KKU Res. J. 2012; 17(4):580-592
http : //resjournal.kku.ac.th

Potential use of *shochu kasu* to produce exopolysaccharides from *Lactobacillus sakei* CY1 and their effect on soil aggregation

Emma Yuliani, Tsuyoshi Imai and Shintaro Tomita

Divison of Environmental Science and Engineering, Graduate School of Science and Engineering, Yamaguchi University, Yamaguchi 755-8611 Japan
** Correspondent authors: imai@yamaguchi-u.ac.jp*

Abstract

Shochu, a distilled beverage from Japan, generate in distillery waste, which contain high concentration of organic matter that can cause environmental problem. In this study, we determine the utilization of *shochu kasu* (distillery waste) as nutrient source for *Lactobacillus sakei* CY1 to produce extracellular polysaccharides in order to make this wastewater be a valuable material. The highest yield of *Lactobacillus sakei* CY1 exopolysaccharides (4.6 g/L) was obtained from the basal medium supplemented with 75% (v/v) of *shochu kasu* at 30°C and pH of 6.2. The polymer produced consisted of 25% of glucose and 13% of galactose and 62% of other sugar. To determine the effectiveness of *L. sakei* CY1 as a biological agent for the improvement of soil quality with *shochu kasu* as culture media, we examined the effect of *L. sakei* CY1 on soil aggregation in indoor experiment. The extent of aggregation was determined after 28 days of culture inoculation on sandy soil by varying concentration of *shochu kasu* on culture media. We compared these results with those obtained using chemical defined media. The application of *shochu kasu* as nutrient source obviously improved the level of aggregation, 70-80% of soil particles adhere to each other and build up macro-aggregates, which have an important role in the soil stabilization. These results indicate than *L. sakei* CY1 with *shochu kasu* as culture media can be potentially be used to improve soil structure.

Keywords: *shochu kasu*, exopolysaccharides, *Lactobacillus sakei* CY1

1. INTRODUCTION

Lactobacillus sakei is a species of *Lactobacillus*. It is a gram-positive bacillus which groups by pair or short chain commonly used as starter culture on fermentation foods. Hammes and Bantleon reported on their study that *L. sakei* has been isolated from several raw fermented food products of plant and animal origin [1]. Other study reported that among mesophilic LAB, *L. sakei* is frequently isolated from food products and widely used in industry for fermented food [2]. It was found in silage, sauerkraut, sourdough, and smoked fish, but is mainly found in meat products. This bacterium is also exceptionally adaptable to changes in environmental

redox states, and proliferates both at refrigeration temperatures and at high salt concentrations [3]. Like another *Lactobacillus*, *L. sakei* can produce bacteriocin that can inhibit another bacteria growth and secrete polysaccharides. These microbial polysaccharides can be produced in either one of two forms; capsular polysaccharides and exopolysaccharides. In this study the main interest is in that of exopolysaccharides (EPS). EPS of microbial origin have unique properties in their capability of forming very viscous solutions at low concentration and their pseudoplastic nature [4]. EPSs are comprised of various monosaccharides, mostly glucose and galactose, but also including rhamnose, fructose, mannose, galactosamine, and other sugars [5]. The viscosity of EPSs depends on the bacterial culture medium. In a liquid medium, the sugars may be highly viscous or gel-like, but on a solid surface, EPSs take on the form of a biofilm. Growth conditions are, therefore, very important to the composition, physical properties, and organisation of the polysaccharides.

A key problem in the industrial production of EPSs by lactobacilli is the high cost of culture medium. For industrial production, the culture medium must contain all the *Lactobacillus*-specific nutrients [6]. Some such media are commercially available but are quite expensive. Therefore, it appears that substitution of chemical media with a less-expensive alternative, such as organic wastes, will allow the development of a cheaper method of EPS production. In this study, we investigate the potential use of sweet potato-*shochu* distillery wastewater as a supplement for *Lactobacillus sakei* growth and EPS production.

Shochu is a popular traditional beverage in Japan, prepared mainly in Kyushu, Okinawa, and the southern islands of Japan from a variety of ingredients such as rice, sweet potatoes, barley, buckwheat, or sugar beets. During *shochu* production, *shochu* kasu (distillery waste), which contains a high concentration of organic matter, is produced. Currently, the disposal of *shochu* distillery wastewater into rivers or fields is prohibited in Japan; therefore, it is mainly dumped into the ocean. However, ocean dumping is not only expensive but also causes marine pollution [7]. Therefore, an effective method of treating, recycling, or otherwise upgrading the disposal of *shochu* distillery waste is highly desirable [8]. Since distilled *shochu* wastewater contains no known harmful substances, its use in cell culture media or its bioconversion to more valuable materials are attractive alternatives for the disposal of this wastewater [9].

