Genome Mining   基因组挖掘

Complete genome sequencing and diversity analysis of lipolytic enzymes in *Stenotrophomonas maltophilia* OUC_Est10

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Abstract: **[Objective]** The aim of this study was to study the diversity of lipolytic enzymes in *Stenotrophomonas maltophilia* OUC_Est10. **[Methods]** Ion exchange chromatography, genome sequencing and heterologous expression were used to study the diversity of lipolytic enzymes in *Stenotrophomonas maltophilia* OUC_Est10. **[Results]** *Stenotrophomonas maltophilia* OUC_Est10 could secret a wide range of lipolytic enzymes (lipases and esterases) as revealed by ion exchange chromatography. The complete genome is of 4668743 bp in length, with an average GC content of 66.25%. Genome annotation indicated the presence of 33 candidate genes whose products possess the predicted lipolytic enzyme activities. Analysis of catalytic features was carried out by expressing five putative lipolytic enzyme genes, and lipolytic enzymes in OUC_Est10 had different catalytic properties. **[Conclusion]** We proved that *Stenotrophomonas maltophilia* OUC_Est10 was a good candidate to produce diverse lipolytic enzymes, with potential applications in various fields.

Keywords: *Stenotrophomonas maltophilia*, separation, lipolytic enzyme, complete genome sequence, catalytic properties

Lipolytic enzymes, including esterases (3.1.1.1) and lipases (3.1.1.3), are members of the α/β hydrolase superfamily, which contain a catalytic triad (Ser-His-Asp/Glu)[1]. With the development of industrial biotechnology, lipolytic enzymes are important catalysts in the biological manufacturing processes, such as food, bioenergy, detergent, pharmaceutical and advanced chemical manufacturing[2–3]. Lipolytic enzymes can catalyze the cleavage and formation of ester bonds, and the catalytic mechanisms for esterases and lipases are similar[4]. In organic media, both esterases and lipases catalyze various reactions, such as esterification, transesterification, aminolysis and interesterification[5]. The increasing demand for novel biocatalysts has prompted the development of new methods which are used to screen for new genes, such as genome sequencing.

Stenotrophomonas maltophilia is a Gram-negative bacterium which, in our previous research, has been demonstrated to secrete esterases and lipases to

prepare free astaxanthin efficiently[6]. This result suggests that *S. maltophilia* OUC_Est10 is a good microbial resource for novel lipolytic enzymes. However, there are only few genome sequences available for *S. maltophilia*[7–8]. They are all focused on the drug resistance of *S. maltophilia,* and to the best of our knowledge, there has been no study on the diversity of lipolytic enzymes. Since lipolytic enzymes have wide application in industry, it is necessary to investigate the diversity of relevant genes in the genome of *S. maltophilia* in order to discover more encoding genes for the biocatalysts.

In this study, the lipolytic enzymes of *S. maltophilia* OUC_Est10 (China General Microbiological Culture Collection Center (CGMCC), 10672) were induced in the fermentation medium (KH_2PO_4, 0.025%; $MgSO_4\cdot7H_2O$, 0.025%; $FeSO_4\cdot7H_2O$, 0.001%; beef extract, 0.1%; peptone, 1.0%; cholesterol oleate, 0.1%; Tween-80, 1.0%; H_2O, 100 mL; pH 7.0). Esterase activity and lipase activity were determined spectrophotometrically at 405 nm. Fermentation liquid of OUC_Est10 was centrifuged at 5439×g for 10 min, and the supernatant was subjected to ammonium sulfate precipitation. The resulting crude enzyme was put onto a DEAE-Sepharose Fast Flow column, which was previously equilibrated with buffer A (20 mmol/L Tris-HCl, pH 8.0). The unbound proteins were washed with buffer A until the absorbance at 280 nm reached the baseline. Furthermore, the bound proteins were eluted by a gradient of 0.1–0.7 mol/L NaCl in buffer A (Figure 1).

The fractions resulted in a peak were analyzed in terms of esterase activity (*p*-nitrophenyl butyrate (*p*NPB) as substrate) and lipase activity (*p*-nitrophenyl palmitate (*p*NPP) as substrate).

