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A fermented, ropy, non-dairy oat product based on the
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Olle Holst.

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PAPER V

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A FERMENTED, ROPY, NON-DAIRY OAT PRODUCT BASED ON THE EXOPOLYSACCHARIDE-PRODUCING STRAIN *Pediococcus damnosus*

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SUMMARY

An attempt has been made to produce a fermented, ropy, non-dairy product (Y2.6) containing the exopolysaccharide(EPS)-producing lactic acid bacterial strain *Pediococcus damnosus* 2.6 in combination with an ordinary yoghurt culture (V2). The fermentation profile, including physical parameters, such as viscosity and ropiness, was measured during a fermentation period of 24 h. The stability of the product was determined during 30 days of cold storage (6 °C). No decrease in viscosity or ropiness was seen during the storage period. The survival of the *P. damnosus* 2.6 strain was high during the whole storage period. Finally, a sensory preference test was done on two differently flavoured Y2.6 products. A traditional, fermented, ropy, dairy product was used as a control product. No significant difference ($P < 0.05$) between the fermented, ropy Y2.6 product and the commercial, fermented, ropy dairy product was shown by the sensory preference test. This study shows the possibility of developing a fermented, ropy, non-dairy product derived entirely from oats and water.

KEYWORDS: Non-dairy, Adavena[®], *Pediococcus damnosus* 2.6, oats, exopolysaccharides (EPSs), lactic acid bacteria (LAB)

INTRODUCTION

Fermented non-dairy products have been developed in recent years to meet the increasing demand from consumers for non-dairy products [1-4]. In comparison to other cereals and legumes, oats is an interesting raw material for fermented food products because of a well-balanced nutritional composition and containing both soluble and insoluble fiber [5]. It is also generally accepted

that the predominant soluble fiber in oats, β -glucans, has a positive effect on blood cholesterol levels [6-7]. High serum cholesterol concentration levels are strongly associated with an increased risk of ischemic heart disease [8]. To increase the interest in oats as a raw material for new functional food products, the use of a fermentation process in combination with new oat-bases (Adavena[®]) (Ceba Foods AB, Lund, Sweden) rich in other things, in dietary fiber, could provide new foods based on oats. The oat-bases are made entirely of oats and water using a patented enzymatic process (US patent No. 5.686.123) [9]. One application is a non-dairy milk substitute (Ceba Foods AB, Lund, Sweden). This product has been reported to have both high acceptance among consumers and a cholesterol lowering effect [10-11]. It has also been shown that this product can be fermented by lactic acid bacteria (LAB) that have the ability to produce exopolysaccharides (EPSs) [12].

Previous studies indicate that these microbial polysaccharides (EPSs) could have a physiological function similar to polysaccharides from plants [13-14]. The EPS-producing bacterial strain used in this study, *Pediococcus damnosus* 2.6, produces a homopolysaccharide with a β -glucan structure [15]. A fermented product that contains both soluble plant polysaccharides, such as β -glucans from oats, and microbial polysaccharides that are produced *in situ* during the fermentation may, therefore, have improved, beneficial physiological effects.

The main objective of this study was to demonstrate the possibility of producing a new type of fermented, ropy, non-dairy product, which is totally derived from oats and water and fermented with a LAB, that has a documented production of an EPS with a β -glucan structure, in combination with a traditional yoghurt culture.

MATERIALS AND METHODS

Starter cultures

The *Pediococcus damnosus* 2.6 was obtained from the UPV culture collection (Universidad de Pais Vasco, San Sebastian, Spain). The strain was stored at $-80\text{ }^{\circ}\text{C}$ in MRS broth [16] plus 25% (v/v) glycerol. Before experimental use the cultures were propagated twice in MRS (Merck, Darmstadt, Germany) at $30\text{ }^{\circ}\text{C}$. The commercial yoghurt culture, V2, which is a 1:1 mixture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (Visby Tønder A/S, Tønder, Denmark) was stored according to the manufacturer recommendations ($-80\text{ }^{\circ}\text{C}$) before use. This yoghurt culture was chosen, as it is a starter culture commonly used in Sweden for yoghurt products.

