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Isolation and Optimisation of Polylactic Acid (PLA)-packaging-degrading Actinomycete for PLA-packaging Degradation

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ABSTRACT

Nowadays polylactic acid (PLA) is used extensively with respect to environmental concern and good practice in solid-waste management, as food packaging. However, the study on biodegradation of PLA-packaging is very limited. In this work, isolation, identification and optimisation of biomass production of PLA-packaging-degrading actinomycete were carried out. The isolate KKU215 gave the highest cell density in basal medium containing 1.0 g/L PLA-packaging as sole carbon source after 4-week incubation. The weight loss of PLA-packaging degraded by the isolate was 2.65%. The results from scanning electron microscopy analysis indicated that the isolate KKU215 could clearly degrade PLA-packaging. The degradation of pure PLA by KKU215 as clear zone formation on emulsified PLA agar plate was observed. The 16S rDNA gene sequence of the isolate KKU215 was related to the genus *Streptomyces*. Thus, the isolate KKU215 was assigned as *Streptomyces* sp. KKU215. Concentration of yeast extract, initial pH value, temperature and agitation speed for PLA-packaging degradation were optimised by response surface methodology with Box-Behnken design. The highest growth of *Streptomyces* sp. KKU215 was found in basal medium with 3.26% yeast extract, initial pH value of 7.69, and agitated at 149 rpm at 33.7°C. Streptomyces sp. KKU215 when cultured in the optimal conditions could enhance PLA-packaging weight loss from 2.65% to 84.04%, which was 81.39% higher than the unoptimised conditions. Up to date, there is no report of *Streptomyces* species, similar with *Streptomyces* sp. KKU215 capable of degrading PLA. Therefore, *Streptomyces* sp. KKU215, a

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novel polylactic acid-packaging-degrading actinomycete is a promising strain for use in biodegradation of PLA-packaging.

Keywords: Biodegradation, bioplastic, Box-Behnken design, optimisation, polylactic acid (PLA)-packaging, response surface methodology (RSM), *Streptomyces* sp. KKU215

INTRODUCTION

Synthetic plastics are considered as non-degradable materials that generate pollution. The pollution from synthetic plastics derived from petroleum has reached 300 million tons (Thompson et al., 2009) and continues to grow therefore posing a global environmental problem. Biodegradable plastics were used as substitutes for synthetic plastics in the reduction of pollutants or waste. Numerous research are concentrated on the development of novel bioplastics that are susceptible to degradation in the environment. Bioplastics are biodegradable polymers derived from renewable resources such as alginic acid, cellulose and cellulose acetate, chitin and chitosan, gellan gum, hyaluronic acid, laminarin and curdlan, polyacrylates, polyamides, poly(amide-enamine)s, polyanhydrides, polycaprolactone (PCL), polyglycolic acid (PGA), polyhydroxyalkanoates (PHA), polyhydroxybutyrate (PHB), polylactic acid (PLA), polyurethanes and polyureas, poly(vinyl alcohol) and poly(vinyl acetate) (PVA), pullulan, soy-based plastics, starch and xanthan (Briassoulis, 2004; Flieger et al., 2003).

Polylactic acid (PLA) is a kind of biodegradable plastic which has a wide range of applications, especially food packaging such as cup, bag, bottle, film and wrap. PLA was synthesised by Théophile-Jules Pelouze by the condensation of lactic acid as early as 1845 (Auras et al., 2010), and was developed as biodegradable plastics. PLA is a linear aliphatic polyester consisting of polymerised lactic acid monomers linked by ester bonds. It is synthesised from renewable resources such as corn, cassava, sugar cane, rice and potato through lactic acid fermentation. Biocompatible, compostable, easy-to-fabricate, high mechanical strength and not toxic or carcinogenic substance are favourable properties of PLA.

The use of PLA has received much attention from the viewpoint of environmental protection and solid-waste management. PLA biodegradation is one of the important developments related to those interests. The mechanisms of PLA degradation include photodegradation (Janorkar et al., 2007), hydrolytic degradation (Elsawy et al., 2017) and microbial degradation (Hanphakphoom et al., 2014).

Many studies in microbial degradation since 1997 have isolated pure PLA (pellet, film and granules) degrading actinomycetes. Pranamuda et al. (1997) found microbial degradation of PLA by *Amycolatopsis* sp. HT-32. Since then, PLA degrading actinomycete was found by several researchers such as *Amycolatopsis* sp. KT-s-9 (Tokiwa et al., 1999), *Amycolatopsis* sp. 3118 (Ikura & Kudo, 1999),

Amycolatopsis sp. K104-1 (Nakamura et al., 2001), *Amycolatopsis* sp. 41 (Pranamuda et al., 2001), *Amycolatopsis*, *Lentzea*, *Kibdelosporangium*, *Streptoalloteichus*, *Saccharothrix* (Jarerat et al., 2002), *Saccharothrix waywayandensis* (Jarerat & Tokiwa, 2003), *Kibdelosporangium aridum* (Jarerat et al., 2003), *Amycolatopsis orientalis* (Jarerat et al., 2006), *Actinomadura* sp. T16-1 (Sukkhum et al., 2009), *Amycolatopsis thailandensis* sp. nov. (Chomchoei et al., 2011), *Pseudonocardia alni* AS4.1531(T) (Konkit et al., 2012) and *Laceyella sacchari* LP175 (Hanphakphoom et al., 2014). However, most studies in the microbial degradation of PLA have focused on pure PLA. A study found that polylactide foil was degraded by *Aspergillus ustus* and *Penicillium verrucosum* (Szumigaj et al., 2008). It shows that the study of PLA-packaging degradation is insufficient.

The Response Surface Methodology (RSM) with Box-Behnken Design is a collection of mathematical and statistical techniques for optimisation of various culture conditions both physical factors (such as agitation rate, pH values and temperature) and culture medium (such as carbon sources, nitrogen sources, mineral elements and growth factors). This method has been successfully applied to enhance the degradation of PLA (Chaisu et al., 2012).