The result showed that esterase activities and lipase activities were both determined in 8 peaks (Table 1), and it was finally proven that OUC_Est10 could secrete a wide range of lipolytic enzymes. *S. maltophilia* OUC_Est10 was isolated from the slaughterhouse soil in Qingdao, China. The slaughterhouse soil was rich in lipids, which might explain the wide range of lipolytic enzymes in OUC_Est10. The feature of multiple lipolytic enzymes in *S. maltophilia* OUC_Est10 contributes to the efficient hydrolysis of lipids in the slaughterhouse soil.
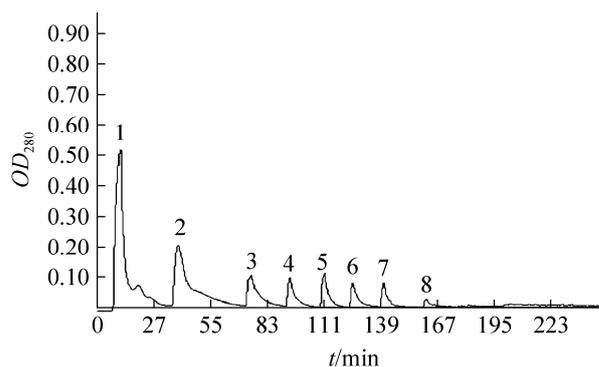
Figure 1.   Ion exchange chromatography map. The elution buffer was 20 mmol/L Tris-HCl buffer (pH 7.0) with NaCl concentration: peak 1, 0 mol/L; peak 2, 0.1 mol/L; peak 3, 0.2 mol/L; peak 4, 0.3 mol/L; peak 5, 0.4 mol/L; peak 6, 0.5 mol/L; peak 7, 0.6 mol/L; peak 8, 0.7 mol/L.

Table 1.   Separation of lipolytic enzymes in the fermentation liquid of OUC_Est10

Peak	c (NaCl)/(mol/L)	Total protein/mg	Total activity/U		Specific activity/(U/mg)	
			*p*NPB	*p*NPP	*p*NPB	*p*NPP
1	0	25.310	148.058	7.535	5.850	0.298
2	0.1	20.278	8.681	10.726	0.428	0.529
3	0.2	9.105	8.183	15.582	0.899	1.711
4	0.3	5.205	2.977	3.769	0.572	0.724
5	0.4	6.612	3.711	4.966	0.561	0.751
6	0.5	6.200	3.588	6.118	0.579	0.987
7	0.6	4.040	2.856	4.989	0.707	1.235
8	0.7	1.390	1.265	1.689	0.910	1.215

To investigate the diversity of lipolytic enzyme genes, the genomic DNA of OUC_Est10 was extracted using Puregene Yeast/Bact. Kit B (QIAGEN, Maryland, USA) and sent to Tianjin Biochip Corporation (Tianjin, China) to sequence. The whole genomic DNA was sequenced on the single-molecule real-time (SRMT) sequencing platform PacBio RS II (Pacific Biosciences, USA). The genomic sequence was obtained after the reads were *de novo* assembled using the RS Hierarchical Genome Assembly Process (HGAP) assembly protocol version 3.0 in SMRT Analysis version 2.3.0 (Pacific Biosciences, USA). The protein coding sequences (CDSs) were predicted using Glimmer 3.0[9]. The procedures of tRNA and rRNA prediction were conducted using tRNAscan-SE[10] and RNAmmer[11], respectively. Functional annotation and metabolic pathway analysis were performed on the Integrated Microbial Genomes-Expert Review (IMG-ER) pipeline[12].

The features of the complete genome sequence of *S. maltophilia* OUC_Est10 are listed in Table 2. The complete genome sequence consists of a single chromosome of 4668743 bp with a GC content of 66.25% (Figure 2), 3315 genes were identified in OUC_Est10, and the average number of genes in sequenced *S. maltophilia* was classified to functional categories according to clusters of orthologous genes (COG) designation (Table 3). Through genome searching, it was found that *S. maltophilia* was rich in lipolytic enzyme genes, which was one of the factors contributing to the virulence in *S. maltophilia*[7]. After detailed analysis, 33 proteins with putative lipolytic enzyme activities are found in the genome sequence of OUC_Est10 (Table 4), and the locations of these genes were also marked in Figure 2. This indicates that OUC_Est10 may be a good candidate used for hydrolyzing lipids.