Preparation of the pre-inoculum

The pre-inoculum for the *P. damnosus* 2.6 strain was made by re-constituting oat powder, Adavena® G40, (Ceba Foods AB, Lund, Sweden) to a final dry matter of 20%. This oat powder was analyzed for protein, fat, different carbohydrates, dietary fiber, various vitamins and minerals by an authorized laboratory (AnalyCen Nordic AB, Lidköping, Sweden) (Table 1). After heat-treatment at $90\text{ }^{\circ}\text{C}$ for 5 min with continuous stirring the medium was cooled to fermentation temperature ($30\text{ }^{\circ}\text{C}$). The oat-base medium was inoculated (5%) with an exponentially growing culture of *P. damnosus* 2.6 in MRS broth. The pre-inoculum was incubated at $30\text{ }^{\circ}\text{C}$ for 20 h.

TABLE 1 - Chemical composition (g/100g) of the non-dairy oat base (G 40 medium).

Components	G40 medium (g/100g)
Dry matter (%)	20
Protein (g)	2.2
Fat (g)	1.6
Glucose (g)	8.8
Maltose (g)	0.6
Maltodextrin (g)	5.4
Total fiber (g)	1.6
β -glucan (g)	0.8
α -tocopherol (mg)	0.2
Thiamin (mg)	0.08
Riboflavin (μg)	19.2
Niacin (mg)	0.2
Folic acid (μg)	6.6
Pyridoxine (mg)	0.02
Iron (mg)	0.2
Magnesium (mg)	9.4
Manganese (mg)	0.2
Phosphorus (mg)	54
Sodium (mg)	22
Zinc (mg)	0.2

Experimental procedures

Comparison of fermentation characteristics of *P. damnosus* 2.6 in the presence and absence of a yoghurt culture (V2). Fermentation characteristics were compared in Erlenmeyer flasks (200 ml) at two incubation temperatures (28 and $37\text{ }^{\circ}\text{C}$) using both the *P. damnosus* 2.6 strain and the V2 culture as pure and mixed cultures. The non-dairy oat base, G40, was prepared to a final dry matter of 16%, as described earlier [3]. After heat treatment at $90\text{ }^{\circ}\text{C}$ for 5 min with continuous stirring the medium was cooled to fermentation temperature. The *P. damnosus* 2.6 inoculated (5% (v/v)) was taken from a fresh (20 h incubation) pre-inoculum. The commercial yoghurt culture (V2) was inoculated directly (0.02% (w/v)) into it. After incubation the characteristics of the final product, such as pH, viable counts, viscosity and ropiness were measured.

Production of the fermented, ropy Y2.6 product

To investigate the fermentation characteristics during the production of a ropy, oat-based product, fermented both by a commercial yoghurt culture and by an EPS-producing strain (*P. damnosus* 2.6), cultivations were performed in a 12 L fermentor (Chemoferm, Sweden) with an 8 L working volume for 24 h. A concentrated liquid form of the Adavena G40 oat-base with a final dry matter of 16% (v/v) was used as medium. The medium was heated to $90\text{ }^{\circ}\text{C}$ for 5 min with continuous stirring and then transferred to the sterile fermentor. The fermentation temperature was kept at $28\text{ }^{\circ}\text{C}$ without pH control and agitation. The fermentor was inoculated with a fresh 5% (v/v) pre-inoculum, described above, together with a commercial yoghurt culture (V2).

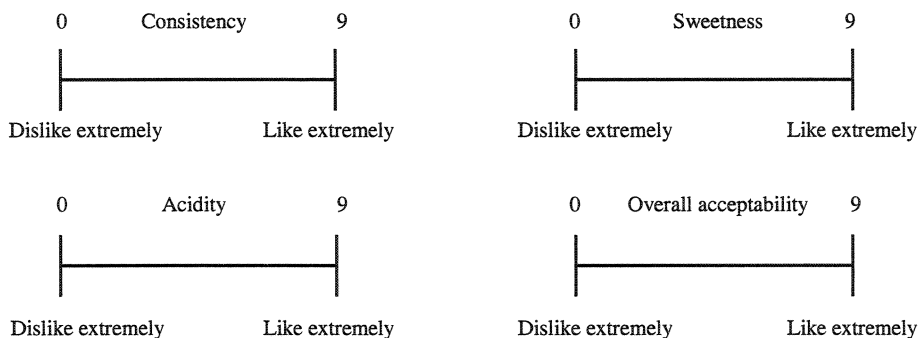
Product stability during refrigerated storage

The storage stability of the Y2.6 products was investigated during cold storage. The products were analysed every fifth day for viscosity, pH, ropiness and bacterial survival of the starter cultures over a storage period of 30 days.

