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A simple approach for the selective enzymatic synthesis of dilauroyl maltose in organic media

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ABSTRACT

A selective synthesis of dilauroyl maltose was developed using lipase-catalyzed condensation of lauric acid and maltose in two-solvent mixtures. The characteristics of different solvent combination were tested and it was found that the combination of acetone with *n*-hexane has a good selectivity for the synthesis of dilauroyl maltose. The highest diester conversion of 69% (i.e. 36.5 g/L of dilauroyl maltose) was obtained under optimal conditions: 25.65 g/L maltose, 60 g/L lauric acid, 60 g/L molecular sieve and 10 g/L lipase at 150 rpm and 50 °C for 72 h in 10 mL of mixed solvent of acetone:*n*-hexane (60:40, v/v).

1. Introduction

Sugar esters are amphiphilic and biodegradable nonionic surfactants widely used in food, cosmetic and medical fields [1–4]. Maltose ester is one of those which attracts much attention. Recently, many scientific studies are focused on the enzymatic synthesis of sugar esters [5–7]. The use of sugar fatty acid esters varies with the characteristics of the substrates, the degree and site of esterification of sugar with fatty acid. The enzymic synthesis offers the possibility of obtaining specific isomers in high yields. A considerable amount of research showed that lipase, particularly lipase B from *Candida antarctica*, was a good catalyst in non-aqueous media [8–11]. It has been shown that the activity and specificity of enzyme in organic solvents is highly dependent on the nature of the solvent [12,13]. The enzyme activity in organic media is often correlated with the solvent hydrophobicity (log *P*) with the highest activities found at high log *P* values [14,15]. In order to ensure the successful condensation of saccharide fatty acid esters, the solvent must have a high hydrophobicity and a good solubility of substrates. Most organic solvents with high log *P* values such as *n*-hexane and *n*-heptane have a good solubility of fatty acid but very poor solubility of sugars, and are not appropriate for reactions where the two substrates greatly differ in terms of polarity. Solvents with low log *P* values that can dissolve both sugars and lipids include dimethyl sulfoxide, pyridine, and dimethylformamide, but these solvents often inactivate the enzyme and are incompatible with food applications [16]. The study has indicated disaccharides such as maltose are soluble in polar solvents but lipase is easily inactivated [17]. To overcome these disadvantages, it is possible to choose suitable mixtures of two or more solvents for lipase-catalyzed esterifications of carbohydrate fatty acid ester.

The maltose monoesters have relatively higher HLB (hydrophilic-lipophile balance) values. To obtain sugar esters of low HLB, the best way is to bring two or more fatty acid groups into one molecule of sugar. Therefore, the selective synthesis of diesters or polyesters is desired.

We have previously reported that a high selectivity of monolauroyl maltose was obtained through the enzymatic synthesis utilizing continuous stirred tank reactors in a single acetone solvent [18]. As a continuation of lipase-selective synthesis of sugar esters [18,19], the present work was aimed at developing a new media for the selective synthesis of dilauroyl maltose, based on the comparison of the selectivity and the conversion of dilauroyl maltose in various mixed solvent systems.

2. Experimental

2.1. Materials

Commercial immobilized *Candida antarctica* lipase B (CALB), Novozym 435, was purchased from Novo Nordisk A/S, Denmark. Maltose, lauric acid, DMSO, *tert*-pentanol, acetone, acetonitrile and

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n-hexane were purchased from Sinopharm Chemical Reagent Co., Ltd. China. The 3 Å 1/16 and 4 Å 1/16 molecular sieves were purchased from Shanghai UOP, China.

2.2. Lipase-catalyzed reactions and purification of maltose esters

Maltose (0.5 mmol, 0.18 g), lauric acid (2 mmol, 0.40 g), molecular sieve 3 Å (0.80 g) and immobilized lipase (0.20 g) were weighed into a glass vial. 10 mL of solvent was added into the vial. The solvent had been dehydrated with molecular sieve 4 Å for at least 24 h. The vial was tightly screw-capped and immersed in a water-bath to conduct condensation. The condensation was carried out with vigorous shaking of 150 rpm at 50 °C for 72 h.