EPS were defined [10] as organic polymers of microbial origin which in biofilm systems are frequently responsible for binding cells and other particulate materials together (cohesion) and to the substratum (adhesion). In soil, the production of polysaccharide-binding materials by bacteria can cause sand particles to adhere to one another and build aggregates [11]. These aggregates determine the mechanical and physical properties of soil, such as water retention, movement of water, aeration, and temperature [12]. Aggregate formation facilitates cultivation, drainage, and aeration; increases the moisture-holding capacity of the soil; and reduces erosion [13]. Some industries have already produced polysaccharides, such as xanthan and chitosan, from microbes but as reported by Sutherland that although many gel-forming water-insoluble microbial polysaccharides are produced by industry, these materials are impractical for use in soil because of their high cost [14]. The use of these polysaccharides for soil aggregation will only become economically reasonable if microorganisms can be grown in situ to directly produce EPS. The production of bacterial exopolymers in situ can be used to modify soil properties [10]. Many studies have reported on the suitability of EPS-producing bacteria for forming aggregate, but few studies have examined the effects of a mass inoculation of *Lactobacillus* on soil

aggregation. In addition, *Lactobacillus* is fast growing, easily available, and safe for the environment. Here, we report our findings on the effectiveness of EPS produced by the facultative anaerobic bacteria, *L. sakei* CY1, in the aggregation of sandy soils with *shochu* distillery wastewater as nutrient source.

2. MATERIAL AND METHOD

2.1 Bacterial isolation and identification

Four different kinds of fermented foods, i.e., fermented cucumber, belacan (Indonesian food), natto, and mixed vegetables, were used as sources of microbes. Samples of about 5.0 g were inoculated into de Man-Rogosa-Sharpe (MRS) medium. This media is specifically designed to the nutrient requirements of *Lactobacillus* spp. Aerobic condition was maintained by shaking at 15 rpm at 30°C for 24 h. Bacterial suspension were then spread on MRS agar and incubated at 30°C for 24 h. Bacterial identification was performed by 16s rRNA gene amplification. After harvesting, the bacterial cells were extracted with QIAamp® DNA Stool kit. The 16S rRNA cistron was amplified using the universal bacterial primers pA (5′-AGA GTT TGA TCC TGG CTC AG-3′) and pH (5′-AAG GAG GTG ATC CAG CCG CA-3′), designed by Edwards et al. [15] with Illustra puReTaq Ready-To-Go™ PCR Beads (GE Healthcare, United Kingdom). PCR conditions were as follows: 94°C for 3 min, followed by 30 cycles of (94°C for 30 s, 57°C for 1 min, and 72°C for 1 min), and then, a final extension at 72°C for 10 min. The results were visualized by gel electrophoresis. Gel bands of 16S amplicons (1,500 bp) were extracted using sterilized blades, and DNA was purified from the gel slices by MagExtractor NPK-601 (Toyobo, Japan). Purified 16S amplicons were subsequently cloned for sequencing by using a pGEM-T Easy kit (Promega, USA) and transformed into competent *E. coli* JM109. Recombinant plasmid clones were selected on Luria Bertani (BR) agar supplemented with ampicillin/isopropyl-β-D-thiogalactopyranoside (IPTG)/X-gal. Plasmid DNA was isolated from host cells by alkaline lysis method. The cloned inserts were sequenced, according to manufacturer's instructions by using an ABI 310 DNA sequencer (Applied Biosystems, USA). The 16S rRNA gene sequence was compared with those available in the GenBank public databases (http://www.ncbi.nlm.nih.gov/GenBank/).

2.2 Determination of optimum growth condition

To monitor growth rate characteristics of *L. sakei* CY1, we used MRS broth (Difco; Becton Dickinson and Company, USA) at pH 6.2 as the culture medium. The growth kinetics of *L. sakei* incubated under shaker conditions at 30°C and 15 rpm were analyzed during the period from 4–56 h of incubation. MRS is a chemically defined medium suitable for *Lactobacillus* spp. that contains proteose peptone (10 g), beef extract (10 g), yeast extract (5 g), dextrose (20 g), polysorbate (1 g), ammonium citrate (2 g), sodium acetate (5 g), magnesium sulfate (0.1 g), manganese sulfate (0.05 g), and dipotassium phosphate (2 g). The density of bacterial cells was determined by streak plate method on MRS agar.

