Lipase from *Bacillius cereus*: A potential solution to alleviate dietary oil pollution

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Abstract. Dietary oil spillage has become a widespread and increasing threat to the environment worldwide. Lipase produced by bacteria provides an efficient, environment-friendly and cost-saving way to degrade dietary oil waste. Thus, selection of highly efficient lipases becomes an urgent need to solve dietary oil-related environmental issues. In this study, with comparison analysis of different bacteria species showing oil degradation abilities, we found *Bacillus cereus* XN12 displayed higher dietary oil degradation efficiency than *Bacillus subtilis* and *Enterobacter cloacae*. *Bacillus cereus* flourishes better in oil-containing medium than non-oil medium, which indicated that *Bacillus cereus* prefers to use dietary oil as their energy source. We then successfully cloned the lipase gene from *Bacillus cereus* XN12 and expressed this *Bacillus cereus* XN12 lipase gene in *Escherichia coli* BL21. The *Escherichia coli* expressing the recombinant *Bacillus cereus* lipase protein displayed significant higher lipase activity and oil degradation ability. These results suggested that lipase from *Bacillus cereus* XN12 provides a potential solution to alleviate dietary oil pollution.

1. Introduction

Dietary oil spillage from both producers and end-users, such as vegetable oil processing facilities and restaurant waste water, has become a major concern in developing and industrialized countries. Dietary oil spillage can cause numerous ecological, environmental and municipal problems including suffocation of animals and plants coated in oil, depletion of oxygen content in water bodies resulting in death of aquatic life, clogging of drainage systems or water treatment facilities, and et al [1]. Thus, there is an urgent need to generate a high efficient, environmental-friendly and cost-saving technology to control dietary oil pollution. Some microorganism species have been discovered to inhabit at the oil-contaminated environment and have the ability to degrade oil and fats with lipases [2-3], which can be a candidate to solve the dietary oil pollution problem.

Lipases (triacylglycerol hydrolases, E.C. 3.1.1.3) are ubiquitous enzymes that act at the interface between hydrophobic lipid substrates and hydrophilic aqueous medium to catalyze the hydrolysis of ester bonds in triglyceride molecule into free fatty acids, diglycerides, monoglycerides, and glycerol [4-5]. Except for hydrolysis activity, lipases are also able to catalyze other reactions including esterification, amidation, alcoholysis, acidolysis, and aminolysis [6]. Lipases have a broad substrate

spectrum, high stability and the ability to catalyze hydrolysis reaction in various harsh conditions without requiring expensive cofactors [7]. By presenting these unique characteristics, lipases have been widely used in pharmaceutical, chemical, food and detergent industries [8-9]. Lipases are naturally produced by animals, plants and microorganisms [7]. Among these sources, lipases from microorganisms draw the most attention and are widely exploited for various applications due to higher stability, higher genetic manipulation capacity, wider availability and lower production cost than other sources [10].

In this study, we aimed to identify a bacterial lipase with high hydrolysis activity on dietary oil spillage. *Bacillus subtilis*, *Enterobacter cloacae* and *Bacillus cereus* genera, which are often found in polluted and contaminated environmental samples, have a strong innate tolerance to pollutants of various sources as well as the ability to metabolize these pollutants [11-15]. By comparing the dietary oil degradation ability of these bacterial strains, we found that *Bacillus cereus* XN12showed higher oil degradation rate on olive oil than *Enterobacter cloacae* YIN and *Bacillus cereus* SUB. *Bacillus cereus* XN12 exhibited higher growth rate in medium containing dietary oil than non-oil based medium, which indicated that dietary oil is a preferred nutrient source for *Bacillus cereus* XN12. To investigate whether the presence of dietary oil significantly shifts the metabolism state of *Bacillus cereus* XN12, we also evaluated the lipid-related metabolites profile. We then cloned *Bacillus cereus*XN12 *lipase* gene and expressed it in *Escherichia coli* cells. The *Escherichia coli* cells expressing *Bacillus cereus*lipase protein exhibited higher lipase activity and dietary oil degradation efficiency than those of control cells. These results suggested that the lipase from *Bacillus cereus* XN12 is a promising biodegradation solution in controlling dietary oil pollution.

