Supplementary Materials

Purification and characterization of a cutinase-like enzyme with activity on Polyethylene terephthalate (PET), from a newly isolated bacterium *Stenotrophomonas maltophilia* PRS8 at mesophilic temperature

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S1. UV PET treatment

The plastic PET film was exposed to UVB light at 280nm for 10 minutes, which was significantly shorter than the UV exposure time (Much longer exposure times, 14 days, can affect the PET structure [1]). The distance between the PET plastic and UV Lamp was 30.5 cm. There was no significant difference when it was exposed to a UV lamp.

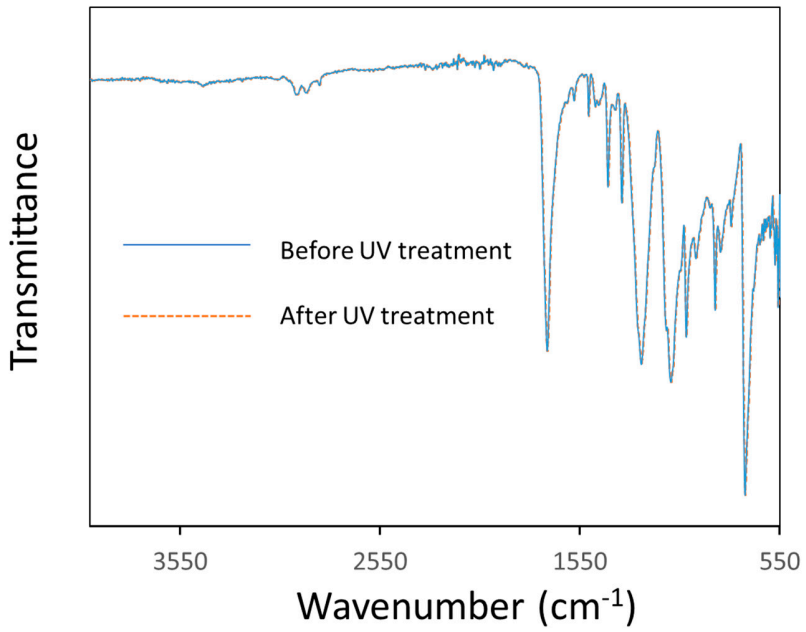

Figure S1. FT-IF spectra of PET flakes before and after UV exposure. The data is normalized at 1410 cm^{-1}. No differences are detectable.

Reference

1. Falkenstein, P.; Gräsing, D.; Bielytskyi, P.; Zimmermann, W.; Matysik, J.; Wei, R.; Song, C. UV pretreatment impairs the enzymatic degradation of polyethylene terephthalate. *Frontiers in Microbiology* 2020, *11*, 689.

S2. Experimental Design

Plackett Burman Design (PDB) for optimizing of nutritional condition

The PBD developed by Design Expert 10.1 software (Stat-Ease Inc. Minneapolis) was used to screen various dietary ingredients for optimum cutinase-like enzyme production from *S. maltophilia* PRS8. The design was mathematically modelled using the first-order polynomial equation listed below (Eq. 1).

$$Y = \beta_0 + \sum \beta_i X_i \qquad \text{(Eq. 1)}$$

Where Y represents specific activity (U/mg) of cutinase of the predicted response; β_0 exhibited the intercept; β_i linear coefficient of the model, while X_i described the level of independent variable. A total of 10 components were optimized by Placket Burman design which included glucose $(NH_4)_2SO_4$, sucrose, $NaNO_3$, K_2HPO_4, Yeast Extract, $MgSO_4.7H_2O$, KCl, $FeSO_4.7H_2O$, cutin and Na_2HPO_4. The effect of these variables was studied on two levels (Tables S1 and S2) of the Design Expert 7 software showed a total of 15 sets of experimentation with varying chemical ingredient concentrations. The cultivation was conducted at 35 °C and 150 rpm. The crude enzyme activity was determined after 24 h intervals up to maximum 72 h and results were recorded to find out specific activity (U/mg) using *p*-NPB as a substrate. All experiments were carried out in triplicates. The significant factors were determined by evaluating the results and factors with *p*-value <0.05 were considered as significant and further analyzed by Central Composite design.

Central Composite Design (CDC) Experiment

Three significant factors (cutin, $NaNO_3$, $(NH_4)_2S0_4$) derived from PBD were further analyzed through Central Composite design (CCD) to achieve a more extended optimization for high enzyme yield. The effect of independent factors such as linear, quadratic, and mutual interactions on the dependent variable enzyme specific activity (U/mg) was investigated. The mathematical relationship between the dependent variable and the significant independent variable is represented by the quadratic polynomial equation provided:

$$Y = \beta_0 + \sum_{i=1}^{6} \beta_i X_i + \sum_{i=1}^{6} \beta_{ii} X_i^2 + \sum_{i<j=2}^{6} \beta_{ij} X_i X_j \qquad \text{(Eq. 2)}$$

In this equation Y, X_i, β_i, β_{ii}, β_{ij}, β_0 are representing the specific activity, significant independent variables, linear regression coefficients, quadratic regression coefficients, interactive regression coefficients and a constant term, respectively (Table S3). ANOVA was used to assess the model's feasibility (Table S4).