Microbiological analysis

For all microbiological counts, a sample (1 ml) was taken and transferred for serial dilution using 9 ml of peptone water, 0.1% peptone (Difco, Detroit, Michigan, US) and 0.8% NaCl (Merck). Enumeration of *S. salivarius* subsp. *thermophilus* was performed using M17-agar plates (Merck, Darmstadt) [17]. The plates were incubated at $37\text{ }^{\circ}\text{C}$ for 72 h under aerobic conditions. Enumeration of *L. delbrueckii* subsp. *bulgaricus* was performed using MRS-agar plates (Merck). The plates were incubated at $37\text{ }^{\circ}\text{C}$ for 72 h under aerobic conditions. *P. damnosus* 2.6 was enumerated by using MRS-agar plates (Merck) containing 6% v/v ethanol. The plates were incubated at $30\text{ }^{\circ}\text{C}$ for 72 h using anaerobic jars (5% CO_2 , 85% N_2 , 10% H_2).

FIGURE 1
Hedonic scales used in the ranking for preference evaluation for the different fermented products using four different sensory parameters, consistency, sweetness, acidity and overall acceptability
 (The unmarked scales ranged from 1 - dislike extremely to 9 - like extremely).



Chemical and physical analysis

Every second hour during a fermentation period of 24 h, samples were withdrawn from the fermentation vessel and analysed for pH, lactic acid concentration, soluble fiber, viscosity and ropiness. Lactic acid concentration (LA) was determined by using a commercial enzymatic kit (Boehringer, Mannheim, Germany). pH measurements were carried out using a digital pH meter, MA235 model (Mettler Toledo, Hightstown, USA). To measure the fraction of soluble fiber in the G40 medium during the fermentation, the pH of the samples was adjusted to 6.2. Proteins were hydrolysed by a combination of Alcalase[®] and Esperase[®] (Novo Nordisk, Bagsværd, Denmark) at a ratio of 1:1 and a concentration of 1% (v/v). After an incubation period of 16 h at 30 °C, the samples were centrifuged for 30 min at 4,000 rpm, the supernatant collected and washed through a filter (200 µm) (Spectra Mesh Industries Inc., Houston, USA). The filtrate was collected and precipitated with 4 volumes of cold ethanol (95%), followed by storage overnight at 4 °C. The precipitate was recovered by centrifugation at 6,000 rpm for 30 min and dissolved and dialysed against distilled water (5 L) for 72 h at 4 °C. After centrifugation the samples were lyophilised and weighed. The amount of soluble polymer from the G40 medium was expressed as polymer dry mass per litre (mg PDM/L). The viscosity was measured using a Brookfield DV-I Viscometer (Brookfield Viscometers LTD, Harlow, UK) with the S63 spindle, at 28 °C for 2 min at 50 rpm and was expressed as mPas. The ropy characteristic of the products was measured by using an Instron 4442 (Instron Ltd, Buckinghamshire, UK). 25 ml of a sample were transferred to a petri dish. A probe with a diameter of 3.8 cm was brought into contact

with the surface of the sample and lifted up to a speed of 100 cm/min. When the product lost its contact with the probe the measurement terminated. The measurements were done in triplicate and at ambient temperature. Maltose content was analysed by high pH anion exchange chromatography (HPHEC) using a CarboPac PA 10 column (Dionex, Jouy-en-Josas, France) with 0.2 M NaOH as the mobile phase at a flow rate of 1.4 ml/min.

Sensory evaluation

Eight panellists of various ages and of both genders determined the organoleptic properties of the Y2.6 product flavoured with two different jams, mixed black berries and exotic fruit jam (Hafi AB, Getinge, Sweden). A fermented, ropy, dairy product, Långfil[®], (Milko, Östersund, Sweden) was used as control product on the basis of its ropy texture. The control products were flavoured in the same manner as the Y2.6 products. All products were filled into identical commercial yoghurt containers (100 ml) and labelled with a three-digit code. The products were presented to the panellists as a double blind test. The evaluation was done twice within a period of 14 days.