2. Materials and methods

2.1. Plasmids, bacterial strains and culture conditions

pMAL-p5X plasmid was purchased from New England Biolabs. *Bacillus cereus* strain XN12 was isolated by our laboratory from activated sludge of Beijing Gaobeidian Sewage Treatment Plant (Beijing, China). *Enterobacter cloacae* (CMCC(B)45301) and *Bacillus subtilis* (CMCC(B)63501) were obtained from Shanghai Luwei Microbial Sci. & Tech. Co.,Ltd. (Shanghai, China). They were named *Enterobacter cloacae* YIN and *Bacillus subtilis* SUB, respectively. Competent *Escherichia Coli*DH5α and BL21 were obtained from Takara Bio. The bacteria strains were retrieved and sub-cultured in Luria–Bertani (LB) agar plates [g/L: 10.0 tryptone, 5.0 yeast extract, 10.0 sodium chloride with 15.0 agar] and incubated at 37 °C overnight. Further optimized conditions were applied to liquid culture preparations by single colony inoculation method using LB broth (g/L: 10.0 tryptone, 5.0 yeast extract, 10.0 sodium chloride, pH: 7.0) and incubated in an orbital shaker (200 rpm) overnight at 37 °C.

2.2. Bacillus cereus growth curve measurement

Bacillus cereus XN12 overnight liquid culture in LB broth was inoculated with 1% (v/v) ratio into 100 mL fresh LB broth with or without adding 0.3 mL olive oil and incubated in an orbital shaker (200 rpm) at 37 °C. 1 mL culture was retrieved every hour to measure optical density at 600 nm (OD600) for 14 hours.

2.3. Cloning lipase gene from Bacillus cereus XN12 genome

The genomic DNA of *Bacillus cereus* strain XN12 was extracted and the lipase gene was amplified by polymerase chain reaction (PCR) using the following primers with incorporated restriction sites for NdeI and EcoRI: in the forward: 5′ - ATCGAGGGAAGGATTTCACATATGCGTACTCCTTTATC CTT TGATAAA-3′ and reverse: 5′ -TATTTAATTACCTGCAGGGAATTCAAAATGAGAAGTCA GA CATGTTTT-3′ , respectively. The primers were designed using primer designing tool with *Bacillus cereus* lipase gene (GeneID: 23126559) as template. The PCR product was purified, digested with NdeI and EcoRI, then ligated into pMAL-p5X plasmid by Seamless Assembly Cloning Kit (Clonesmarter, # C5891), and transformed into *E. coli* DH5α competent cells. The transformants were

spread on LB agar plate supplemented with ampicillin (100 μg/mL). After overnight incubation at 37 °C, several single colonies were picked for initial screening of lipase insert with PCR reaction using the above lipase primers. For further confirmation, the recombinant plasmid (pMAL-p5X-lipase) was extracted and DNA sequencing was performed.

2.4. Lipase gene expression and western blot analysis

pMAL-p5X-lipase plasmid and the control plasmid pMAL-p5X were transformed respectively into competent *E. coli* BL21 cells. LB medium with ampicillin (100 μg/mL) was inoculated with 1 % (v/v) overnight culture of *E. coli* BL21 carrying the plasmids and grown aerobically at 37 ° C until the culture reached an optical density of 0.4 at 600 nm. 0.2 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) was then added to the culture, which was continued to grow with agitation at 200 rpm for 6 h at 37 ° C to induce lipase protein expression. The cells were harvested by centrifugation and washed with 50 mM Tris-buffer (pH 7.0). The cell pellet was resuspended in 10 volumes of lysis buffer (50 mMTris [pH 6.8], 0.5 M NaCl) and disrupted by sonication. The cell lysate was then centrifuged at 4 degrees for 10 minutes. The supernatant was harvested and the pellet was dissolved in 8M urea. The proteins were separated on SDS-polyacrylamide gels and transferred onto PVDF membrane (BIO-RAD, #162-0177). The blots were probed with anti-His-Tag antibody (Proteintech, #66005) and DyLight800 labeled goat anti-mouse antibody (KPL,#072-07-18-06). Signals were detected by LI-COR Infrared Imaging system (LI-COR, Inc).

