16S rRNA gene in the treatment of potato processing wastes. Digested by *Hha*I (**A**), *Msp*I (**B**), and *Hae*III (**C**).

this peak. Therefore, 4 days of fermentation is suitable for achieving a high yield and quality of SCP products with low processing costs.

A large batch fermentation (165 m^3) was performed to obtain enough SCP products for the animal feeding experiments. The sample was sent to Harbin Medical University for acute toxicity testing and for 30 days of SCP product feeding to rats. All test groups across various acute toxicity criteria (Tables S1 and S2, and Fig. S1) showed no significant difference ($p > 0.05$) to the control group. These results indicated that the SCP product was non-toxic and can thus be used for commercial processing.

Microbial Community Analysis

In an unsterilized fermentation process, microbial community analysis was conducted to determine the community composition and the role of the domain and other species. Bacterial 16S rRNA genes were extracted from treatment samples and digested with *Hha*I, *Hae*III, and *Msp*I every day. The relative T-RFs are shown in Fig. 2. The feature of the *Hae*III-digested T-RF of 306–308 bp and the *Msp*I-digested T-RF of 151–152 bp were similar to those of the *Hha*I-digested T-RF of 232–233 bp. The populations of *Hha*I-digested T-RF of 366–369 bp were predominant in the first 2 days, but became undetectable after the third day, and were again detected in the last 2 days. The *Msp*I-digested T-RF of 491–493 bp showed a similar trend. The bacterial community formed during the fermentation process differed significantly from the community found in the original waste (day 0). Our results suggest that the bacterial community quickly changed during the start of the fermentation process (day 1) because of their high growth rates. Some bacterial communities stayed predominant throughout the fermentation process, whereas others became detectable only after the fourth day. This suggests that the predominant bacteria species grew rapidly until the fourth day, repressing the growth of other bacteria. After the fourth day, the predominant bacterial species entered a declining phase, allowing other bacteria to grow, albeit under a limited nutrition condition.

The genera of the bacteria in the samples could be identified based on T-RF sizes, although some T-RFs were

Table 5. Presumptive identification of bacteria during the fermentation according to T-RFLP patterns to sequences retrieved from databases.

T-RF size (bp)			Phylogenic group
*Hha*I	*Hae*III	*Msp*I	
232–233	306–308	151–152	*Bacillus* sp.
366–369	-	491–493	*Paenibacillus*/ *Bacillus*
238–240	231–234	207	-
238–240	231–234	166–169	-
238–240	231–234	518–519	-
227	220	166–169	-
227	220	207	-
227	220	518–519	-

not found in the database (Table 5). The population of *Hha*I-digested T-RF of 232–233 bp (*Hae*III-digested T-RF of 306–308 bp, *Msp*I-digested T-RF of 151–152 bp) was inferred to be *Bacillus* sp., which was the dominant species in the first 4 days. *Bacillus* can hydrolyze and degrade potato processing wastes into single-cell detritus. *Bacillus* is reportedly useful for the conversion and degradation of chitin wastes as well [11, 22]. They produce SCP and various extracellular enzymes that can catalyze reactions ranging from degradation of cellulose or starch to biofuel production. The population with *Hha*I-digested T-RF of 366–369 bp (*Msp*I-digested T-RF of 491–493 bp) was inferred to be *Paenibacillus*/*Bacillus*. The *Paenibacillus* spp. has gained increasing attention because of their importance [4] in agriculture and horticulture, as well as industrial and medical applications. Various *Paenibacillus* spp. also produce antibiotics that affect a wide spectrum of microorganisms [21] such as anaerobic pathogens and plant pathogenic bacteria. We speculate that *Paenibacillus* spp. in the treatment system may help in suppressing the growth of harmful bacteria.

Acknowledgments

The authors wish to thank the Chinese government for the financial support of this study under the National High Technology Research and Development Program 2012AA021404 and Heilongjiang Province Technological Project GA08C201.