2.3 Isolation, purification, and quantification of EPS

We used an isolation method that was slightly modified from previous descriptions [16]. Selected isolates were grown on basal media with 0%, 25%, 50%, 75%, or 100% *shochu* distillery wastewater as an addition. Cells were precipitated by centrifugation for 10 min at 12000 × g. Cell dry weights were determined by drying the cells at 105°C. After washing with distilled water, 2 volumes of cold ethanol were added

to the supernatant, and it was stored overnight at 4°C. The precipitate was collected by centrifugation (20 min at 1500 × g), re-suspended in distilled water, and mixed with 2 volumes of cold ethanol. Samples were centrifuged at 1500 × g, and the pellets were dried at 105°C. The total carbohydrate content of the EPS was colorimetrically determined by adding 1 mL anthrone reagent (50 mL H_2SO_4, 5 mL H_2O, and 0.1 g anthrone) to 0.25 mL of dialyzed supernatant. The samples were boiled until the reaction was complete, allowed to cool, and then, the absorbance was measured at OD_{620nm}.

2.4 Monosaccharide analysis

Glucose, galactose, mannose, rhamnose, xylose and arabinose concentrations in the dialyzed supernatant (described above) were determined by HPLC (Hitachi Elite LaChrom, Japan), in conjunction with the analytical software; detector (model L-2490) with temperature 35°C; an auto sampler (L-2200) with 5mL volume sample after diluted 10-fold in H_2O. and a pump (model L-2100) with 0.3 mL/min flow rate

2.5 Culture conditions

In all EPS production experiments and dry cell weight measurements, the flasks were incubated for 24 h at 30°C. EPS was obtained during the stationary phase of growth. 150 mL flasks containing 50 mL of the medium were inoculated with 50 mL of primary bacteria culture and incubated on a shaker at 20 rpm (EYELA, Japan).

2.6 Bacterial Growth Culture Media

2.6.1 De Man, Rogosa, Sharpe (MRS) broth (Difco, Becton, Dickinson and Company, USA), pH 6.2 was used for isolating Lactobacillus. The composition of MRS agar and MRS broth has shown on table 3.1. MRS media, a chemical defined media, suitable for *Lactobacillus.*

2.6.2 The basal culture medium for EPS production contained peptone 5 $g \cdot L^{-1}$, glucose 10–50 $g \cdot L^{-1}$ (to determine the effect of glucose concentration), yeast extract 5 $g \cdot L^{-1}$, and sodium chloride 5 $g \cdot L^{-1}$ at pH 6.2.

2.7 *Shochu* wastewater preparation

Sweet potato-*shochu* distillery wastewater was obtained from a *shochu*-making facility in Yamaguchi prefecture, Japan. The wastewater was centrifuged at 15,000 rpm for 10 min; the pH of the supernatant was adjusted by adding NaOH. Chemical oxygen demand (COD) values and nitrogen and phosphorus levels in the wastewater were measured, as described previously [17]. Protein concentrations were determined by Lowry-Folin method [18].

Table 1. Soil sample characteristic and nutrients composition

	Value
Soil characteristic:	
Type	sandy soil
pH	6.9
Water content (%)	14
Porosity	0.54
Void ratio	1.17
Bulk density (g/cm^3)	1.21
Particle density (g/cm^3)	2.65
Nutrient composition:	
T-N ($g \cdot Kg^{-1}$)	87.8
T-P (g·Kg-1)	5.4
NO_3 ($g \cdot Kg^{-1}$)	15.09
NH_4 ($g \cdot Kg^{-1}$)	6.37

2.8 Aggregation of soil media

After the EPS-producing *L. sakei* CY1 (LSCY1) culture was grown in *shochu* distillery wastewater at pH 6.2 with varying concentration from 0 – 100%, there were inoculated in 100 g of sandy soil and left undisturbed for 28 days. Cultures made with broth were taken during the stationary phase of growth. Decho in theirs studies reported that slime EPS are produced mainly during stationary phase of growth and were harvested at that time [19]. Sandy soil, collected from a field around Ube city, Yamaguchi, Japan, was passed through a 2-mm mesh sieve. This soil was sterilized using an autoclave (SV-302 II; Advantec Toyo, Ltd, Japan) at 138 kPa for 30 min. Sterilized soil was allowed to cool to room temperature before use. Soil without nutrient sources or organic content was maintained as control. After incubation, the soils were sieved under water [20] using different mesh sizes. Wet samples were placed at the top of the sieve set, the uppermost sieve having a 2000-μm mesh, following with 1000-and 500-μm mesh sieves were placed, respectively. The soil samples were dried at 105°C for 24 h before they were weighed. As a comparison to the LSCY1 cultures, we also tested De Man, Rogosa, Sharpe (MRS) broth (Difco, Becton, Dickinson and Company, USA) as chemical defined media and soil without inoculation as control.