The reaction optimization was performed in a system composed of maltose (0.5–1.5 mmol, 0.18–0.54 g), lauric acid (2.0–6.0 mmol, 0.40–1.20 g), molecular sieve 3 Å (0.2–1.0 g), immobilized lipase (0.05–0.40 g) and 60:40 acetone:*n*-hexane (10 mL). The temperature was 50 °C, and the samples were shaken on an orbital shaker at 150 rpm for 72 h.

The reaction mixture was filtered to remove the immobilized enzyme and molecular sieves after completion of the reaction, and the filtrate was concentrated by a rotary evaporator. The residue was applied to a silica gel column chromatography to purify the product. The eluent was chloroform/methanol (4:1, v/v), and the flow rate of the eluent was 18 mL/h.

All the condensation reactions were triplicated. The mean of data are illustrated in figures, and the deviations were not more than 6%.

2.3. Analytical methods

2.3.1. High-performance liquid chromatography

The reaction mixture was analyzed by HPLC using a symmetry-C18 column (3.9 mm × 150 mm Waters, USA) eluted with acetonitrile/water (85:15, v/v) at 0.8 mL/min, and the eluate was monitored by a Waters 2996 PDA detector at 210 nm for dilauroyl maltose and a Waters 2420 evaporative light scattering detector (ELSD) at drift-tube temperature 45 °C, sprayer temperature 36 °C and carrier gas pressure 137.8 kPa for monolauroyl maltose, respectively. The calibration curves for the monolauroyl maltose and dilauroyl maltose were prepared using the esters purified according to the methods described above. The conversion was defined as the molar ratio of the amount of esters to that of maltose at the beginning of reaction.

2.3.2. FT-IR, NMR and mass spectrometry analysis

The infrared spectra were taken on Nicolet Nexus FT-IR. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian INOVA (600 and 150 MHz) spectrometer at 30 °C in CD_3OD. Chemical shifts were referred to the methanol multiplet, centered at 3.30 ppm for ^{1}H NMR and 49.0 ppm for ^{13}C NMR. The ^{1}H NMR spectra showed chemical shifts and coupling constants within ±0.005 and ±0.5 Hz, respectively. Mass spectra were obtained by Mass Spectrometry (Waters Platform ZMD 4000, Milford, MA, USA) with positive EI mode.

2.4. Determination of the residual enzyme activity

The lipases were recovered after reaction and their residual activity was determined. After decanting the solvent, the lipase and the molecular sieves were washed 4 times with warm (50 °C) methanol followed by drying in vacuum for 2 h. Additional 3 Å molecular sieve (0.4 g) was added before reuse. The residual activity was determined in a reaction system composed of maltose (0.25 mmol, 0.09 g), lauric acid (1 mmol, 0.2 g), and acetone (5 mL). The temperature was 50 °C, and the samples were shaken on an orbital shaker at 150 rpm for 72 h. The residual activity was expressed as the number of moles of lauroyl maltoses of the recovered enzyme relative to that of the fresh enzyme in the same reaction system. The data presented were averaged from three experiments.

2.5. Determination of maltose solubilities

To determine the solubility of maltose in various solvents, 0.05 g of maltose was dissolved in 1.0 mL of water, acetonitrile, acetone, *tert*-pentanol or *n*-hexane, and 0.5 g of maltose in 1.0 mL of DMSO. These solutions were incubated at 50 °C for 6 h, and 0.8 mL of each were subsequently passed through a 0.2 μ filter. 0.5 mL of each filtered sample were dried by a vacuum concentration dryer. The dry sugar in each tube was re-suspended in 0.5 mL de-ionized water. 20 μL of each sample were analyzed by HPLC with water as the mobile phase and detected with an evaporative light scattering detector (Waters 2420) at 36 °C with a nitrogen gas pressure of 137.8 kPa. The amount of maltose in each sample was determined by comparing peak areas to the maltose-in-water control. The data presented in tables were the mean of three experiments, and the deviations were not more than 6%.

3. Results and discussion

3.1. Product identification

The condensation products were analyzed by FT-IR, ^{1}H NMR, ^{13}C NMR and MS and identified to be 6′-*O*-lauroylmaltose and 6,6′-di-*O*-lauroylmaltose. In selected samples an α/β mix of maltose was present. Assignment of hydrogen and carbon resonances in NMR can get from our previous publication [18].