2.5. Oil degradation rate measurement

Oil degradation rate was determined using the standard gravimetric method (Association et al., 1989). Briefly, overnight bacterial culture was inoculated with 1% (v/v) ratio into 100 mL LB medium containing 0.5 g olive oil (5 g/L) and cultivated at 37 °C with agitation (200 rpm). The same medium without bacteria inoculation was incubated at the same condition to serve as control. After 48 hours, sulfuric acid (1+1) was added to the medium until pH<2, followed by adding 2-3 g NaCl. The oil in the culture medium was extracted three times using 25 mL petroleum ether (mainly consisting of hexane and pentane and boiling in the range 60-90 °C) each time and transferred to a distilling flask with a specified weight. Then the petroleum ether was evaporated at 65 °C temperature, and the flask was reweighted. The difference between the two weights of the flasks is the weight of the oil in the culture medium. The difference between the oil amounts in sample medium and that in control medium is the amount of oil that was degraded by bacteria. Oil degradation rate was calculated by the ratio of degraded oil amount and the oil amount in control medium.

2.6. Oil metabolites profiling

To define the metabolites produced by *Bacillus cereus* XN12 during degrading olive oil (the weight percentage of fatty acids in olive oil are: oleic acid (C18:1), 66%; palmitic acid (C16:0), 17%; linoleic acid (C18:1), 11%; stearic acid (C18:0), 3%; palmitoleic acid (C16:1), 2%; linolenic acid (C18:3), 0.6%; and peanut acid (C20:0), 0.4%), 150 μL liquid was removed into a 15 mL centrifuge tube from the top oil layer. After adding 3 mL hexane, the samples were methylated with 2 mL 1mol/L MeOH-HCl and incubated at 55 ºC for 20 minutes. After the samples cooled down, 2 mL saturated NaCl solution was added. The samples were then vortexed for 1 minute, followed by standing still to separate the layers. 1 mL top layer liquid was removed into a fresh eppendorf tube and centrifuged for 5 min at 5939 g. The supernatant was injected into a GC3440 gas chromatograph (Beijing Beifen-Ruili Analytical Instrument (Group) Co. Ltd.) with flame ionization detector (FID). Separations were performed on a DB-WAX column (3 m × 0.320 mm internal diameter × 0.25 μm film thickness) from Thermo Scientific. Injector and detector temperature were 250 °C and 280 °C, respectively.The gas chromatograph oven was maintained at 160 °C for 5 min following injection and was then raised at 3 °C min^{-1} to 220 °C and maintained for 2 minutes. Quantitative and qualitative fatty acids analyses were performed by the external standard method based on peak areas using ChemStation software for the GC3440.

2.7. Fatty acids assessment

To access fatty acids concentration in bacterial cultures supplemented with olive oil, the culture medium was centrifuged at 344 g for 5 minutes. The supernatant was injected into a gas chromatograph with flame ionization detector (Beijing Beifen-Ruili Analytical Instrument Co., Ltd.). The conditions for the analysis were as follows: injector temperature was 250 °C and detector temperature was 150 °C. The initial gas chromatograph oven temperature was 60 °C and was raised at 10 °C min^{-1} to 130 °C, followed by being raised at 5 °C min^{-1} to 240 °C and maintained for 10 minutes.Quantitative and qualitative fatty acids analyses were performed by the external standard method based on peak areas using ChemStation software for the GC3420.

2.8. Lipase activity measurement

Overnight culture of *Escherichia coli* BL21 harboring pMAL-p5X or pMAL-p5X-lipase plasmids was inoculated with 1% (v/v) ratio to 10 mL LB medium containing 100 μg/mL ampicillin, 0.3% glucose and grown aerobically at 37 °C until the culture reached an optical density of 0.6 at 600 nm. IPTG was added to the culture to the final concentration of 0.2 mmol/L. After 2 hours, 1 mL medium was retrieved for lipase activity measurement with bacterial lipase activity quantification kit (GENMED, #GMS15020.1) according to manufacturer's protocol.