This research, therefore, aimed to isolate, identify and optimise culture conditions of PLA-packaging-degrading actinomycete for bioplastic waste management purposes and the development of the bioplastic industry applications in the future.

MATERIALS AND METHODS

Isolation and Screening of the PLA-packaging-degrading Actinomycete

PLA-packaging was obtained from Dairy Home Co., Ltd, in Nakhon Ratchasima province, Thailand. Prior to use, PLA-packaging was cut (about 1×1 cm) and surface sterilised with 70% (v/v) ethanol and allowed to air dry.

Microbial source samples were collected from liquid biofertilisers, botanical garden soils, cattle pen soils, composts and rubbish dump soils to isolate the PLA-packaging-degrading actinomycete. The samples were collected in pre-sterilised sample bottle following aseptic conditions. The labeled samples were stored at 4°C for further analysis.

The Basal Medium (BM) had the following composition: (per litre of distilled water) 2 g K_2HPO_4; 2 g KH_2PO_4; 0.5 g $MgSO_4.7H_2O$; and 4 g $(NH_4)_2SO_4$, per 1 L of distilled water (pH value 7.0) (Tomita et al., 1999). The media was autoclaved at 121°C and 15 lbs. pressure. The BM was supplemented with PLA-packaging (1.0 g/L) as sole carbon source.

The sample (10 g) was suspended in 100 mL of BM for 10 min and then settled for 30 min. The supernatant (10 mL) was transferred into 100 mL of BM with 0.1 g of PLA-packaging followed by incubation at 37°C, 180 rpm for 7 days in a rotary shaker. The subcultures were made five times by inoculating 10 mL of the original culture into fresh BM containing PLA-packaging as a carbon source. After five sub-culturing stages, each PLA-packaging was placed on

to BM Agar plates for 7 days of incubation at 37°C (Jeon & Kim, 2013). After incubation, colonies that formed around them were purified by re-streaking on Nutrient Agar (NA) contained (g/L): 3 g beef extract; 5 g peptone and 15 g agar per l L of distilled water. Pure isolates were maintained on NA slants at 4C, until used.

PLA-packaging-degrading actinomycete was screened by evaluating the growth in BM with PLA-packaging as a carbon source using the optical density (OD) at wavelength 600 nm (Lee et al., 2013). Spore suspension of the isolates was prepared by streaking each isolate on NA plate and then cultivated for 10 days at 37°C. Spores were harvested by adding sterilised BM to each plate and scrapping the spores using sterilised blade. Spore concentration was adjusted to 1 x 10^7 spores/mL using haemacytometer. Spore suspension (1 mL) of each isolate was inoculated into 100 mL of BM with 0.1 g of PLA-packaging and then incubated at 37°C for 4 weeks under shaking at 180 rpm. BM, BM with PLA-packaging and BM with the cell were used as controls. All treatments were done in triplicate.

Degradation of PLA-packaging by the Isolated Strain

The degradation of PLA-packaging by PLA-packaging-degrading actinomycete was analysed by measuring weight loss and surface changes of the individual samples after 4 weeks of incubation in BM with PLA-packaging as sole carbon source.

PLA-packaging of each treatment was recovered, washed with distilled water 3 times to remove loosely adhered cell and dried in an electronic desiccator (Boekel Dricycler 1344412, Boekel Scientific, Feasterville, Pennsylvania, USA) to ensure a constant weight. Weight loss of the PLA-packaging was calculated according to Equation [1] (Vey et al., 2007).

$$\% \text{ Weight loss } = \frac{m_{ini} - m_{dry}}{m_{ini}} \times 100\%$$

Therein m_{ini} is the initial weight of the PLA-packaging before degrading and m_{dry} is the dry weight of the PLA-packaging after degrading. The surface changes of PLA-packaging was examined using a Desktop Scanning Electron Microscopes (MiniSEM) (SEC, model SNE-4500M). Prior to the analysis, the sample was dried overnight and coated with gold to protect the sample morphology against electron beam.

PLA Degradation of the PLA-packaging-degrading Actinomycete

PLA-packaging-degrading actinomycete was studied for the ability to degrade PLA by the clear zone formation around the colony on emulsified PLA agar plate. Emulsified PLA agar was prepared as follows: 0.5 g of PLA pellet (2003D grade, average molecular weight 200,000 g/mol, melting temperature 210°C, purchased from NatureWorks LLC, U.S.A.) was dissolved in 25 mL of dichloromethane and 25 µL of tween 20, and sonicated using an ultrasonicator (VC 505-VC 750; Sonics &

Materials Inc., Newtown, USA) with 50 % power for 5 min in 200 mL of sterilised distilled water. Emulsified PLA solution was mixed with melted BM (added 1 g of yeast extract per 1 L of BM) before pour plate. PLA degradability was investigated after 2 weeks of incubation at 37°C (modified from Penkhrue et al., 2015).

Identification of PLA-packaging-degrading Strain

Macroscopic colony morphology of form, colour, surface, edge, opacity and elevation was observed. The shape, endospore-forming and gram staining characteristic were observed by microscope after gram staining (Wang et al., 2013). The pH values (1 to 10), temperatures (20, 25, 30, 35, 40, 45, 50, 55 and 60°C) and salt concentrations (0 to 14 % NaCl (w/v)) were determined for the range of PLA-packaging-degrading actinomycete growth (Chen et al., 2015).