Table 2.   Genome features of *Stenotrophomonas maltophilia* OUC_Est10

Feature	Value
Genome size/bp	4668743
G+C content/%	66.25
Protein coding genes (CDS)	4189
rRNA (5S, 16S, 23S)	13
tRNA	73
Miscellaneous RNA	38
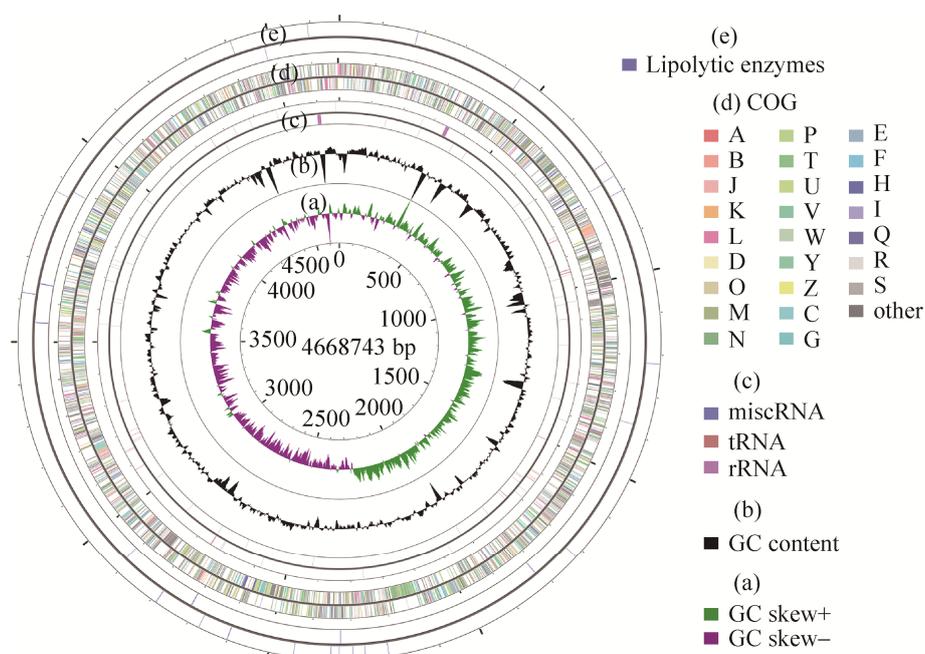
Figure 2.   Circular diagram of the main features of OUC_Est10.

Table 3.　Number of genes of functional categories

Functional category	Average of *S. maltophilia*	OUC_Est10
General function prediction only	265.2±25.7	276
Transcription	241.2±27.9	261
Cell wall/membrane/envelope biogenesis	211.4±20.9	224
Signal transduction mechanisms	203.9±21.9	220
Amino acid transport and metabolism	207.6±18.3	218
Translation, ribosomal structure and biogenesis	207.3±15.3	218
Function unknown	198.6±20.9	214
Inorganic ion transport and metabolism	185.4±22.8	201
Energy production and conversion	184.9±15.6	190
Posttranslational modification, protein turnover, chaperones	147.1±12.7	155
Coenzyme transport and metabolism	146.4±14.7	152
Carbohydrate transport and metabolism	144.4±14.2	148
Lipid transport and metabolism	136.5±12.4	144
Cell motility	110.1±9.6	116
Defense mechanisms	97.0±11.2	111
Replication, recombination and repair	107.2±14.9	105
Secondary metabolites biosynthesis, transport and catabolism	80.3±7.7	82
Intracellular trafficking, secretion, and vesicular transport	77.5±10.2	73
Nucleotide transport and metabolism	67.8±5.1	71
Mobilome: prophages, transposons	30.2±16.7	51
Extracellular structures	47.2±6.6	48
Cell cycle control, cell division, chromosome partitioning	32.9±4.2	34
RNA processing and modification	1.0±0.4	1
Chromatin structure and dynamics	1.0±0.2	1
Cytoskeleton	1.0±0.2	1
Total genes	3133.0±283.4	3315