2.9. Statistics

Statistical analysis was done by student's t-test when comparing the means between two groups. When comparing the means of more than two groups, two-way ANOVA and Fisher's LSD were performed. Differences at $p < 0.05$ were considered significant.

3. Results

3.1. Bacillus cereus showed higher oil degradation rate than Enterobacter cloacae and Bacillus subtilis

To select a bacterial lipase with high hydrolysis activity on dietary oil, we first selected the bacterial strain that have higher dietary oil degradation activity. *Bacillus subtilis*, *Enterobacter cloacae* and *Bacillus cereus* genera are often found in oil polluted and contaminated environmental samples and have the ability to metabolize these pollutants [11-15]. We cultured these bacteria strains in LB medium supplemented with 5 g/L olive oil and measured oil degradation rate 48 hours later. *Bacillus cereus* strain XN12 showed significant higher oil degradation rate (71.31±3.42) than those of *Enterobacter cloacae* YIN (55.57±0.80) and *Bacillus subtilis* SUB (66.37±2.40) (figure1). Thus, we focused on *Bacillus cereus* strain XN12 to investigate its lipolytic activity.

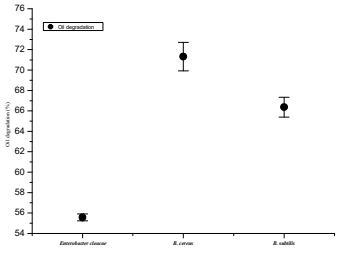

Figure 1. *Bacillus cereus* XN12 showed higher oil degradation rate than *Enterobacter cloacae* YIN and *Bacillus subtilis* SUB.

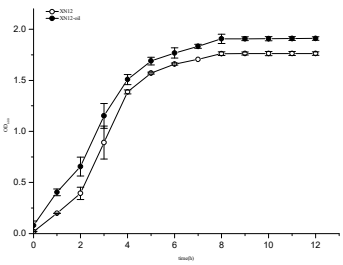

Figure 2. Olive oil boosts *Bacillus cereus* XN12 growth.

3.2. Olive oil boosts Bacillus cereus XN12 growth

We next checked whether the presence of dietary oil influenced the growth of *Bacillus cereus* XN12. Overnight *Bacillus cereus* XN12 culture was inoculated into either LB medium or LB medium

supplemented with olive oil (3 g/L). OD_{600} was recorded every hour for 14 hours. Under both conditions, *Bacillus cereus* XN12 displayed standard growth curve [16] (figure 2). As the *Bacillus cereus* XN12 cells were taken from the overnight culture, which was in stationary phase, into fresh medium, they could easily adapt to the environment and rapidly moved into log phase [17]. The *Bacillus cereus* XN12 cells cultured in LB medium containing olive oil displayed higher OD_{600} values during both log phase and stationary phase than those cultured in LB medium. These results indicated that dietary oil is able to boost *Bacillus cereus* XN12 growth and suggested that dietary oil is a preferred nutrient source for *Bacillus cereus* XN12. Similar conclusions were reached by other laboratories. Stathopoulou, P. M. et al. indicated that olive oil was able to induce significant lipase production levels and proved to be the optimal carbon source for strain SP75, which was isolated from the volcanic area of Santorini, Aegean Sea, Greece [18].

3.3. Metabolites profiling of Bacillus cereus XN12 in oil-containing medium

Lipid metabolism is initiated with hydrolysis of triglycerides into glycerol and fatty acids by lipase [19-20]. Glycerol can readily be converted into dihydroxyacetone phosphate, which is an intermediate of glycolysis[21]. Fatty acids are oxidized through beta-oxidation, which removes two carbons at a time as acetyl-CoA in a spiral type reaction. The generated Acetyl-CoA is condensed with oxaloacetate to form citric acid, which is ready to feed in the tricarboxylic acid cycle to generate energy [2]. To investigate whether the presence of dietary oil significantly shifts the metabolism state of *Bacillus cereus* XN12, we evaluated the lipid-related metabolites profile of the culture supernatant after 48 hours' cultivation in 2 g/L and 4 g/L olive oil-supplemented LB medium.