Genomic DNA of PLA degrading strains was isolated using "Genomic mini kit" (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instruction. The 16S rDNA gene was amplified by PCR using the universal two primers, 20F and 1500R (Brosius et al., 1981) with DNA Engine Dyad® Thermal Cycler (Bio-Rad Laboratories). The PCR products were analysed by 0.8% (w/v) agarose gel electrophoresis and purified with a GenepHlow™ Gel/PCR Kit (Geneaid Biotech Ltd., Taiwan). Direct sequencing of purified PCR products was carried out on an ABI Prism® 3730 XL DNA Sequence (Applied Biosystem, Foster City, California, USA). Single and double strand 16S rDNA sequencing used 2 (27F or 800R and 518F or 1492R) and 4 (27F, 518F, 800R and 1492R) primers, respectively. The sequences were aligned with EzTaxon-e server (http://eztaxon-e-ezbiocloud.net/; Kim & Park, 2010) together with those of genera possessing sequences similar to those of the isolated strain using CLUSTAL X (version 1.8) (Thompson et al., 1997) in BioEdit program (Hall, 1999). The phylogenetic tree was inferred with the neighbor-joining method of Saitou and Nei (1987) using MEGA version 6.0 (Tamura et al., 2013).

Optimisation of Biomass Production for PLA-packaging Degradation using Response Surface Methodology

Enhancement of the degradation of PLA-packaging by optimisation of *Streptomyces* sp. KKU215 growth conditions was determined. The optimal conditions of the four factors including concentration of yeast extract, initial pH value, temperature and agitation speed for PLA-packaging degradation were investigated by response surface methodology (RSM) with Box-Behnken design using Design-Expert® Software Version 10 (trial version). The ranges of each independent variable are as follows: concentration of yeast extract (1.3-5.3 %), initial pH value (6.0-10.0), temperature (25-45°C) and agitation speed (100-200 rpm), and are shown in Table 1. The cell dry weight was measured after 2 days of cultivation.

Table 1
Experimental code and levels of factors in Box-Behnken design

Independent variables	Code	Levels		
		-1	0	1
Concentration of yeast extract (%)	X_1	1.3	3.3	5.3
Initial pH value	X_2	6.0	8.0	10.0
Temperature (°C)	X_3	25	35	45
Agitation rate (rpm)	X_4	100	150	200

The optimisation results were confirmed by cell dry weight after 2 days of cultivation and degradation of PLA-packaging after 4 weeks of cultivation under the optimal conditions based on the results of RSM.

RESULTS AND DISCUSSION

Isolation and Screening of the PLA-packaging-degrading Actinomycete

Isolation of PLA-packaging-degrading actinomycete among liquid biofertiliser and soil from botanical garden, cattle pen, compost and rubbish dump samples found that only one actinomycete strain from botanical garden soil no. KKU215 exhibited increasing OD in BM supplemented with PLA-packaging as sole carbon source. The increasing OD of KKU215 in the culture medium after 4 weeks as opposed to no increase in OD of the controls (BM, BM with PLA-packaging and BM with KKU215) (Figure 1) demonstrated that KKU215 was able to use PLA as their carbon and energy source.

The growth of tested actinomycete in BM with PLA-packaging as sole carbon source by using the OD at wavelength 600 nm was assessed for their ability to degrade PLA-packaging. The increasing OD of the PBS-2 strain in poly(butylene succinate)-

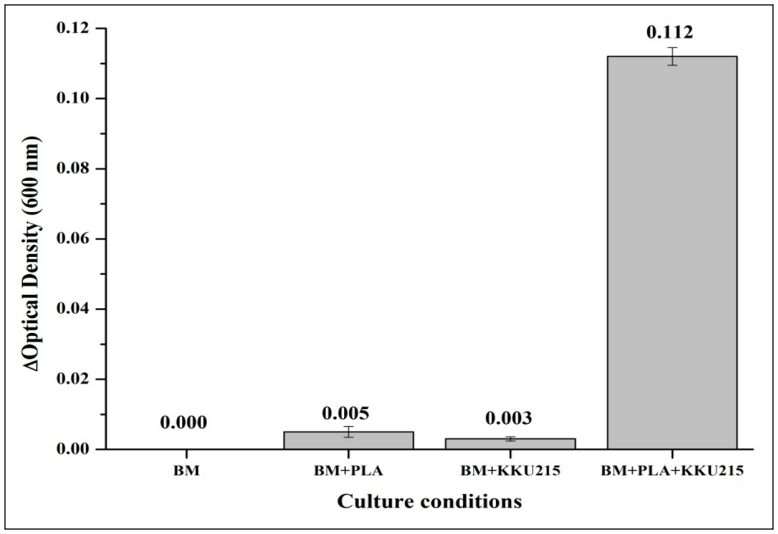


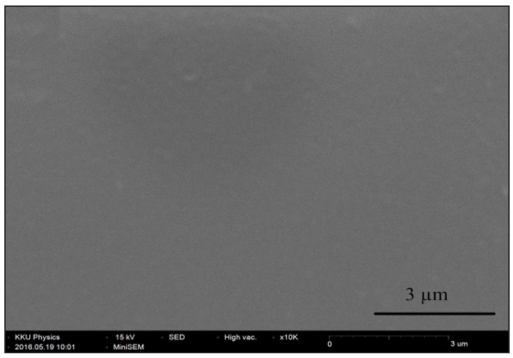
Figure 1. ΔOptical density of PLA-packaging-degrading strain KKU215 in various conditions for 4 weeks at 37°C

tryptone basal liquid medium indicated that PBS-2 could degrade poly(butylene succinate) as carbon source (Lee et al., 2013).