References

1. Alcaide EM, Ruiz DRY, Moumen A, Garcia AIM. 2003. Ruminal degradability and *in vitro* intestinal digestibility of sunflower meal and *in vitro* digestibility of olive by-products supplemented with urea or sunflower meal: comparison between goats and sheep. *Anim. Feed Sci. Technol.* **110:** 3-15.
2. Aharoni Y, Brosh A, Orlov A, Shargal E, Gutman A. 2004. Measurements of energy balance of grazing beef cows on Mediterranean pasture, the effects of stocking rate and season - 1. Digesta kinetics, faecal output and digestible dry matter intake. *Livest. Prod. Sci.* **90:** 89-100.
3. Cibis E, Kent CA, Krzywonos M, Garncarek Z, Garncarek B, Miskiewic T. 2002. Biodegradation of potato slops from a rural distillery by thermophilic aerobic bacteria. *Bioresour. Technol.* **85:** 57-61.
4. Choi KK, Park CW, Kim SY, Lyoo WS, Lee SH, Lee JW. 2004. Polyvinyl alcohol degradation by *Microbacterium barkeri* KCCM 10507 and *Paenibacillus amylolyticus* KCCM 10508 in dyeing wastewater. *J. Microbiol. Biotechnol.* **14:** 1009-1013.
5. Cibis E, Krzywonos M, Miskiewicz T. 2006. Aerobic biodegradation of potato slops under moderate thermophilic conditions: effect of pollution load. *Bioresour. Technol.* **97:** 679-685.
6. Dalluge JJ, Smith S, Sanchez-Riera F, McGuire C, Hobson R. 2004. Potential of fermentation profiling *via* rapid measurement of amino acid metabolism by liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* **1043:** 3-7.
7. Fang C, Boe K, Angelidaki I. 2011. Biogas production from potato-juice, a by-product from potato-starch processing, in upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactors. *Bioresour. Technol.* **102:** 5734-5741.
8. Giannoutsou EP, Meintanis C, Karagouni AD. 2004. Identification of yeast strains isolated from a two-phase decanter system olive oil waste and investigation of their ability for its fermentation. *Bioresour. Technol.* **93:** 301-306.
9. Giannoutsou EP, Katsifas EA, Geli A, Karagouni AD. 2012. Protein increase and lysine production by a *Paecilomyces variotii* strain grown on two-phase olive mill waste. *World J. Microbiol. Biotechnol.* **28:** 849-856.
10. Hewitt D, Ford JE. 1985. Nutritional availability of methionine, lysine and tryptophan in fish meals, as assessed with biological, microbiological and dye-binding assay procedures. *Br. J. Nutr.* **53:** 575-586.
11. Haddar A, Hmidet N, Ghorbel-Bellaaj O, Fakhfakh-Zouari N, Sellami-Kamoun A, Nasri M. 2011. Alkaline proteases produced by *Bacillus licheniformis* RP1 grown on shrimp wastes: application in chitin extraction, chicken feather-degradation and as a dehairing agent. *Biotechnol. Bioprocess Eng.* **16:** 669-678.
12. Klingspohn U, Bader J, Kruse B, Kishore PV, Schugerl K, Krackehelm HA, *et al*. 1993. Utilization of potato pulp from potato starch processing. *Process Biochem*. **28:** 91-98.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265-75.

14. Lasik M, Nowak J, Krzywonos M, Cibis E. 2010. Impact of batch, repeated-batch (with cell recycle and medium replacement) and continuous processes on the course and efficiency of aerobic thermophilic biodegradation of potato processing wastewater. *Bioresour. Technol.* **101:** 3444-3451.
15. Liu BN, Song JZ, Li Y, Niu J, Wang ZY, Yang Q. 2013. Towards industrially feasible treatment of potato starch processing waste by mixed cultures. *Appl. Biochem. Biotechnol.* **171:** 1001-1010.
16. Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31:** 426.
17. Mayer F, Hillebrandt JO. 1997. Potato pulp: microbiological characterization, physical modification, and application of this agricultural waste product. *Appl. Microbiol. Biotechnol.* **48:** 435-440.
18. Nowak J, Lasik M, Czarnecki Z. 2005. Prediction of the metabolic activity of a mixed *Bacillus* culture during the biodegradation of wastewater from the potato industry. *Eng. Life Sci.* **5:** 458-465.
19. Parawira W, Murto M, Read JS, Mattiasson B. 2005. Profile of hydrolases and biogas production during two-stage mesophilic anaerobic digestion of solid potato waste. *Process Biochem.* **40:** 2945-2952.
20. Suzuki S, Fukuoka M, Tada S, Matsushita-Morita M, Hattori R, Kitamoto N, *et al*. 2010. Production of polygalacturonase by recombinant *Aspergillus oryzae* in solid-state fermentation using potato pulp. *Food Sci. Technol. Res.* **16:** 517-521.
21. von der Weid I, Alviano DS, Santos AL, Soares RM, Alviano CS, Seldin L. 2003. Antimicrobial activity of *Paenibacillus peoriae* strain NRRL BD-62 against a broad spectrum of phytopathogenic bacteria and fungi. *J. Appl. Microbiol.* **95:** 1143-1151.
22. Wang SL, Chen TR, Liang TW, Wu PC. 2009. Conversion and degradation of shellfish wastes by *Bacillus cereus* TKU018 fermentation for the production of chitosanases and bioactive materials. *Biochem. Eng. J.* **48:** 111-117.
23. Xue F, Zhou ZM, Ren LP, Meng QX. 2011. Influence of rumen-protected lysine supplementation on growth performance and plasma amino acid concentrations in growing cattle offered the maize stalk silage/maize grain-based diet. *Anim. Feed Sci. Technol.* **169:** 61-67.