In the culture medium without olive oil supplement, the culture supernatant contained acetic acid (2.61±0.39 g/L), propionic acid (0.19±0.03 g/L) and succinic acid (2.15±0.12 g/L), but no glycerol (Table 1). The *Bacillus cereus* culture supernatant with 2 g/L and 4 g/L olive oil displayed quite similar metabolites profiles, although higher oil degradation rate was observed in the culture with 2 g/L (84.70±0.87) olive oil than that with 4 g/L (76.90±2.38) olive oil (figure 3 and Table 1).

When compared to the culture supernatant without adding olive oil, the culture supernatant containing olive oil displayed similar acetic acid levels, higher propionic acid levels, lower succinic acid levels and the appearance of glycerol (Table 1). These results suggested a shift of metabolic state in *Bacillus cereus* XN12 cells when they were adapting to the environment containing dietary oil.

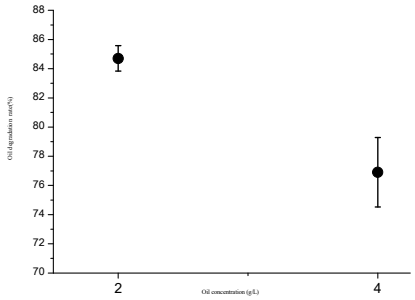

Figure 3. Higher initial oil concentration lowered oil degradation rate by *Bacillus cereus* XN12.

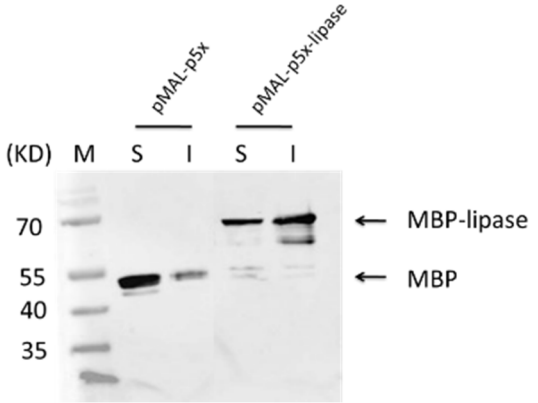

Figure 4. Expression of recombinant Bacillus cereus XN12 lipase protein in *Escherichia coli* BL21.

Table 1.Metabolites profile and oil of *Bacillus cereus*[a].

Olive oil-concentration	Metabolites profile(g/L)	Oil degradation rate(%)

in LB medium (g/L)	Acetic acid	Propionic acid	Succinic acid	Glycerol	
0	2.61±0.39	0.19±0.03	2.15±0.12	0	/
2	3.31±0.75	0.38±0.05	1.55±0.27	0.57±0.12	84.70±1.95
4	2.76±0.61	0.42 ±0.08	1.58±0.17	1.08±0.13	76.90±5.31

[a]Note: Values of metabolites profile are means ± SEM. n=4.

3.4. Cloning and expression of Bacillus cereus lipase gene

Because *Bacillus cereus* XN12 displayed efficient dietary oil degradation ability, we hypothesized that the lipase produced by this strain has high lipolytic activity. We then cloned the lipase gene from *Bacillus cereus* XN12 genome. Sequencing results revealed that the coding sequence (CDS) for *Bacillus cereus* XN12 lipase gene is 720 bp long, with 39.3% GC content and shares 100% similarity with the lipase gene from *Bacillus cereus* NC7401 (AP007209). Next the *Bacillus cereus* XN12 lipase clone was inserted into pMAL-p5x plasmid to generate pMAL-p5x-lip, which will produce a maltose-binding protein (MBP)-lipase fusion protein. pMAL-p5x-lip and the control pMAL-p5x plasmids were transformed into *Escherichia coli* BL21 for recombinant protein expression.

The molecular weight for MBP protein is 42.5 KD. The deduced molecular weight for *Bacillus cereus* XN12 lipase is 26.5 KD as it contains 240 amino acids. Thus, the MBP-lipase fusion protein was expected to be 68 KD. Western blot analysis showed that the MBP-lipase fusion protein was successfully overexpressed in *Escherichia coli* BL21cells after IPTG induction with quite a lot in the soluble fraction, when compared that without IPTG induction or transformed with control plasmid (figure 4).