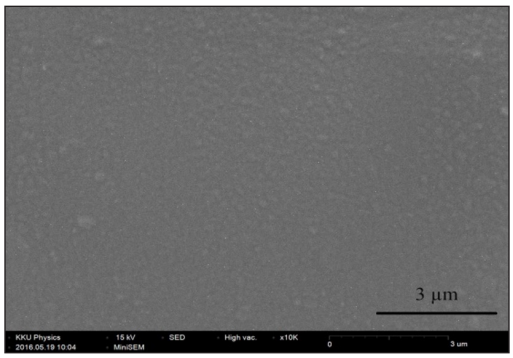
Degradation of PLA-packaging by the Isolated Strain

The percentage of reduction in weight of PLA-packaging by KKU215 was 2.65%, while the weight of the PLA-packaging did not change during incubation in BM without inoculation for 4 weeks. These implied that KKU215 had ability to degrade the PLA-packaging. Although, *Pseudonocardia alni* AS4.1531(T) could degrade PLA film with weight loss of 71.5% within 8 days (Konkit et al., 2012) while weight loss of PLA-packaging in BM by KKU215 was rather lower. This probably was caused by the differences of culture medium which had impact on the growth and all ability. In case of *Pseudonocardia alni* AS4.1531(T), this strain was grown in BM supplemented with 1.0 g yeast extract and 1.0 g gelatin per litre while the KKU215 was grown without any supplementation. Yeast extract had an influence on the growth of *Streptomyces* and their products (Zhou et al., 2017) because yeast extract increases *Streptomyces* mycelium biosynthesis (Suutari et al., 2002). In addition, gelatin could induce PLA degrading activity of actinomycetes (Jarerat et al., 2003; Jarerat & Tokiwa, 2003; Konkit et al., 2012).

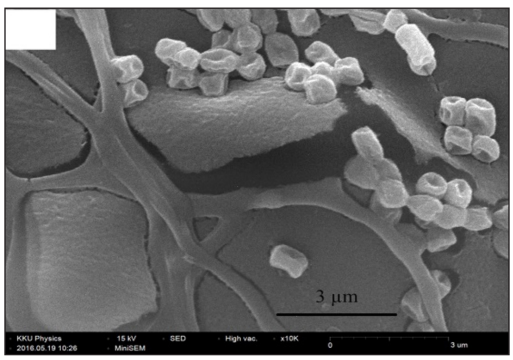
To elucidate the degradation of PLA-packaging induced by KKU215 more clearly, it was important to consider the change of morphology during the degradation. The SEM images of the degraded PLA-packaging were shown in Figures 2a-c. For the inoculated samples, Figure 2c showed a porous structure on the surface and the filaments of KKU215



(a)



(b)



(c)

Figure 2. Scanning electron micrographs of original PLA-packaging surface: (a) after incubation without inoculation of the strain, (b) and after degradation by KKU215, (c) after 4 weeks

able to penetrate the surface of the PLA-packaging. In contrast, the PLA-packaging degraded in control medium (without inoculation of the strain) remained intact (Figure 2b), and very similar to the original PLA-packaging surface (Figure 2a). The penetration of KKU215 filament into PLA-packaging showed the capability to degrade PLA-packaging. Similar to Szumigaj et al. (2008) who investigated the filamentous fungi degradation of commercial poly(lactic acid) foil provided by the Chair of the Food Packaging and Biopolymers Department of the University of Agriculture in Szczecin, Poland. They found that *Aspergillus ustus* and *Penicillium verrucosum* could degrade the PLA food packaging, in which structural changes were observed under the SEM microscope.

The penetration of KKU215 into PLA-packaging and weight loss of PLA-packaging by KKU215 indicated that KKU215 was a PLA-packaging-degrading actinomycete.

PLA Degradation of the PLA-packaging-degrading Actinomycete

The pure PLA degrading ability of KKU215, which is PLA-packaging-degrading actinomycete, was confirmed by the standard clear zone method. KKU215 showed the ability to form clear zone around its colony on emulsified PLA agar plate after 2 weeks of incubation at 37°C (Figure 3). The standard clear zone method was generally used for isolation of biopolymer degrading microorganism including PLA (Hanphakphoom et al., 2014; Penkhrue et al., 2015). The microorganism that could degrade suspended biopolymer in emulsified agar medium would produce clear zone around the colony.

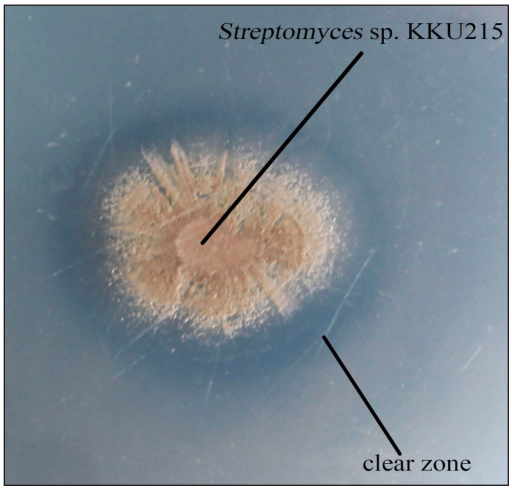


Figure 3. Clear zone formation around the colony of KKU215 on emulsified PLA agar plate for 2 weeks

Identification of PLA-packaging-degrading Strain

KKU215 is a gram-positive, aerobic, filamentous, light white substrate mycelium, grey aerial mycelium, smooth long chain spore. The colony of KKU215 is circular, spore formed in the aerial mycelium and water droplets on colonies. Growth occurs in NA medium at NaCl concentrations, pH values and temperatures at 1 to 6 % (w/v), 6 to 10 and 25 to 45°C, respectively. The phylogenetic relationships of KKU215 were studied based on 16S rDNA gene sequences. The Gene-Bank database was used to search for 16S rDNA sequences similarity to the 16S rDNA sequences of isolates. The results showed that similarity sequencing of KKU215 related to the genus *Streptomyces* and we named it *Streptomyces*

sp. KKU215 (Figure 4). The 16S rDNA sequence of *Streptomyces* sp. KKU215 showed similarity with *Streptomyces tendae* (99.31%), *Streptomyces violaceorubidus* (99.31%), *Streptomyces tritolerans* (99.24%), *Streptomyces malachitofuscus* (99.24%), *Streptomyces griseoincarnatus* (99.10%), *Streptomyces variabilis* (99.10%), *Streptomyces erythrogriseus* (99.10%), *Streptomyces rubrogriseus* (99.10%), *Streptomyces griseorubens* (99.10%), *Streptomyces flavoviridis* (99.10%), *Streptomyces labedae* (99.09%), *Streptomyces pilosus* (99.09%) and *Streptomyces lienomycini* (99.02%). KKU215 formed a cluster within the genus *Streptomyces* and similarity more than 99% with numerous *Streptomyces*. Nevertheless, these *Streptomyces* species had not been reported on PLA degradation yet. Therefore,