Table S1. Placket Burman design of factors with specific enzyme activity (U/mg) as response

Run	Factor Glucose (%)	Factor Yeast Extract (%)	Factor Sucrose (%)	Factor $NaNO_3$ (%)	Factor K_2HPO_4 (%)	Factor $MgSO_4$ $7H_2O$ (%)	Factor KCl (%)	Factor $FeSO_4$. $7H_2O$ (%)	Factor Cutin (%)	Factor Na_2HPO_4 (%)	Factor $(NH_4)_2SO_4$ (%)	Response 1 Specific Activity U/mg
1	0.7	0.9	0.50	0.50	0.20	0.02	0.90	0.01	0.60	0.20	0.30	247.59
2	0.7	0.3	1.10	0.10	0.20	0.08	0.03	0.01	0.60	0.60	0.30	131.65
3	0.7	0.9	1.10	0.50	0.08	0.02	0.03	0.01	0.20	0.60	0.50	162.54
4	1.3	0.9	0.50	0.10	0.08	0.08	0.03	0.01	0.60	0.20	0.50	222.53
5	0.7	0.3	0.50	0.50	0.08	0.08	0.90	0.00	0.60	0.60	0.50	211.55
6	1.3	0.9	1.10	0.10	0.08	0.02	0.90	0.00	0.60	0.60	0.30	169.97
7	0.7	0.3	0.50	0.10	0.08	0.02	0.03	0.00	0.20	0.20	0.30	46.85
8	0.7	0.9	1.10	0.10	0.20	0.08	0.90	0.00	0.20	0.20	0.50	93.1
9	1.0	0.6	0.80	0.30	0.14	0.05	0.47	0.00	0.40	0.40	0.40	111.51
10	1.0	0.6	0.80	0.30	0.14	0.05	0.47	0.00	0.40	0.40	0.40	119.65
11	1.0	0.6	0.80	0.30	0.14	0.05	0.47	0.00	0.40	0.40	0.40	113.09
12	1.3	0.3	0.50	0.10	0.20	0.02	0.90	0.01	0.20	0.60	0.50	146.20
13	1.3	0.3	1.10	0.50	0.08	0.08	0.90	0.01	0.20	0.20	0.30	131.46
14	1.3	0.3	1.10	0.50	0.20	0.02	0.03	0.00	0.60	0.20	0.50	313.96
15	1.3	0.9	0.50	0.50	0.20	0.08	0.03	0.00	0.20	0.60	0.30	155.26

Table S2. ANNOVA for Placket-Burman Design

Source	Sum of Squares	Degree of Freedom	Means Square	F Value	p-value prob>F
Model	54429.80	10	5442.98	9.56	0.0445
A-Glucose	5046.98	1	5046.98	8.87	0.0587
B-Yeast extract	400.49	1	400.49	0.70	0.4631
C-Sucrose	62.07	1	62.07	0.11	0.7629
D-$NaNO_3$	14149.43	1	14149.43	24.86	0.0155
E-K_2HPO_4	1700.50	1	1700.50	2.99	0.1823
F-$MgSO_4.7H_2O$	713.02	1	713.02	6.60	0.124
G-KCl	90.24	1	90.24	0.16	0.7171
H-$FeSO_4.7H_2O$	219.18	1	219.18	0.39	0.5788
I-$(NH_4)_2SO_4$	5944.86	1	5944.86	10.45	0.0481
J-Cutin	26304.84	1	26304.84	46.22	0.0065
K-Na2HPO4	511.21	1	511.21	0.90	0.4132

Table S3. Central Composite design of factors with specific activity (U/mg)

Run	Factor 1 Cutin (%)	Factor 2 $NaNO_3$ (%)	Factor 3 $(NH_4)_2SO_4$ (%)	Response 1 Specific Activity U/mg
1	0.90	0.60	0.80	307.95
2	0.80	1.14	0.68	253.05
3	0.90	1.00	0.55	369.93
4	0.80	0.80	0.68	275.63
5	0.90	0.60	0.55	113.50
6	0.80	0.80	0.68	224.54
7	0.80	0.80	0.68	187.81
8	0.90	1.00	0.80	290.91
9	0.70	1.00	0.55	339.32
10	0.97	0.80	0.68	194.22
11	0.70	0.60	0.80	102.15
12	0.80	0.46	0.68	87.35
13	0.80	0.80	0.68	283.91
14	0.70	0.60	0.55	179.21
15	0.80	0.80	0.68	289.95
16	0.80	0.80	0.46	217.34
17	0.80	0.80	0.68	242.56
18	0.80	0.80	0.89	206.63
19	0.70	1.00	0.80	123.63
20	0.63	0.80	0.68	139.29