3. RESULT AND DISCUSSION

3.1 Bacterial screening

Isolates of *Lactobacillus* named CY1, were obtained from a cucumber fermentation since no good *Lactobacillus* isolate can be taken from other microbial sources after cultivation. Actually, this isolate also can found on natto but the concentration was lower than isolate from cucumber fermentation. The partial sequence 658 bp of CY1 isolate 16s rRNA amplicon was aligned and compared with GenBank database. The blast result showed that CY1 is identical to *Lactobacillus sakei* strain PSH-313 (100%), *Lactobacillus sakei* strain 23K (99%) and *Lactobacillus sakei* strain LSJ618 (99%). The phylogenetic tree was constructed using the consensus sequence of CY1 and other member belongings to *Lactobacillus* genus in which information were retrieved from Genbank and Ribosomal database project. A neighbour-joining tree (Figure 1) was indicated that bacterial isolate CY1 clustered closely with *Lactobacillus sakei* strain PSH-313 (DQ989236) with a bootstrap percentage 23.4%.

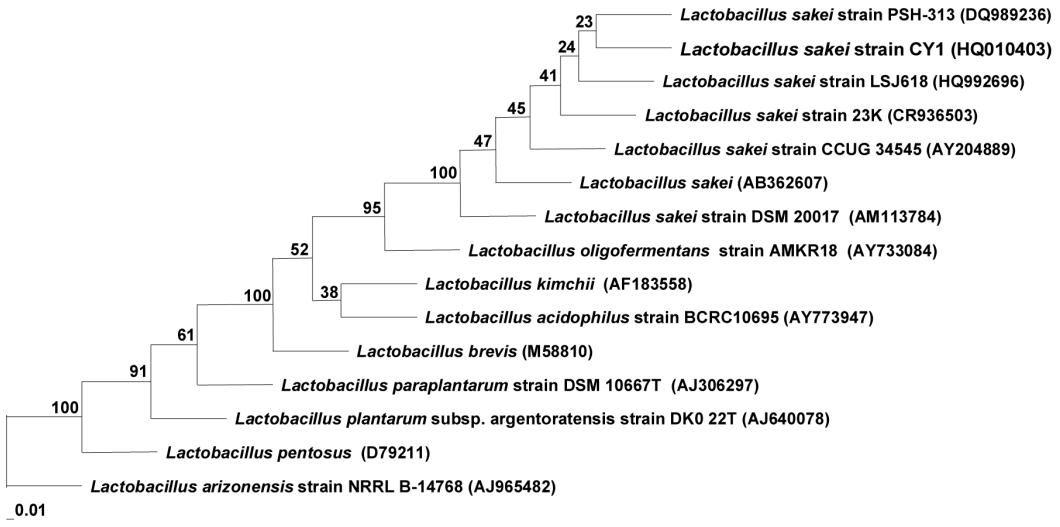

Figure 1. Phylogenetic tree indicating estimated relationship between strain *Lactobacillus sakei* CY1 and other members of the genus *Lactobacillus* based on 16s rRNA sequence. Numbers at nodes represent the bootstrap percentages from 1000 replicates. The scale bar indicates substitutions per nucleotide position. Nucleotide accession numbers are given in parentheses.

The 16S rRNA gene sequence of CY1 isolate using in this study has been deposited in the GenBank database under the accession number HQ010403 as the name *L. sakei* CY1. This strain is a class of gram-positive bacteria that are rod-shaped, facultatively anaerobic, non-spore-forming, and facultatively heterofermentative. The biochemical characteristics of the isolate included nitrate reduction, gelatin liquefaction, and fermentation of a variety of carbohydrates

3.2 Effect of wastewater concentration

Cromwell et al. reported that *shochu* distillery wastewater is a valuable source of protein, water-soluble vitamins, and minerals [21]. In our study, nitrogen and phosphorus contents were shown to be high, but vitamin content was not measured. Based on this reason we conduct *shochu* distillery wastewater as alternative media for *L. sakei* CY1. The composition of sweet potato-*shochu* distillery wastewater is shown in Table 2. To know whether the wastewater has potential to serve as a medium for EPS production, EPS yield in the presence of wastewater was compared with the yield in the absence of wastewater (as control), and with those from cultures grown in MRS medium. The basal medium (pH 6.2) supplemented with sweet potato-*shochu* distillery wastewater at concentrations from 0% to 100% were evaluated.

Table 2. Composition of sweet-potato *shochu* distillery wastewater

Total nitrogen ($g \cdot L^{-1}$)	3.47
NO_3^- ($g \cdot L^{-1}$)	0.63
$NH4^+$ ($g \cdot L^{-1}$)	0.02
Total phosphorus ($g \cdot L^{-1}$)	0.52
Suspended solids ($g \cdot L^{-1}$)	28
Volatile suspended solids($g \cdot L^{-1}$)	25
Total organic carbon ($g \cdot L^{-1}$)	32
Total sugar ($g \cdot L^{-1}$)	2.7
CODcr ($g \cdot L^{-1}$)	80
Protein ($g \cdot L^{-1}$)	40.5
pH	3.8

Figure 2 shows that until 75% wastewater was added, both dry cell weight and EPS production by *L. sakei* CY1 increased with increase in wastewater concentration. However, supplementing with 100% wastewater led to decreased EPS production. *Shochu* wastewater has high protein content, and the addition of a carbon source such as glucose present in basal media, has the effect of increasing the growth rates of *L. sakei* CY1. The highest yield of EPSs (about 4.6 $g \cdot L^{-1}$) was attained by using a medium containing 75% wastewater; this yield was higher than those reported in other published report.