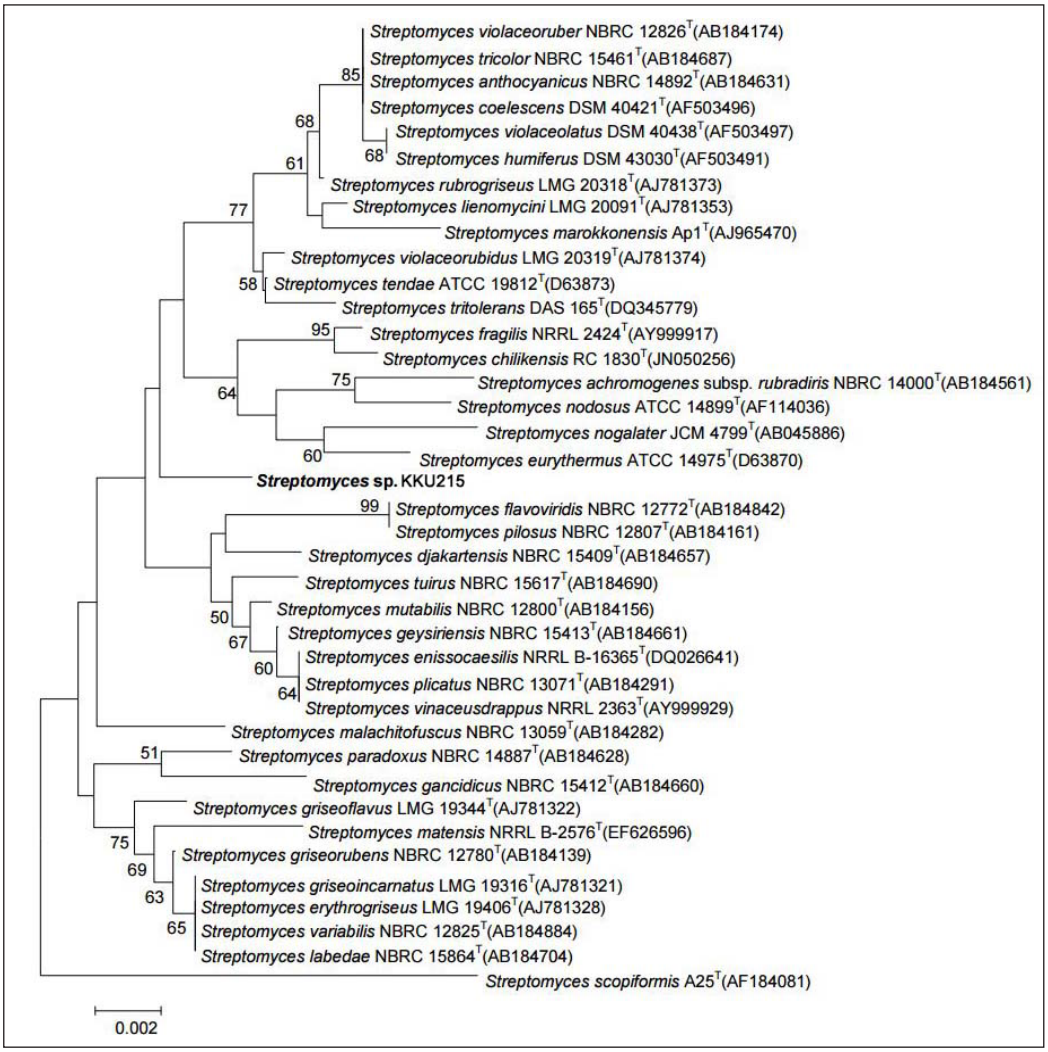


Figure 4. Phylogenetic tree based on 16S rDNA gene sequences of KKU215 and related species of the genus *Streptomyces*. Numbers at nodes are percentage bootstrap values based on 1000 replicates

Streptomyces sp. KKU215 is a novel strain for PLA degradation.

Optimisation of Biomass Production for PLA-packaging Degradation using Response Surface Methodology

A Box-Behnken design under RSM was used to analyse the main and interactive effect of concentration of yeast extract, initial pH value, temperature and agitation speed to reach the optimum level in relation to dry weight. Twenty-eight experiments and its responses are shown in Supplementary Table 1.

The experimental results gained from Box-Behnken design were fitted to a second order polynomial model to explain the dependence of dry weight on the four factors. The regression equation is shown as follow:

$$Y = - 2892.57853 + (84.41313X_1) + (338.44792X_2) + (66.18875X_3) + (7.32177X_4) + (0.5625X_1X_2) + (0.1125X_1X_3) + (0.07825X_1X_4) + (0.6125X_2X_3) - (0.03475X_2X_4) - (0.0052X_3X_4) - (16.09479X_1^2) - (23.13229X_2^2) - (1.04217X_3^2) - (0.023917X_4^2) \quad [2]$$

where Y is the predicted dry weight, and X_1, X_2, X_3 and X_4 are the coded variables of concentration of yeast extract, initial pH value, temperature and agitation speed, respectively.