Sensory properties (appearance, consistency, acidity and sweetness) of the various products were compared and ranked using hedonic scales (Figure 1). The panellists were asked to number the products from 1 to 9 according to their preference, using the same sensory qualifications. The closer the rank sum was to the highest reference score (36.0) the higher was the overall preference for the product.

Statistical analysis

Values were expressed as the means and the standard deviation. The mean values of the treatments were compared by Student's *t* test. Differences were considered significant at $P < 0.05$. Results concerning the different products in the sensory preference test were analysed by paired *t*-tests. The significance of the ranking in the preference tests was analysed using Kramer's table [18].

RESULTS

Fermentation characteristics of *P. damnosus* 2.6 (2.6) in the presence and the absence of a yoghurt culture (V2)

There was an increase in both viscosity and ropiness when the *P. damnosus* 2.6 strains were grown in the G40 oat-base (Table 2). This increase did not change when the yoghurt culture was included. The change of incubation temperature had the strongest impact on the final viscosity and ropiness of the product. This was most obvious when the strains were grown at the higher incubation temperature (37 °C), which gave a much lower value for viscosity and ropiness. The *P. damnosus* 2.6 strain showed high viability ($> 10^8$ cfu/ml) at both incubation temperatures. It was observed that the viability of the two cultures (V2 and *P. damnosus* 2.6) was not decreased when they were grown as mixed cultures.

Fermentation of the ropy Y2.6 product

The fermentation profile of the Y2.6 product is shown in Figure 2. Both viscosity and ropiness increased during the fermentation period and gave the highest value after 24 h. There was a continuous decrease in pH during the whole period. However, it was found that this decrease

declined towards the end of the period. The glucose and the β -glucan concentrations were measured before and after the fermentation period (Table 3). It was shown that most of the glucose in the oat-base was not used by the bacterial strains during the fermentation process and consequently was left unfermented.

Stability and bacterial survival during refrigerated storage

The stability of the product in terms of its consistency (viscosity and ropiness), pH and the bacterial survival of the starter cultures were measured (Figure 3). There was an evident increase in viscosity and ropiness during the storage period. The survival of *P. damnosus* 2.6 was high (10^9 cfu/ml) during the whole storage period. The survival of the two yoghurt bacterial strains, *L. bulgaricus* subsp. *delbrueckii* and *S. salivarius* subsp. *thermophilus*, decreased during the storage period to a value of 10^3 cfu/ml after 30 days. The pH of the product was constant during the whole storage period.

Sensory evaluation

The result of the sensorial preference test is shown in Figure 4. The two control products, the ropy dairy products, obtained the highest total in the preference test. The difference, however, is not significant. The two flavoured Y2.6 products received a lower score mainly in terms of the consistency of the product in comparison to both dairy control products. In the two other sensory parameters (sweetness and acidity) there were no significant differences between the two Y2.6 products and the two ropy, dairy control products. It was also observed that the product containing the black berry jam was given the highest total score of the products evaluated in the preference test for both the Y2.6 case and the ropy, dairy control case.

TABLE 2
Fermentation characteristics of the oat-based G40 medium when using a yoghurt culture (V2) consisting of *L. delbrueckii* subsp. *bulgaricus* (Ldb) and *S. salivarius* subsp. *thermophilus* (Sst) and an EPS-producing strain, *P. damnosus* 2.6 (2.6), as pure or mixed cultures.

Culture	Temperature (°C)	Viscosity (mPas) ^a	Ropiness (cm) ^b	pH	Viable count (log (cfu/ml))		
					Ldb	Sst	2.6
V2	28	546 ± 42	1.5 ± 0.1	5.4 ± 0.20	6.3	6.0	ND ^c
V2	37	450 ± 36	1.8 ± 0.1	3.9 ± 0.10	7.5	8.0	ND
2.6	28	2,400 ± 0	28.2 ± 2.4	4.1 ± 0.10	ND	ND	8.2
2.6	37	2,181 ± 169	16.7 ± 3.1	5.7 ± 0.20	ND	ND	8.2
V2 + 2.6	28	2,400 ± 0	22.6 ± 7.6	4.1 ± 0.10	6.3	6.0	9.0
V2 + 2.6	37	958 ± 175	3.1 ± 0.2	3.6 ± 0.10	7.9	7.0	8.5

^a Viscosity measured after 2 min of shear thinning at 50 rpm and 28 °C. - ^b Ropiness measured at a speed of 100 cm/min. - ^c ND=Not determined.