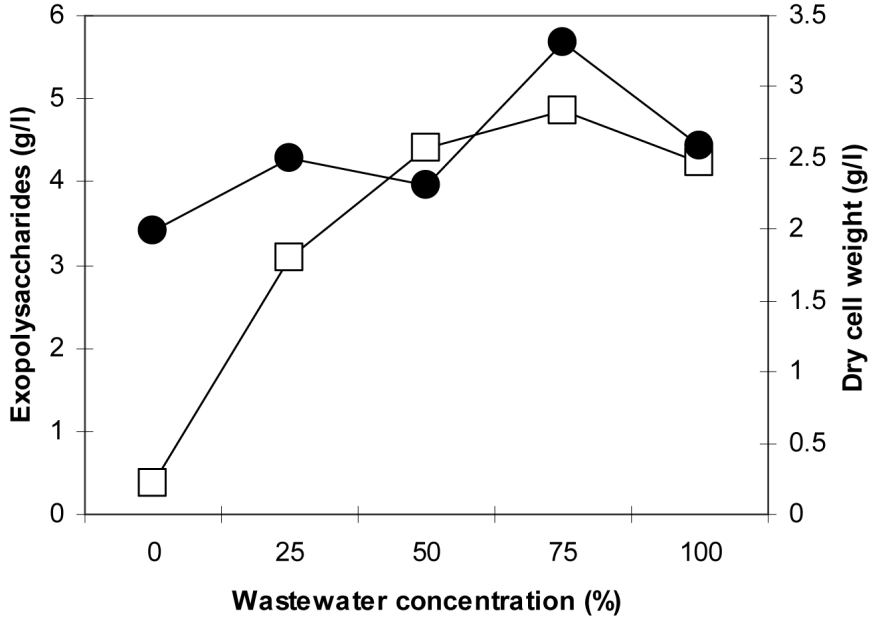

Figure 2. Effect of *shochu* distillery wastewater concentration on exopolysaccharides production using *Lactobacillus sakei* sp. CY1 on 24 hour. The basal medium (pH 6.2) contained 30g·L glucose. (●) dry cell weight, (□) exopolysaccharides ($mg \cdot L^{-1}$)

The highest EPS yield previously reported was 2.77 $g \cdot L^{-1}$ by *Lactobacillus rhamnosus* RW-9595 [22]. It had been indicated that the organic compounds contained in wastewater may act as a sufficient carbon source for this strain. The only medium component necessary for growth of the strain was glucose in addition to the distillery wastewater. This means, that the conventionally used and expensive nitrogen and vitamin sources which is available almost free of charge. To examine this, the dry cell weight of and EPS production

by the strain grown in pure basal medium (as control) and MRS medium were determined and compared (Table 3).

Table 3. Biomass and exopolymer production of *L. sakei* CY1 on different media at optimum condition

	BM	SDWW	MRS
Dry cell weight (gL^{-1})	1.99	3.31	2.98
Exopolysaccharides (gL^{-1})	0.38	4.6	2.51

BM = basal medium contain peptone, glucose, salt, and yeast; SDWW = 75% *shochu* distillery wastewater presence on basal medium; MRS = de Man, Rogosa, Sharpe medium

The results show that when compared to the chemically defined media designed to supply all the required nutrients, the medium supplemented with *shochu* distillery wastewater still gave the highest yield, in terms of both biomass and EPS production.

3.3 Effect of glucose concentration

Even sucrose given the best result of exopolysaccharide production but glucose were used as carbon source, because glucose has a lower tendency, relative to other hexose sugars, to react non-specifically with the amino groups of proteins. This reaction (glycation) reduces or destroys the function of many enzymes. Basal media containing sweet potato-*shochu* distillery wastewater were analyzed for glucose concentration. Figure 3 shows that maximum EPS production occurred at the 3% glucose concentration. Increasing glucose concentration resulted in increased EPS production until the added glucose concentration reached 4%.