The adequacy of the model was checked through analysis of variance (ANOVA). The results of the second order response surface model fitting in the form of ANOVA are given in Supplementary Table 2. The model was found highly significant having Fischer test value of 25.67 and *P*-value of <0.0001. The coefficient of determination R^2 showed the appropriateness of the adequate model. The coefficient of determination $R^2 = 0.9651$ or 96.51% indicated that about 3.49% variations were not determined by the model. The adjusted determination coefficient $R^2 = 0.9275$ or 92.75% also showed that the model was highly significant. Consequently, the regression model was believed to accurately and reliably predict and analyse the dry weight of *Streptomyces* sp. KKU215.

The linear effect of pH (p-value = 0.0002) and temperature (p-value = 0.0007) was highly significant. The quadratic effect of all factors showed great impact on the dry weight according to the significance of corresponding p-values (<0.0001), indicating that the change of the response value (dry weight) was complex. The effect of the factors on the dry weight of *Streptomyces* sp. KKU215 was not a simple linear relationship but a significant surface effect (Yun et al., 2018). The interaction effect of all four factors was not significant, indicating that there are no interactions between variables.

Regression models generated the three-dimensional response surface plots to determine the impact of independent variables and interaction effect of each variable , and discover the optimal level of each variable for maximal response (Figures 5 and 6). Each of 3D response surface graphs and contour plots was generated using two test variables at one time while keeping the other two variables at their centre value. The convex response surfaces suggest that there

are well-defined optimal variables. Every response surface plot clearly indicated that the optimal condition should occur with medium levels of concentration of yeast extract, initial pH value, temperature and agitation speed.

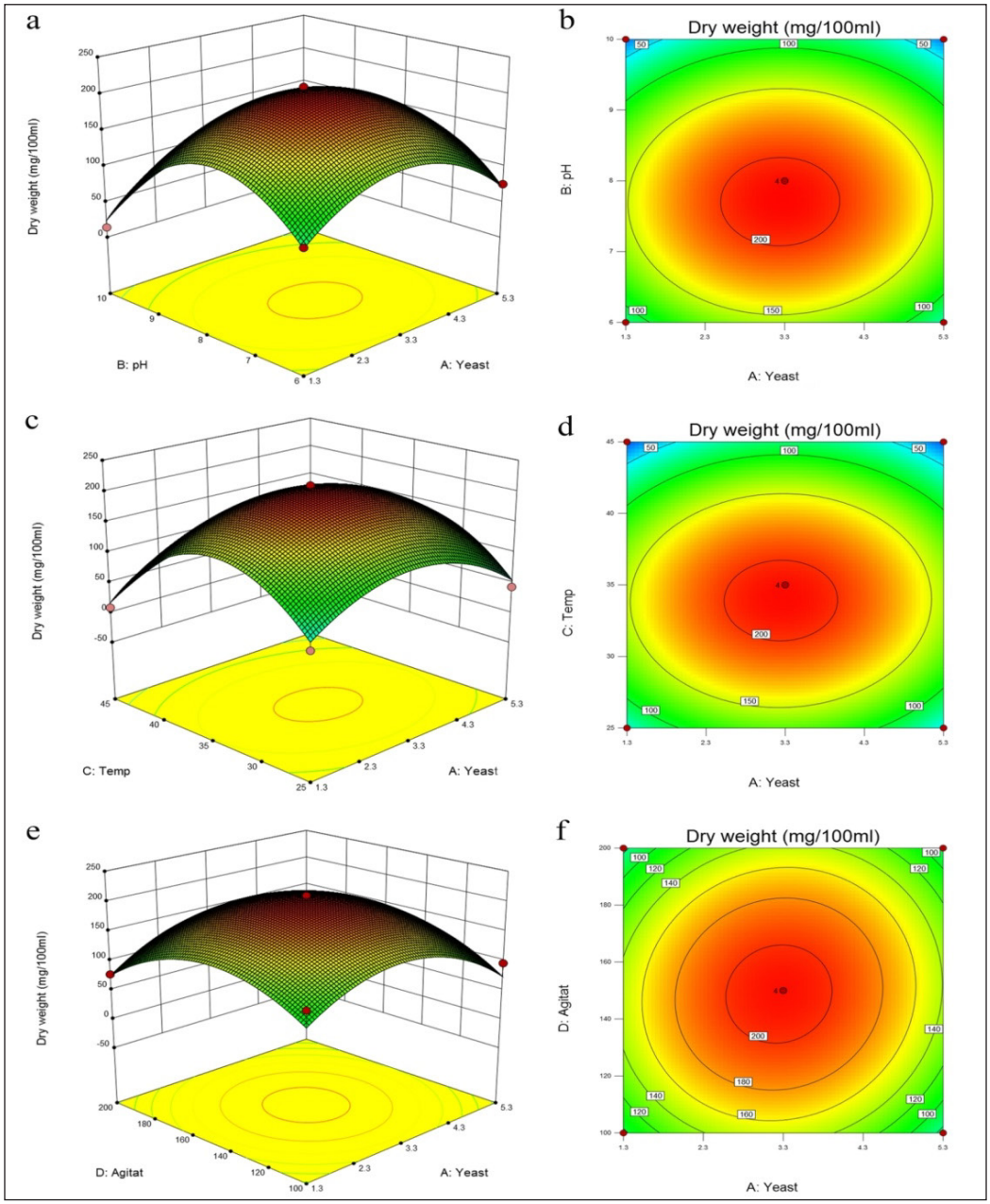


Figure 5. Response surface plots (3D; a, c, e) and contour plots (2D; b, d, f) showing the individual and interactive effects of variables on the dry weight of *Streptomyces* sp. KKU215 [(a, b) effects of concentration of yeast extract and pH; (c, d) effects of concentration of yeast extract and temperature; (e, f) effects of concentration of yeast extract and agitation speed]

By referring to Figure 5, the increase of yeast extract concentration from 1.30% to 3.26% (optimal concentration of yeast extract) increased the dry weight of *Streptomyces* sp. KKU215. Yeast extract is an excellent organic nitrogen source, contains high protein and amino acid content, vitamins, minerals and growth factors that are essential for *Streptomyces* growth (Chen et al., 2012). However, an increase in concentration of yeast extract higher than optimal condition decreased the dry weight.