Table 4.　Genes with predicted lipolytic enzyme activities

No.	Length/bp	Function	No.	Length/bp	Function
LEn1	435	Esterase YdiI	LEn18	1188	Lipase_GDSL_2
LEn2	891	Pimeloyl-ACP ME carboxylesterase	LEn19	960	Pimeloyl-ACP ME carboxylesterase
LEn3	813	Pimeloyl-ACP ME carboxylesterase	LEn20	825	Pimeloyl-ACP ME carboxylesterase
LEn4	1260	Putative esterase	LEn21	1911	Predicted acyl esterase
LEn5	927	Pimeloyl-ACP ME carboxylesterase	LEn22	1329	Lipase (class 3)
LEn6	645	Lipase_GDSL_2	LEn23	789	Lipase_GDSL_2
LEn7	702	Lipase_GDSL_2	LEn24	939	Esterase
LEn8	942	Pimeloyl-ACP ME carboxylesterase	LEn25	843	Esterase
LEn9	795	Pimeloyl-ACP ME carboxylesterase	LEn26	1203	Secretory lipase
LEn10	837	carboxylesterase	LEn27	1854	Outer membrane lipase/esterase
LEn11	696	Pimeloyl-ACP ME carboxylesterase	LEn28	618	Lipase_GDSL_2
LEn12	1308	Lipase_GDSL_2	LEn29	831	Esterase
LEn13	1029	Esterase	LEn30	660	carboxylesterase
LEn14	954	Pimeloyl-ACP ME carboxylesterase	LEn31	780	Pimeloyl-ACP ME esterase
LEn15	1038	Fermentation-respiration switch protein FrsA	LEn32	702	Pimeloyl-ACP ME carboxylesterase
LEn16	1005	Esterase, PHB depolymerase family	LEn33	855	Pimeloyl-ACP ME carboxylesterase
LEn17	879	Pimeloyl-ACP ME carboxylesterase			

ACP represents acyl-carrier protein; ME represents methyl ester.

To prove the diversity and the potential use of lipolytic enzymes in *S. maltophilia* OUC_Est10, 16 pairs of primers were designed. The result of nucleic acid electrophoresis is shown in Supplementary material Figure S1. After being ligated to the pET-28a (+) vector, LEn4, LEn6, LEn12, LEn27 and LEn30 were successfully expressed in BL21 (DE3). Substrate specificity determination was carried out using *p*-nitrophenyl (*p*NP) esters with different acyl chain length. The results showed that they had different preferences for the length of fatty acid. LEn4, LEn12 and LEn27 had a preference for short-chain fatty acids, while LEn6 and LEn30 had a preference for medium-chain fatty acids (data not shown). It was worth noting that LEn27 was able to hydrolyze *p*NP esters with acyl chain length from 4 to 16, which indicated that LEn27 could be widely used for hydrolysis or synthesis of esters with different acyl chain lengths.

The organic solvents tolerance experiment showed that LEn4, LEn6, LEn12 and LEn27 had good organic solvent resistant properties, while LEn30 was highly denatured by organic solvents. LEn4, LEn6, LEn12 and LEn27 could all be used to synthesize ethyl esters (such as cinnamyl acetate) in a non-aqueous system (Figure 3). The determination of cinnamyl acetate was carried out using an HP-5 capillary column (30 m×0.25 mm×0.25 mm).
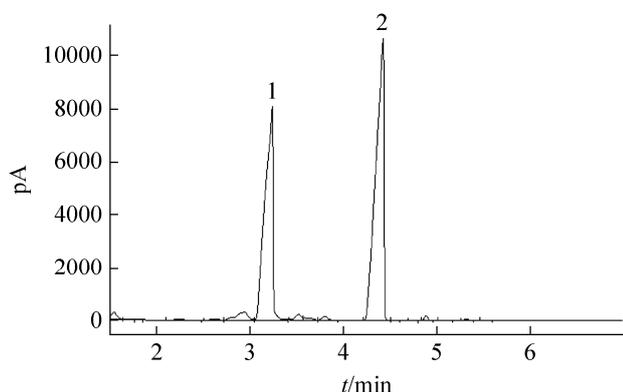
Figure 3. GC analysis of cinnamyl alcohol and cinnamyl acetate. Peak 1, the substrate (cinnamyl alcohol); Peak 2, the product (cinnamyl acetate).

These experiments indicated that lipolytic enzymes in OUC_Est10 had varied catalytic properties, which could broaden the application range of OUC_Est10. Our further studies will focus on expressing these putative lipolytic enzymes, and they will be used in many fields according to their catalytic properties.

Nucleotide sequence accession number

The complete genome sequence of *S. maltophilia* OUC_Est10 is available at the IMG database under the accession number Ga0114270 and the GenBank database under the accession number CP015612.

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嗜麦芽窄食单胞菌 OUC_Est10 全基因组测序及脂类水解酶多样性分析

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摘要： OUC_Est10

OUC_Est10

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4668743 bp GC 66.25%

33 OUC_Est10 5

OUC_Est10

关键词：

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