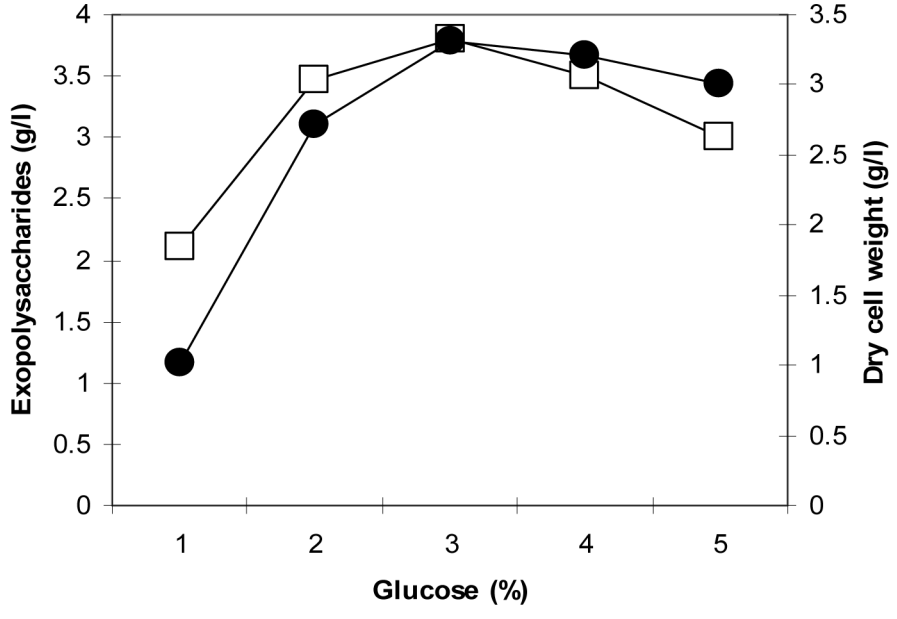

Figure 3. Effect of concentration of glucose on exopolysaccharides production using *Lactobacillus sakei* sp. CY1 on 24 hour. The basal medium (pH 6.2) contained 75% (v/v) sweet potato-*shochu* distillery wastewater. (●) dry cell weight, (□) exopolysaccharides (mg/l)

The culture grown in 75% sweet potato-*shochu* distillery wastewater and basal medium containing 5% glucose yielded 3 $g \cdot L^{-1}$ of polymer, while the 3% glucose medium gave higher yields, about 3.8 $g \cdot L^{-1}$.

3.4 EPS characterization

Purified EPSs were insoluble in cold 99% (v/v) ethanol. After precipitation, a sticky pellet was observed in all media except control. Examination of the monosaccharides and of the chemical composition of the EPS precipitate (Table 4) showed that on the basal medium combining with 75% wastewater had higher concentrations of carbohydrate than any other media (basal media and MRS media) whereas had lower amounts of protein compare MRS media.

Table 4. Monosaccharides and chemical composition of the polymers produced by *L. sakei* CY1

	Monosaccharides composition (%)					
	glucose	galactose	mannose	arabinose	xylose	rhamnose
Basal media	6.4	3.5	0.9	0.0	5.6	0.0
Shochu wastewater	25.5	13.2	0.1	0.1	0.2	0.0
MRS media	5.7	3.2	0.1	0.7	0.0	0.0

	Chemical composition (μg/mL)	
	carbohydrate	protein
Basal media	63	23
Shochu wastewater	256	39
MRS media	146	53

BM = basal medium contain peptone, glucose, salt, and yeast; SDWW = 75% *shochu* distillery wastewater presence on basal medium; MRS = de Man, Rogosa, Sharpe medium

HPLC analysis revealed that glucose and galactose were the dominant monosaccharides in *L. sakei* CY1 exopolymers. These results were similar to the findings of several studies, which reported that glucose and galactose occur frequently in bacterial exopolymers [23, 24, 25, 26]. Besides glucose and galactose, another monosaccharide had also been reported in several studies. Verges et al. reported that *L. sakei* EPSs generally contain glucose and rhamnose at the ratio 3:2 [27]; Cerning et al. reported that arabinose and xylose are also present in bacterial EPSs [24]. Glucose and galactose concentrations in the exopolymers produced by cells grown in 75% *shochu* distillery wastewater-supplemented media were higher than those produced by cells grown in either basal or chemically defined media (25.5% and 13.2%, respectively). Generally, the sugar composition of the 3 types of media was similar. Even the carbon source of the culture medium had no remarkable effect on the monosaccharide composition of the EPSs; only the carbon source influenced EPS yield. This confirms the findings of Torriani et al., who found that different energy sources in the medium do not alter the composition of the EPSs produced [28].