TABLE 3
Polymer dry mass (PDM) ($g\ l^{-1}$) and glucose (%) content in the Y2.6 product before (initial value) and after 24 h of fermentation

	Initial value	Value after 24h of fermentation
PDM ($g\ l^{-1}$)	4.9 ± 0.1	5.1 ± 0.1
Glucose (%)	7.3 ± 0.1	5.7 ± 0.1

FIGURE 2
Fermentation profile of the Y2.6 product, consisting of *P. damnosus* 2.6 and V2, during 24 h at 28 °C. Viscosity (x), ropiness (O), pH (●), lactic acid (*). The results are the mean values of two fermentations.

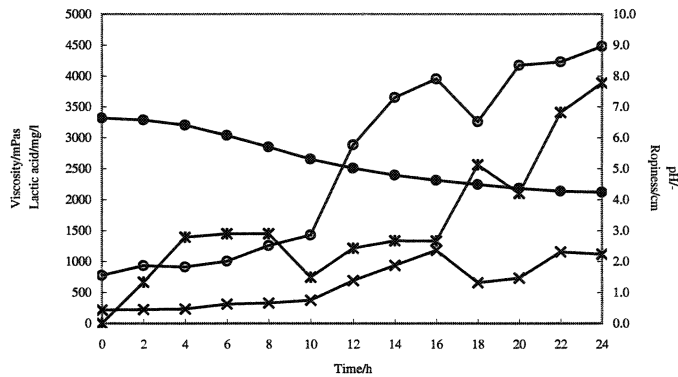


FIGURE 3
Storage stability of the fermented, ropy Y2.6 products containing black berry or exotic fruit jam, during 30 days in cold storage (6 °C). Survival of *Pediococcus damnosus* 2.6 (◆), *Lactobacillus delbrueckii* subsp. *bulgaricus* (▲), *Streptococcus salivarius* subsp. *thermophilus* (■), viscosity (x), ropiness (O) and pH (●). The results are the mean values of three determinations.

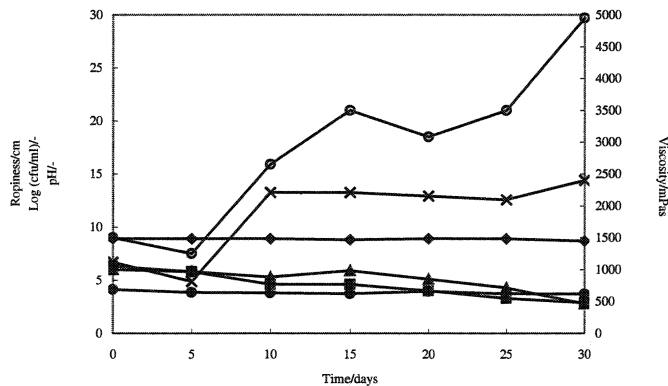
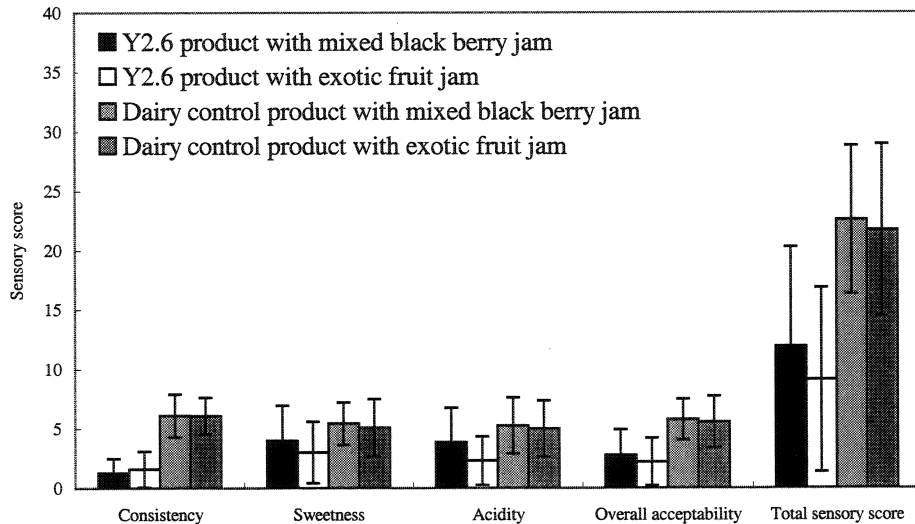