6′-*O*-Lauroylmaltose: IR (υ, cm^{-1}) 3346 (br, O–H), 1731 (C=O); ^{1}H NMR (δ, ppm): 5.08 (d, 3H, J = 2.9 Hz), 4.47 (d, 1H, J = 2.9 Hz), 4.36 (d, 2H, J = 11.7 Hz), 4.15 (dd, 2H, J = 11.7, 6.3 Hz), 3.83 (m, 8H), 3.60 (m, 3H), 3.42 (m, 6H), 3.24 (t, 2H, J = 9.7 Hz), 3.17 (t, 1H, J = 9.4 Hz), 2.35 (t, 2H, J = 7.3 Hz), 2.24 (t, 2H, J = 7.3 Hz), 1.58 (m, 2×2H), 1.26 (m, 2×16H), 0.87 (t, 2×3H, J = 6.8 Hz); ^{13}C NMR (δ, ppm) 175.95, 103.52, 98.26, 94.23, 82.98, 82.52, 78.28, 77.18, 76.21, 75.48, 75.10, 74.68, 74.51, 73.83, 72.71, 72.17, 72.08, 65.4, 62.8, 35.51, 35.37, 33.52, 31.20, 31.18, 31.09, 31.06, 30.92, 30.90, 30.69, 26.57, 26.45, 24.18, 14.94; MS m/z 559.6 (M^++Cl), 523.9 (M^+−1), 403.7 (M^+-7OH-2), 339.6 (M^+-$C_{11}H_{23}CO$-2).

6,6′-di-*O*-Lauroylmaltose: IR (υ, cm^{-1}) 3335 (br, O–H), 1746, 1712 (C=O); ^{1}H NMR (δ, ppm) 5.05 (d, 3H, J = 3.4 Hz), 4.46 (d, 1H), 4.41 (m, 1H), 4.34 (m, 2H), 4.21 (dd, 1H, J = 11.7, 4.9 Hz), 4.16 (dd, 1H, J = 11.7, 5.4 Hz), 4.12 (dd, 2H, J = 10.3, 3.4 Hz), 4.02 (m, 1H), 3.93 (m, 1H), 3.85 (m, 4H), 3.60 (m, 3H), 3.44 (m, 3H), 3.39 (dd, 1H, J = 3.4, 9.7 Hz), 3.32 (dd, 1H, J = 3.4, 9.7 Hz), 3.24 (t, 2H, J = 10.5 Hz), 3.17 (t, 1H, J = 8.8 Hz), 2.31 (t, 2×2H, J = 7.3 Hz), 2.25 (t, 2×2H, J = 7.3 Hz), 1.58 (m, 4×2H), 1.27 (m, 4×16H), 0.87 (t, 4×3H, J = 6.6 Hz); ^{13}C NMR (δ, ppm) 175.80, 175.53, 104.07, 98.60, 94.35, 83.72, 83.30, 78.04, 76.00, 75.30, 75.13, 75.01, 74.61, 74.47, 74.16, 73.68, 72.77, 72.32, 71.89, 71.04, 69.48, 65.19, 65.00, 35.40, 35.28, 33.48, 33.45, 31.17, 31.12, 31.07, 31.00, 30.89, 30.85, 30.81, 30.64, 30.59, 26.51, 26.47, 26.42, 24.14, 24.11, 14.87, 14.84; MS m/z 742.2 (M^++Cl), 706.0 (M^+−1), 403.5 (M^+-6OH-$C_{11}H_{23}CO_2$-2), 339.4 (M^+-2×$C_{11}H_{23}CO$-1).

3.2. Esterification in single solvent

It is well known that the hydrophobicity of solvent is a key factor for esterification owing to the fact that the lipase catalyzes hydrolysis in aqueous environment [20]. The hydrophobicity is expressed by the value of log P which is defined as the logarithm of the partition coefficient of a given compound in the standard two-phase system of octanol/water [14,15]. The higher the log P is, the stronger

Table 1
Log *P* values, lauroyl maltose conversion, maltose solubility and residual enzyme activity of different solvents.