3.5.Recombinant lipase displayed remarkable lipolytic activity

To determine whether the recombinant lipase is functional, we measured the lipase activity and the long chain fatty acids degradation rate of *Escherichia coli* BL21 harboring pMAL-p5x-lip. The *Escherichia coli* BL21 cells overexpressing recombinant MBP-lipase fusion protein (+IPTG) displayed significant higher lipase activity (101.72 ± 21.02 μmol DMPTB/min) than those without IPTG induction (37.04±26.86 μmol DMPTB/min) (Table 2).

Table 2. Lipase activity[a].

Strain	Lipase activity (μmolDMPTB/min)
***E.Coli* BL21 harboring pMAL-p5x-lip with IPTG**	101.72 ± 21.02
***E.Coli* BL21 harboring pMAL-p5x-lip without IPTG**	37.04 ±26.86
***E.Coli* BL21 harboring pMAL-p5x with IPTG**	19.42

[a]Values of recombinant strain with or without IPTG are means ± SEM. n=8. *p < 0.05, t-test.

Consistently, *Escherichia coli* BL21 expressing recombinant MBP-lipase fusion protein showed higher oleic acids degradation rate as well (89.97% vs. 87.24%) (Table 3). Because lipase can also hydrolyze fatty acids in oils and fats [22], we used oleic acid, the major fatty acid component of olive oil, as the substrate to measure the hydrolytic activity of the recombinant lipase protein. These results indicated that the recombinant lipase displayed remarkable lipolytic activity.

Table 3. Oleic acids degradation rate of recombinant strain.

Strain	Oleic acids concentration (g/L)	Oleic acids degradation rate (%)
***E.Coli* BL21 harboring pMAL-p5x-lip**	3.77	89.97
***E.Coli* BL21**	4.79	87.24

4. Discussion

In the present study, we found that *Bacillus cereus* XN12 outperformed *Bacillus subtilis* SUB and *Enterobacter cloacae* YIN on degrading dietary oil. The presence of olive oil not only boosted the growth but also shifted the metabolic status of *Bacillus cereus* XN12. The lipase gene we cloned from *Bacillus cereus* XN12 highly aligned perfectly with various previously published *Bacillus cereus* lipase genes. We successfully overexpressed recombinant *Bacillus cereus* XN12 lipase protein in *Escherichia coli* and found the recombinant lipase protein displayed significant lipolytic activity. These results suggested that the recombinant *Bacillus cereus* XN12 lipase treatment is a promising solution for dietary oil spillage.

Lipases of microbial sources (yeast, fungi and bacteria) have been exploited for a long time and have been used in a wide range of commercial applications. The most important genera of yeasts that have been investigated for their production of lipases are *Candida* and *Yarrowia*. *Candida antarctica* lipase B (CALB) is the most frequently used enzyme in numerous biocatalytic processes due to its specificity, high enantio-selectivity against secondary alcohols and primary amines, and extraordinary stability in organic solvents and at extreme temperatures. Another example is *Candida rugosa* lipase (CRL), which is commercially available as a mixture of different isoforms, it is widely used in the food industry [3]. Among the lipases from fungi, Rhizopus oryzae lipase (ROL), *Rhizomucor miehei* lipase (RML), *Thermomyces lanuginosus* lipase (TLL) and *Fusarium heterosporum* lipase (FHL) are the ones that are typically used in biodiesel production [23]. Lipases from *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium roqueforti*, *Penicillium camemberti*, *Penicillium cyclopium* and *Rhizomucor javanicus*, together with ROL and RML, are used to generate fatty acids for food processing and nutraceutical synthesis [24]. For bacteria, lipase from *Pseudomonas*, *Bacillus* and *Streptomyces*, *Burkholderia*, *Chromobacterium*, *Achromobacter*, *Alcaligenes* and *Arthrobacter* have been used in industrial processes including biodiesel production, oil degumming, synthesis of nutraceuticals, and in detergent formulations. For example, lipases from *Pseudomonas fluorescen*, *Burkholderia cepacia* and *Bacillus thermocatenulatus* are used in biodiesel production [20], while lipases from *Pseudomonas mendocina*, *Pseudomonas alcaligenes*, *Pseudomonas glumae* and *B. cepacia* are used as detergent additives [7].