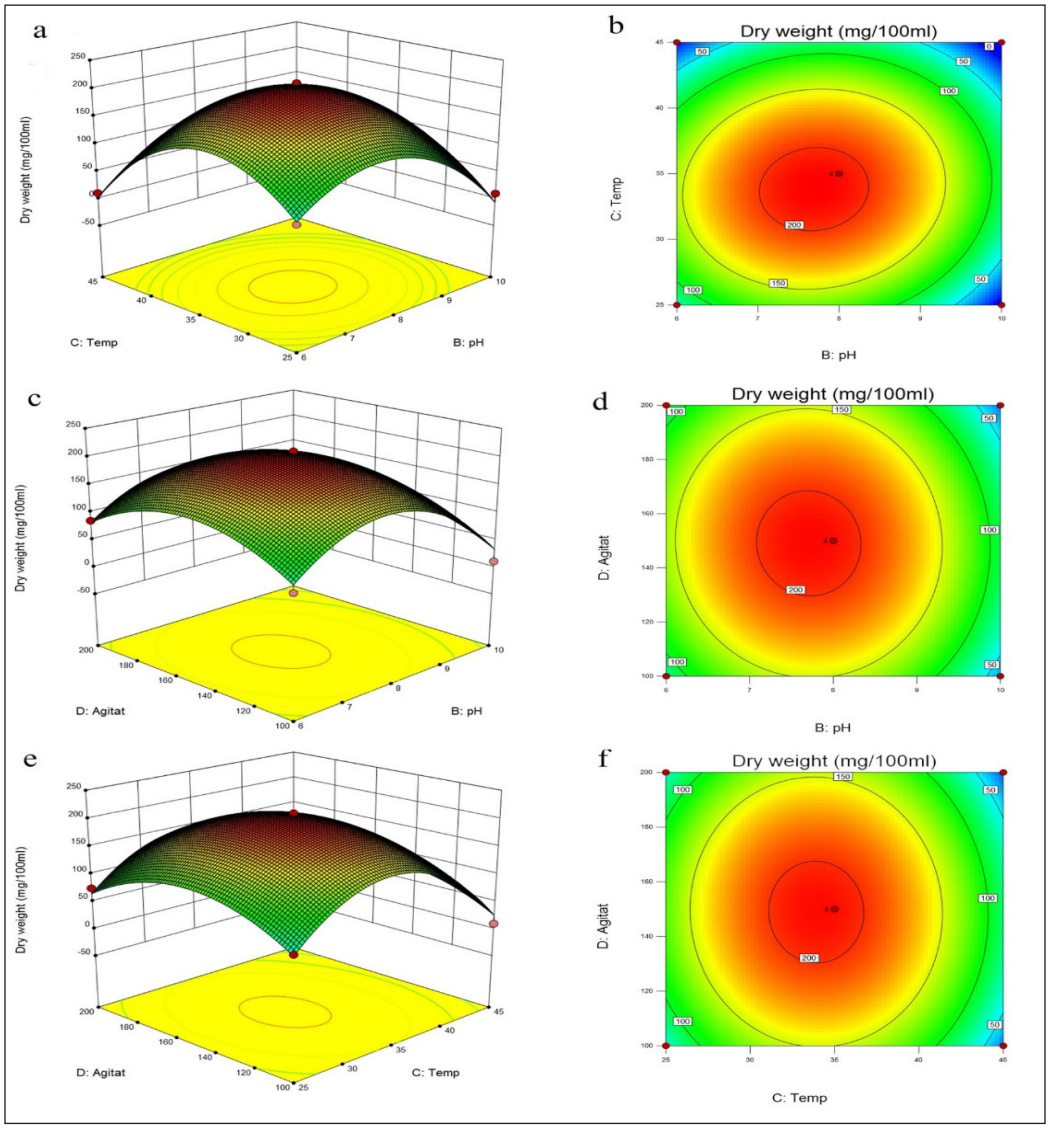


Figure 6. Response surface plots (3D; a, c, e) and contour plots (2D; b, d, f) showing the individual and interactive effects of variables on the dry weight of *Streptomyces* sp. KKU215 [(a, b) effects of pH and temperature; (c, d) effects of pH and agitation speed; (e, f) effects of temperature and agitation speed]

On the one hand, *Streptomyces* sp. KKU215 could grow best at neutral initial pH value (7.69). On the other hand, the acidic and alkaline initial pH values could decrease the dry weight of *Streptomyces* sp. KKU215 (Figures 5a, 5b, 6a, 6b, 6c and 6d). The optimal pH of *Streptomyces* was neutral (pH 6.5-8.0) (Liu et al., 2018; Praveen Kumar et al., 2017; Srivastava et al., 2018; Yamamura et al., 2014).

Dry weight of *Streptomyces* sp. KKU215 increased with increasing temperature up to 33.66°C (optimal temperature). Above the optimal temperature, a negative effect of the temperature on *Streptomyces* sp. KKU215 dry weight was discovered (Figures 5c, 5d, 6a, 6b, 6e and 6f). The temperatures were higher or lower than optimal temperature reduced growth, metabolite production and enzyme activity of *Streptomyces* (Praveen Kumar et al., 2017; Srivastava et al., 2018; Yun et al., 2018).

Agitation speed affects oxygen transfer rate, which plays an important role in aerobic microbial metabolism. Nevertheless, different microorganisms have different oxygen requirements. In this study, the optimal agitation speed of *Streptomyces* sp. KKU215 was 149.13 rpm (Figures 5e, 5f, 6c, 6d, 6e and 6f) as reported by Viana et al. (2010) who found that the highest clavulanic acid production by *Streptomyces* DAUFPE 3060 was achieved at agitation speed of 150 rpm.