Solvent	Log *P*	Conversion of monoester (%)	Conversion of diester (%)	Solubility of maltose (mmol/L)	Residual activity (%)
DMSO	−1.30	0	0	972	0
Acetonitrile	−0.36	17	0	0.35	17
Acetone	−0.26	54	12	0.59	65
tert-Pentanol	1.30	19	0	4.78	9
n-Hexane	3.50	0	0	0	85

the hydrophobicity of solvents and the lower the solubility of sugars in the solvent will be [12]. Furthermore, the stability of the enzyme is usually higher in the more hydrophobic solvents, on the other hand, a hydrophobic solvent is not always a good choice if the solubility of a hydrophilic substrate has to be taken into account [13].

Five solvents (DMSO, *tert*-pentanol, acetone, acetonitrile and *n*-hexane) with different hydrophobicity were tested. The results were shown in Table 1 together with their log *P* values [12], the conversion of lauroyl maltose monoester and diester, the solubility of maltose and the residual enzymatic activity.

As depicted in Table 1, *n*-hexane has the highest hydrophobicity and DMSO has the lowest. No esterification occurred in both the two solvents. In *tert*-pentanol and acetonitrile, the monoester formation was poor and no diester detected. Acetone seems to be the best solvent, for not only the monoester conversion was relatively high but also the amount of diester was significant.

It is interesting to observe the relationship between the conversion and the solubility of maltose. DMSO has the highest solubility of maltose and there was no ester formed. This could be explained by the strong polarity of the pure DMSO which deactivated rapidly the catalyst. Though *tert*-pentanol had a higher maltose solubility than acetone, it did not get notable conversion because of the lower activity of enzyme. On the other hand, the lipase has higher enzyme activity in *n*-hexane, but no lauroyl maltose ester was formed because the maltose solubility in it was almost negligible. Thus, both the enzyme activity and the substrate solubility play an important role in the esterification.

Among the five solvents, acetone was the most suitable medium, but the selectivity was not satisfactory. In particular, the proportion of diester was still low. The use of a mixed solvent system seems to be necessary for the production of diester.

3.3. Esterification in mixed solvents

In order to find the suitable reaction media for the production of diester, various combinations of above solvents were investigated. Due to the difference in solubility of maltose in the solvents, the initial substrate concentration in these experiments was superfluous. The log *P* values of mixed solvents can be estimated by the following formula [15]:

$$\log P = x_1 \log P_1 + x_2 \log P_2 = x_1 \log P_1 + (1 - x_1) \log P_2$$

where x_1 and x_2 are the mole fractions of two solvents, respectively.

First we tested the acylation of maltose in *tert*-pentanol containing 2–25% DMSO (Fig. 1), the log *P* values of which was within a large range. As is shown in Table 1, acetone was the best reaction medium as a single solvent with log *P* values at −0.26. And according to the above mentioned formula, we can carried out that when the mole fraction of DMSO reached 0.60 corresponding to 49% DMSO, the log *P* values of mixture is corresponding to that of acetone. But in fact, the highest conversion of monoester and diester were detected using 5% DMSO corresponding to mole fraction of 0.075, and diester conversion was below 13%. With the increase of proportion of DMSO, the esterification rate sharply declined, indicating a complete deactivation of the lipase. It have been reported [13] that the addition of DMSO to other solvents resulted in an increase of enzyme activity and that there was a optimum concentration of DMSO at which the highest activity was achieved.

Fig. 2 showed the esterification in acetonitrile/*n*-hexane solvent system. With varying the proportion of acetonitrile from 20% to 70% corresponding to log *P* value of 2.8–1.0, the formation of monoester was appreciable (maximum 10.4%) and no diester formed. This presented an opposite relationship between the conversion and the hydrophobicity of solvent mixtures, compared to the combination of DMSO/*tert*-pentanol. An explanation of the results is that the two solvents have both a poor solubility of maltose which limits obviously the esterification. Thus, according to Figs. 1 and 2, the log *P* is apparently not the only factor of importance. The search of a solvent system suitable for diester esterification should be in another orientation.