3.5 Effect of organic wastewater on soil aggregation

Aggregation in the soil is produced by the coalescence of particles to form aggregates [29]. Organic wastes can used as a source of organic matter for fermenting and exopolysaccharide-producing microorganisms in large scale applications to diminish the cost of soil aggregations [30]. Arcenegue et al. found a positive correlations between the stability of soil aggregates and the produced microbial biomass in soil [31]. Furthermore, the degree of aggregation was greatly influenced by the nutrient sources. In the soil 10% v/w *L. sakei CY1* with *shochu* wastewater as a substrate application gave an increase of 70 -80% aggregation within 28 days incubation. Exopolysaccharides produced large aggregates (210 μm) 6-7 times compare untreated soil, and for totally compare with untreated soil, inoculation of *L. sakei* CY1 was grown on 100% SDW, gave increasing aggregation until two times, whereas with *L. sakei* CY1 was grown on MRS media, gave increasing until 33% (Table 5). Even the aggregate formation from *shochu* wastewater as substrate with chemical defined media (MRS) as substrate had no significant different but from economically reason maybe the use of *shochu* wastewater was more suitable.

Table 5. Particle size distribution of inoculated soil with *L. sakei* CY1 (LSCY1) with *shochu* wastewater as carbon source compared with control and MRS media in macro aggregate

	500μm	1000μm	2000μm	Total
		(%)		
Untreated	12.13	12.96	10.96	36.05
MRS	9.36	11.66	40.36	61.38
25% SDW	3.88	6.71	58.77	69.36
50% SDW	2.75	8.92	61.90	73.57
75% SDW	2.96	7.66	62.81	73.43
100% SDW	2.12	11.80	65.32	79.24

The percentage of macro-aggregate formation had increase with increasing wastewater concentration on culture media as shown on Figure 4. These results provide evidence that the nutrient contain on *shochu* distillery wastewater was suitable for *L. sakei* CY1 to produce exopolysaccharides that had effect on aggregate forming.

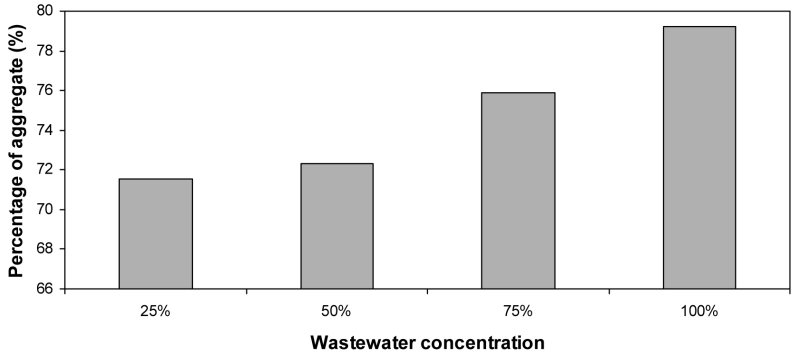

Figure 4. Influence of *shochu* distillery wastewater concentration on aggregate formation

4. CONCLUSION

From this study, we concluded that sweet potato-*shochu* distillery wastewater contains ingredients that can be used by *L. sakei* CY1 to produce commercially important polymers such as EPSs. The culture conditions developed are not expensive, because they do not require the addition of any nutrients to the medium. Moreover, we determined that in 100% *shochu* distillery wastewater without nutrient addition, this strain could grow and produce EPS. This strongly suggests that the currently used and relatively expensive additives such as peptone and yeast extract can be replaced by sweet potato-*shochu* distillery wastewater. The use of *L. sakei* CY1 was grown on *shochu* distillery wastewater gave increasing on aggregate formation until two times compared untreated soil, in addition, the percentage of macro-aggregate formation had increase with increasing wastewater concentration on culture media

5. REFFERENCES

(1) Hammes W.P., Bantleon A., and Min. S. Lactic acid bacteria in meat fermentation, *FEMS Microbiol. Rev.* 1990; 87: 165-174

(2) Tallon R, Philippe Bressolier, Urdaci M.C. Isolation and characterization of two exoplysaccharides produced by *Lactobacillus plantarum* EP56. *Research in Microbiology*. 2003; 154:705-715

(3) Chaillou S., Champomier-Verges M.C., Cornet M., Crutz-Le Coq A.M. The complete genome sequence of the meat bone lactic acid bacterium *Lactobacillus sakei* 23K. *Nature Biotechnology*. 2005; 23: 1527-1533

(4) Sutherland I.W. Biotechnology of microbial exopolysaccharides. Cambridge University Press. 2008.

(5) Berg D.J.C., Robijn G.W., Janssen A.C. Production of a novel extracellular polysaccharide by *Lactobacillus sake* 0-1 and characterization of the polysaccharide. *Applied and Environmental Microbiology*. 1995; 61(8) : 2840-2844

(6) Lauret F., Morel-Deville F., Berthier M.C., Champomier-Vergès P.W., Postma S.D., Ehrlich M., Zagorec. Carbohydrate utilization in *Lactobacillus sake*. *Applied and Environmental Microbiology*. 1996; 62: 1922–1927

(7) Cerning J., Marshall V.M.E. Exopolysaccharides produced by the dairy lactic acid bacteria. *Recent Results and Developments in Microbiology*. 1999; 3: 195-209

(8) Yamasaki T., Tshunehiro Aki., Shinozaki M. Utilization of *shochu* distillery wastewater for production of polyunsaturated fatty acids and xanthophylls using thraustochytrid. *Journal of Bioscience and Bioengineering*, *The Society for Biotechnology*, *Japan*. 2006; 102(4): 323-327

(9) Savadogo A, Ouattara C.A.T., Savadogo P.W. Identification of exoploysaccharide-producing lactic acid bacteria from Burkina Faso fermented milk samples. *African Journal of Biotechnology*. 2003; 3(3): 189-194

(10) Volodymyr Ivanov and Jian Chu. Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Rev. Environ. Sci. Biotechnol. 2008.