Table S4. ANNOVA for Central Composite design

Source	Sum of Squares	Degree of Freedom	Means Square	F Value	p-value prob>F
Model	8031.59	6	15778.90	6.70	0.002
A-Cutin	535.90	1	13561.64	5.75	0.0322
B- $NaNO_3$	45.66	1	35845.94	15.21	0.0018
C-$(NH_4)_2SO_4$	1667.59	1	2794.08	1.19	0.2960
AB	1432.53	1	417.48	0.18	0.0468
AC	13.99.10	1	20825.80	8.84	0.0108
BC	406.85	1	21228.48	9.01	0.0102

Fourier-transform infrared spectra.

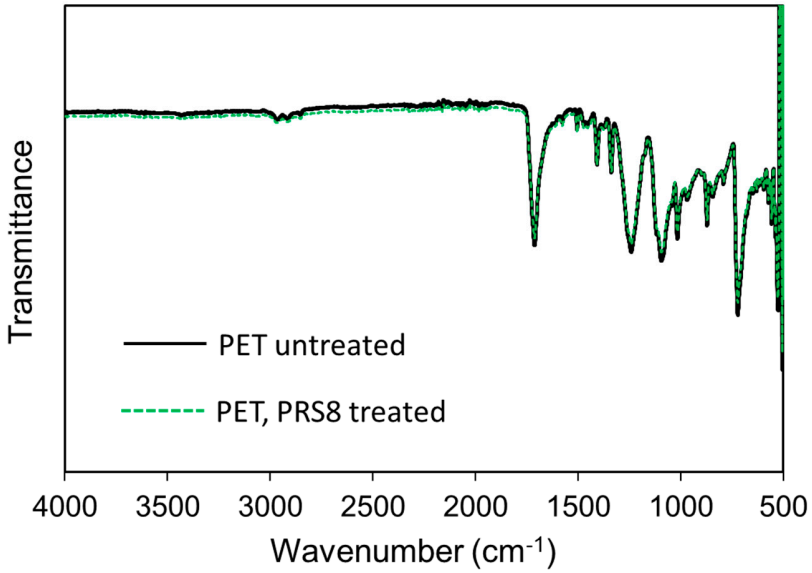

Figure S2: FT-IR spectra of PET (500-4000 cm-1) after 28 days abiotic control untreated (black) and PET treated with *S. maltophilia* PRS8 strain (Green dotted line).

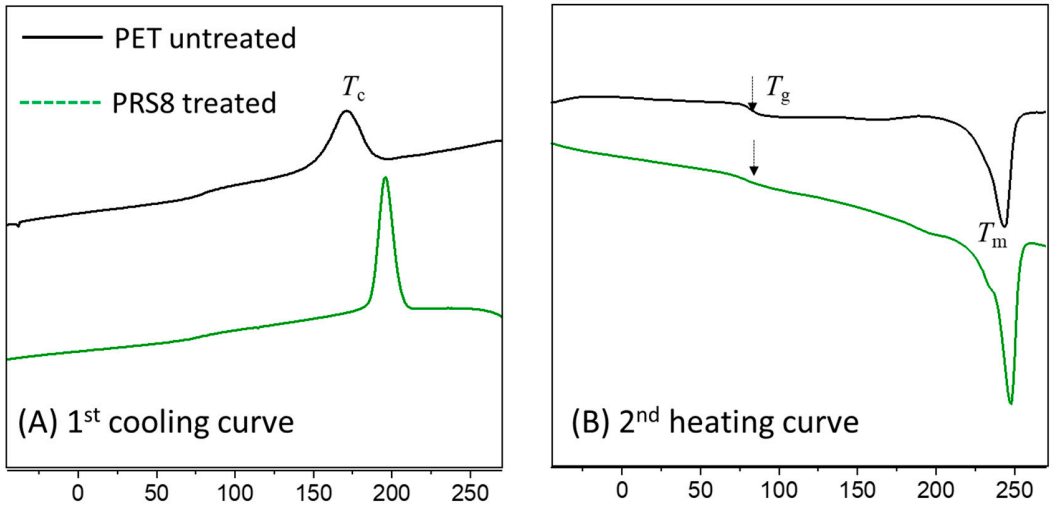

Figure S3: 1st cooling curve (A) and 2nd heating curve (B) from DSC measurements for powdered PET samples i.e. untreated PET powder (black) and PET treated with PRS8 strain (Green). Peak of crystallization (T_c) during first cooling and melting peak in second heating cycle (T_m) is shown for untreated PET sample. T_g in second heating cycle is shown with black arrows for both PET samples.