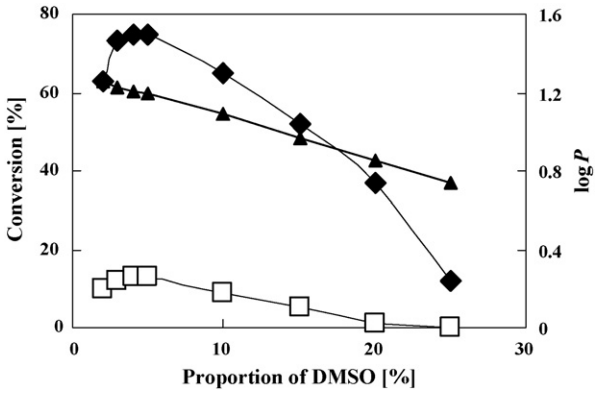

Fig. 1. Effect of the content of DMSO on esterification in the DMSO/*tert*-pentanol solvent system. Reaction conditions: 50 mmol/L maltose, 200 mmol/L lauric acid, 20 g/L lipase and 80 g/L 3 Å molecular sieves in 10 mL of mixed solvent, 150 rpm, 50 °C, 72 h. The symbols (▲, ♦ and □) indicate the log *P* values of mixed solvents and the conversions of monolauroyl maltose and dilauryl maltose, respectively.

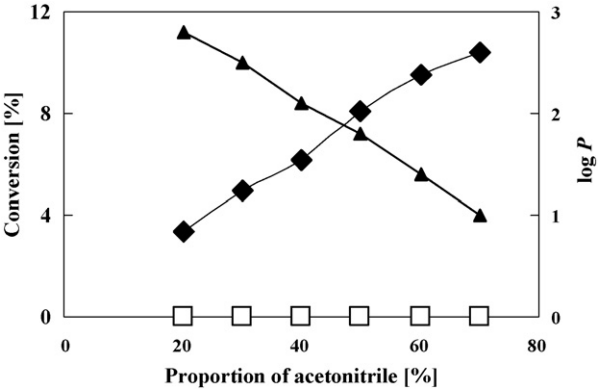

Fig. 2. Effect of the content of acetonitrile on esterification in the acetonitrile/*n*-hexane solvent system. Reaction conditions: 50 mmol/L maltose, 200 mmol/L lauric acid, 20 g/L lipase and 80 g/L 3 Å molecular sieves in 10 mL of mixed solvent, 150 rpm, 50 °C, 72 h. The symbols (▲, ♦ and □) indicate the log *P* values of mixed solvents and the conversions of monolauroyl maltose and dilauryl maltose, respectively.

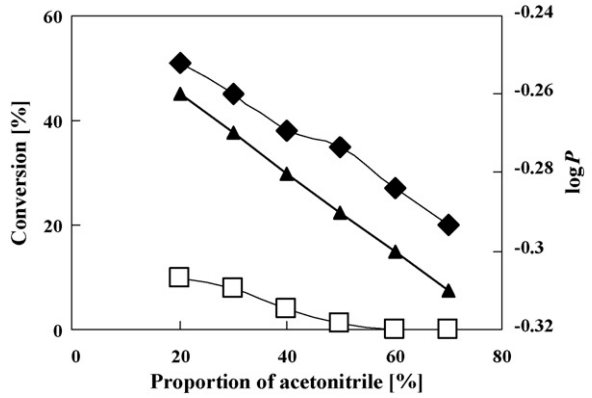

Fig. 3. Effect of the content of acetonitrile on esterification in the acetonitrile/acetone solvent system. Reaction conditions: 50 mmol/L maltose, 200 mmol/L lauric acid, 20 g/L lipase and 80 g/L 3 Å molecular sieves in 10 mL of mixed solvent, 150 rpm, 50 °C, 72 h. The symbols (▲, ♦ and □) indicate the log *P* values of mixed solvents and the conversions of monolauroyl maltose and dilauryl maltose, respectively.

The previous experimental result had point out that acetone was the solvent in which the highest conversion of lauryl maltose was obtained among the five solvents. The solvent hydrophobicity decreases with the addition of acetonitrile to acetone. Unfortunately, the results in Fig. 3 showed that monoester and diester were both formed at a low level. The addition of acetonitrile resulted in lower conversion. Poor solubility was observed in a system containing only acetonitrile and substrate. Hence, the decrease of solubility may be an explanation to the decrease of ester yield.