(11) Godinho A.L., and Saroj Bhosle. Sand aggregation by exopolysaccharide-producing microbacterium arborescens-AGSB. *Current Microbiol.* 2009; 58: 616-621

(12) Dickson E.L., Rasiah V., and Groenevelt P.H. Comparison of four prewetting techniques in wet aggregate stability determination. *Can. J. Soil Sci.* 1990; 71:67-72

(13) Geoghegan and Bria. Aggregate formation in soil: Influence of some bacterial polysaccharides on the binding of soil particles. *Biochem. J.* 1948; 43:5-14

(14) Edwards U., Rogall T., Blocker H.E., Bottger E.C. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Research.* 1989; 17: 7843–7853

(15) Sutherland I.W. Biotechnology of microbial exopolysaccharides. Cambridge University Press, Cambridge. 1990.

(16) Savadogo A, Ouattara C.A.T., Savadogo P.W. Identification of exoploysaccharide-producing lactic acid bacteria from Burkina Faso fermented milk samples. *African Journal of Biotechnology.* 2003; 3(3): 189-194

(17) *Standard Methods for the Examination of Water and Wastewater*: 19th ed, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA. 1995.

(18) Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry.*1951;193: 265-275

(19) Decho A.W. and Lopez G..R. Exopolymer microenvironments of microbial flora. *Limnol. Oceanog.* 1993; 38:1633-1645

(20) Spizizen J. Transformation of a biochemically deficient strain of *Bacillus subtilis* by deoxyribonucleate. Proceedings of the National Academy of Sciences of the United States of America. 1958; 44:1072-1078

(21) Cromwell G. L., Herkelman K. L., Stahly T. S. Physical, chemical, and nutritional characteristics of distillers dried grains with soluble for chicks and pigs. *Journal of Animal Science.* 1993; 71: 679-686

(22) Macedo, M.G. Effect of the medium supplementation on exopolysaccharide production by *Lactobacillus rhamnosus* RW-9595M in whey permeate. *International Dairy Journal.* 2002; 12: 419-426

(23) de Vuyst L., Degeest B. Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol. Rev.*1999; 23: 153-177

(24) Cerning J. Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. *Le Lait.* 1995; 75: 463-472

(25) Gruter M., Leeflang B.R., Kuiper J., Kamerling J.P., Vliegenthart J.F.G. Structural characterization of the exopolysaccharide produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* RR grown in skimmed milk. *Carbohydrate Research.* 1993; 239: 209-226

(26) Korkeala H., Suortti T., Makela P. Ropy slime formation in vacuum-packed cooked meat product caused by homofermentative lactobacilli and a *Leuconostoc* species. *International Journal of Food Microbiology.* 1988; 7: 339-347

(27) Verges M.C.C., Chailou S., Cornet M., Zagorec M. *Lactobacillus sakei*, recent developments and future prospects. *Research in Microbiology. 2002;* 153: 115-123

(28) Torriani, S., van Reenen C.A., Klein G., Reuter G., Dellaglio F., Dicks L.M.T. *Lactobacillus curvatus* subsp. *curvatus* subsp. nov. and *Lactobacillus curvatus* subsp. *melibiosus* subsp. nov. and *Lactobacillus sake* subsp. *sake* subsp. nov. and *Lactobacillus sake* subsp. *carnosus* subsp. nov., new subspecies of *Lactobacillus curvatus* Abo-Elnaga and Kandleri 1965 and *Lactobacillus sake* Katagiri, Kitahara, and Fukami 1934 (Klein

et al. 1996, emended descriptions), respectively. *International Journal of Systematic Bacteriology*. 1996;. 46: 1158-1163

(29) Skinner F.A. Rothamsted studies of soil structure VII. The effects of incubation on soil aggregate stability. *Journal of Soil Science* 30. 1979; 473-481

(30) Volodymyr Ivanov and Jian Chu. Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Rev. Environ. Sci. Biotechnol. 2008.

(31) Lynch J.M. Promotion and inhibition of soil aggregate stabilization by micro-organisms. *Journal of General Microbiology*. 1981; 126: 371-375