FIGURE 4

Ranking for preference of the Y2.6 product to evaluate four different sensory parameters: consistency, sweetness acidity and overall acceptability, and the comparison with a fermented, ropy dairy product. Four different products were used, two Y2.6 products containing black berry jam (■) or an exotic fruit jam (□) and two variants of fermented, ropy dairy control products containing black berry jam (▨) or exotic fruit jam (▩). Figures are mean values ($N = 8$) of distances marked by the panellists from 1 (dislike extremely) to 9 (like extremely). The total sensory score is the sum of the ranking for preference from 8 ($N = 8$) panellists. The results are the mean values of two different evaluation occasions



DISCUSSION

The objective of this study was to investigate the possibility of producing a fermented, ropy, non-dairy product made from oats by including the EPS-producing bacterial strain, *P. damnosus* 2.6, as starter culture in combination with an ordinary commercial yoghurt culture (V2). Fermented, ropy, dairy products have been studied earlier because of their unique textures and consistencies [19-21]. A report has also indicated that fermented, ropy dairy product would have cholesterol-lowering activity [22]. In this study we investigated the fermentation characteristics of *P. damnosus* 2.6, with and without the V2 culture, in an oat-base with a high glucose content (G40) at two different temperatures. An increase in viscosity after fermentation was seen only when *P. damnosus* 2.6 was used in the G40 medium. The ropy characteristic of the oat-base after fermentation was lower at a higher incubation temperature (37 °C). It was shown that a lower incubation temperature (28 °C) gave a higher increase in both viscosity and ropiness after a fermentation period of 24 h. This follows earlier findings that the optimal growth for the EPS production by *P. damnosus* 2.6 would be in the range of 25-30 °C [15]. Although this temperature is far from optimal (37 °C) for the V2 culture, A flavour, sub-

jectively described as a palatable aroma recognised as yoghurt flavour, resulted at this incubation temperature (data not shown).

During a fermentation of 24 h at 28 °C using both the *P. damnosus* 2.6 strain and the V2 culture as starter cultures it was seen that both viscosity and ropiness increased during the whole fermentation period. No significant change could be seen in total polymer dry mass (PDM), indicating that the EPS are produced in amounts too small to be detected analytically in this complex, oat-based medium. The significant change in the physical character of the oat-base during the fermentation period is more likely to be due to the interaction between the microbial polysaccharide and macronutrients, such as proteins in the growth medium. This kind of interaction has been described earlier in milk [23].

It was shown that the Y2.6 products retained their stability during 30 days of storage at 6 °C. A high survival of the *P. damnosus* 2.6 strain was also seen during the time of storage, suggesting that the oat-base used is a good support for this strain during a storage period of 30 days. The

survival of the V2 culture was found to be lower. However, these strains have a higher optimal growth temperature (37 °C) than the *P damnosus* 2.6 strain, with its optimal incubation temperature of 30 °C.

In the sensorial preference test four sensory parameters were investigated, consistency, sweetness, acidity and overall acceptability. The panellists evaluated the ropy consistency to have low acceptability. However, the acceptability for this ropy structure was higher when black berry jam was added to the product, as it had a major impact on the colour of the final product. The total rank sum was lower for both of the two Y2.6 products in comparison to the two fermented, ropy, control products. However, the differences between the products were not significant ($P < 0.05$).

In this study it is shown that it is possible to produce a fermented, ropy, non-dairy product using both a commercial yoghurt culture and an EPS-producing bacterial strain, *P. damnosus* 2.6. This kind of study can facilitate the development of new, fermented, non-dairy, nutritionally well-balanced food products with unique physical properties.

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