Overall, the model predicted that the optimal values of the test factors including concentration of yeast extract, initial pH value, temperature and agitation speed were 3.26%, 7.69, 33.66°C and 149.13 rpm, respectively. The predicted maximum dry weight of *Streptomyces* sp. KKU215 was 210.49 mg/100 mL (Table 2).

Table 2
Optimal values of the test variables, and predicted maximum dry weight

Variables	Value
X_1: Concentration of yeast extract (%)	3.26
X_2: Initial pH value	7.69
X_3: Temperature (°C)	33.66
X_4: Agitation rate (rpm)	149.13
predicted maximum dry weight (mg/100 mL)	210.49

In order to validate the model, the optimisation results were confirmed by cell dry weight after 2 days of cultivation and degradation of PLA-packaging after 4 weeks of cultivation under the optimal conditions based on the results of RSM. The model predicted a maximum response of 210.49 mg/100 mL of *Streptomyces* sp. KKU215 dry weight. At optimal conditions, a maximum *Streptomyces* sp. KKU215 dry weight of 209.25 mg/100 mL was obtained. Thus, the model proved adequate. Moreover, PLA-packaging weight loss under the optimal conditions by *Streptomyces* sp. KKU215 was 84.04%.

CONCLUSION

KKU215, which was isolated from botanical garden soil, was able to degrade PLA-packaging including PLA. Phylogenetic study by 16SrRNA sequence analysis of KKU215 indicated that it related to the genus *Streptomyces*. *Streptomyces* sp.

KKU215 was a novel PLA-packaging-degrading actinomycete. The optimal conditions of concentration of yeast extract, initial pH value, temperature and agitation speed were determined through RSM to be 3.26%, 7.69, 33.66°C and 149.13 rpm, respectively. The optimisation resulted in PLA-packaging weight loss of 84.04%, which was 81.39% higher than that before optimisation (2.65%). The weight loss of PLA-packaging in optimal conditions decreased almost thirty-two-fold than before optimisation. Based on the present findings, *Streptomyces* sp. KKU215 can be used as a potent microbial strain to degrade PLA-packaging. The environmental degradation of PLA-packaging by *Streptomyces* sp. KKU215 will be investigated in further study.

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APPENDIX

Supplementary Table 1
The Box–Behnken experimental design and results

Run No.	Model	Concentration of yeast extract (%)	Initial pH value	Temperature (°C)	Agitation rate (rpm)	Dry weight (mg/100 mL)	
						Actual value	Predicted value
1	+00+	5.3	8	35	200	83.0	84.6
2	0+-0	3.3	10	25	150	10.0	-6.5
3	0000	3.3	8	35	150	204.0	207.0
4	+-00	5.3	6	35	150	76.0	71.7
5	0-0+	3.3	6	35	200	86.0	82.6
6	0-0-	3.3	6	35	100	68.1	81.1
7	00--	3.3	8	25	100	68.1	65.9
8	-+00	1.3	10	35	150	14.0	23.9
9	0++0	3.3	10	45	150	2.0	-27.4
10	+00-	5.3	8	35	100	97.0	74.5
11	-0+0	1.3	8	45	150	9.0	16.7
12	00-+	3.3	8	25	200	75.5	65.5
13	0--0	3.3	6	25	150	68.0	72.4
14	-00-	1.3	8	35	100	123.3	96.7
15	0+0+	3.3	10	35	200	15.0	21.3
16	-0-0	1.3	8	25	150	54.0	66.6
17	0+0-	3.3	10	35	100	11.0	33.7
18	00+-	3.3	8	45	100	10.0	25.6
19	00++	3.3	8	45	200	7.0	14.9
20	0000	3.3	8	35	150	210.0	207.0
21	++00	5.3	10	35	150	15.0	21.9
22	--00	1.3	6	35	150	84.0	82.8
23	+0-0	5.3	8	25	150	44.0	55.6
24	0000	3.3	8	35	150	208.0	207.0
25	0-+0	3.3	6	45	150	11.0	2.4
26	-00+	1.3	8	35	200	78.0	75.5
27	+0+0	5.3	8	45	150	8.0	14.6
28	0000	3.3	8	35	150	206.0	207.0

Supplementary Table 2
Analysis of variance (ANOVA) for the regression equation

Source	SS	df	MS	*F*-value	*P*-value
Model	115700.00	14	8262.47	25.670	<0.0001
Concentration of yeast extract	128.71	1	128.71	0.400	0.5381
pH	8861.77	1	8861.77	27.540	0.0002
Temperature	6192.56	1	6192.56	19.240	0.0007
Agitation rate	90.75	1	90.75	0.280	0.6044
Concentration of yeast extract* pH	20.25	1	20.25	0.063	0.8059
Concentration of yeast extract* Temperature	20.25	1	20.25	0.063	0.8059
Concentration of yeast extract* Agitation rate	244.92	1	244.92	0.760	0.3988
pH* Temperature	600.25	1	600.25	1.870	0.1952
pH* Agitation rate	48.30	1	48.30	0.150	0.7047
Temperature* Agitation rate	27.04	1	27.04	0.084	0.7765
Concentration of yeast extract* Concentration of yeast extract	24868.06	1	24868.06	77.270	<0.0001
pH*pH	51369.88	1	51369.88	159.620	<0.0001
Temperature* Temperature	65166.68	1	65166.68	202.490	<0.0001
Agitation rate* Agitation rate	21450.26	1	21450.26	66.650	<0.0001
Lack of Fit	4163.68	10	416.37	62.460	0.0029
Pure Error	20.00	3	6.67		
Cor Total	119900.00	27			
$R^2 = 0.9651$ Adjusted $R^2 = 0.9275$ Predicted $R^2 = 0.7996$					