The introduction of *n*-hexane modifies the hydrophobicity in another direction. From Fig. 4, it is interesting to note that dilauroyl maltose was remarkably and selectively formed in acetone/hexane system. Compared with pure acetone system, the addition of *n*-hexane to acetone caused a notable acceleration of the reaction, resulting in a 66.8% conversion of diester in 30% hexane. When the content of *n*-hexane was 30–50% in the mixture, the formation of diesters was significant and that of monoester was inhibited. This result presents that the mixture of a solvent with a high solubility of maltose such as acetone and another one with a good stability of enzyme such as *n*-hexane may be optimal for an enzyme-catalyzed selective synthesis of sugar ester. But it should be further remarked since this result was not a universal law until now.

Both acetone and *n*-hexane was allowed by the EEC for the production of food additives. They can also be easily separated from the products. This solvent system offers a good reaction media for the selective synthesis of maltose diesters. We would employ the mixture of acetone and *n*-hexane (60:40, v/v) solvent as a reaction system for synthesis of dilauryl maltose in following work.

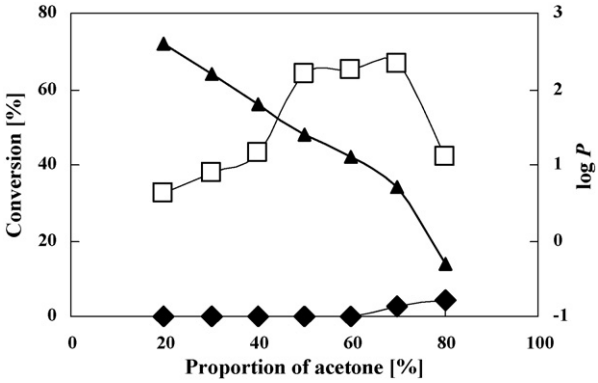

Fig. 4. Effect of the content of acetone on esterification in the acetone/*n*-hexane solvent system. Reaction conditions: 50 mmol/L maltose, 200 mmol/L lauric acid, 20 g/L lipase and 80 g/L 3 Å molecular sieves in 10 mL of mixed solvent, 150 rpm, 50 °C, 72 h. The symbols (▲, ♦ and □) indicate the log *P* values of mixed solvents and the conversions of monolauroyl maltose and dilauryl maltose, respectively.

3.4. Esterification optimization

In order to acquire optimum reaction conditions for the selective synthesis of dilauroyl maltose, the effect of the concentrations of substrates, molecular sieve and lipase on the esterification was further investigated in the acetone:*n*-hexane (60:40, v/v) mixed solvent system. The optimization was conducted step by step, that is, firstly the different enzyme amount was tested with other conditions fixed and the optimal amount was taken in further experiments. The initial reaction conditions were 5 g/L lipase, 50 mmol/L maltose, 4:1 molar ratio of lauric acid to maltose and 80 g/L molecular sieve in 10 mL mixed solvent for 72 h at 50 °C. Lipase concentration (from 5 to 10, 20, 30, 40 g/L), maltose concentration (from 50 to 75, 100, 125, 150 g/L), molar ratio of lauric acid to maltose (from 3 to 4, 5, 6) and molecular sieve concentration (from 20 to 40, 60, 80, 100 g/L) were examined in sequence. The conversion of dilauroyl maltose varied from 38% to 69%. The highest conversion of 69% (i.e. 36.5 g/L of dilauroyl maltose) was obtained in 10 mL of 60:40 acetone:*n*-hexane with 25.65 g/L maltose, 60 g/L lauric acid, 60 g/L molecular sieve and 10 g/L lipase for 72 h at 50 °C.

4. Conclusion

The esterification product of sugar and fatty acid is usually a mixture of esters because of the presence of multiple hydroxyl groups at the sugar molecule. This makes it necessary to develop a reaction system with high selectivity. The enzymatic synthesis meets all the need of such system. The selectivity is related to the hydrophobicity of the solvent but the hydrophobicity is not the only factor of importance for the selectivity. For the synthesis of diester the binary solvent mixtures is necessary. The mixture of acetone and *n*-hexane gives a good selectivity for the synthesis of lauroyl maltose diester. A high conversion of 69% (i.e. 36.5 g/L of dilauroyl maltose) was obtained under following condition: 25.65 g/L maltose, 60 g/L lauric acid, 60 g/L molecular sieve, 10 g/L lipase, and condensation for 72 h at 50 °C.

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