For microbial lipases used in industrial applications, the production of these enzymes from their native host has several drawbacks. First, a complicated culture system is necessary to reach high yields of enzyme production. Because the microorganism has to be grown in the presence of lipids as the carbon source, and as most of the microorganisms that produce lipases are aerobic, the culture medium needs to include lipids, biomass, water, organic solvent, and air [25]. Furthermore, microorganisms, especially yeasts and fungi, often produce a mixture of different lipase isoforms with different catalytic behaviors. To purify a single isoform from a mixture of isoforms with high amino-acid similarity is expensive and time-consuming, because of which, commercial preparations often are mixtures of isoforms, which might lead to irreproducible results and undesirable side effects. Nevertheless, these drawbacks can be overcome by switching to heterologous expression systems. There are tremendous advantages in producing recombinant lipase with heterologous expression system. First of all, with the availability of highly efficient expression systems, the enzyme yields and reproducibility can be greatly increased. Moreover, some enzymes' natural producers are microorganisms that cannot be easily grown by traditional methods, while heterologous expression can remove this obstacle and allow the production of these enzymes. Another huge benefit in using heterologous expression is its ability to produce not only natural but also engineered enzymes. Through the manipulation of the gene encoding a specific enzyme, the feathers of that enzyme can be improved to coordinate with various requirements [26]. Our study was the first approach to express a recombinant Bacillus cereus lipase in *Escherichia coli* and will shed light on improving the strategy for solving dietary oil spillage problem.

Dietary oil spillage, which mainly comes from improper restaurant and household waste water disposal, contains various lipids species. As each lipase has its own substrate specificity [3], to thoroughly degrade dietary oil wastes, a mixture of lipase with different substrate preferences may be

necessary. Several bacterial strains have been reported to have efficient dietary oil degradation capacity. For *Raoultella planticola* strain 232-2, the 24-h degradation rate for 3,000 ppm commercial vegetable oil, lard, beef tallow, mixed lipids, and oleic acid was 71.8 %, 58.7 %, 56.1 %, 55.3 ± 8.5 %, and 91.9 % at pH 4 and 30 °C, respectively [27]. For *Acinetobacter* sp. strain SS-192, the degradation rate of 3,000 ppm of salad oil, lard, and beef tallow was 79.9 ± 2.6%, 63.6 ± 1.9%, and 70.1 ± 1.2%, respectively, during a 24-h cultivation at pH 8.0-9.0 and 37 °C [28]. The degradation rate of 3,000 ppm of salad oil, lard, and beef tallow by *Pseudomonas aeruginosa* strain SS-219 was 82.3 ± 2.1%, 71.9 ± 2.2%, and 71.0 ± 1.1%, respectively, during a 24-h cultivation at pH 8.0-9.0 and 37 °C [23]. Lipases from these strains need to be characterized and can be suitable candidates to use with *Bacillus cereus* XN12 lipase together to get higher degradation rate on dietary oil.

Bacillus subtilis have been extensively studied for biosurfactant production. Biosurfactants are chemically active surface agents produced by various groups of microorganisms that utilize substrates like simple sugars, oils, hydrocarbons from contaminated environment [29-30]. Biosurfactants have the ability to reduce surface and interface tension between two liquids or a liquid and a solid and resulting in diffusing them as emulsions in liquids [31]. Therefore, biosurfactants are widely used in various applications in petroleum, food, cosmetic and pharmaceutical industries. More importantly, as environmental compatability becomes an increasing concern, the use of biosurfactants in environmental applications, such as bioremediation and dispersion of oil spillage, greatly increases [32-33]. Because *Bacillus cereus* displayed higher dietary oil degration ability than *Bacillus subtilis*, it will be interesting to investigate whether *Bacillus cereus* XN12 outperforms *Bacillus subtilis* in biosurfactants production.

Acknowledgments
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