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Searching for Useful Enzymes from Insect Symbiotic Microbes

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Objectives

Isolation of useful enzyme producing microbes from insect symbiotic microorganisms and development of the enzymes for industrial use.

Materials and Methods

○ Materials

Insect - More than 30 species of insect including Coleoptera and Lepidoptera were collected and used for the sources of microbes.

Media - Isolation media : R2A, PCA

Screening & cultivation media : M9 minimal media + 0.5% yeast extract + 0.5% carbon source (olive oil, xylane, pectin etc.)

○ Methods

Endosymbiotic microbes were isolated and secured from the homogenate of insect gut. Useful enzyme producing strains were screened against isolated microbes by agar plate assay and microplate assay. Identification of selected microbes were performed by 16S rDNA sequence analysis. Enzyme producing activities of the microbes at different carbon sources were tested against selected strains. Purification of the enzyme was performed by traditional column chromatography and/or adsorption/desorption method using polypropylene matrix. Properties of the enzyme were investigated for substrate specificity, optimum temperature and pH, metal ion effect, and stabilities at different conditions. The genes coding for the enzyme was cloned by PCR amplification using primers designed based on the information of N-terminal amino acid sequencing and sequence homology comparison of known bacterial lipases followed by DNA walking technique.

Results and Discussion

More than 500 bacterial strains, 76 fungi, and 60 yeast were isolated as insect symbiotic microbes and screened for useful enzymes such as lipase, xylanase, and pectinase etc. Through the screening, we selected several lipase, xylanase, and pectinase

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producing strains. Among them, a high lipase producing strain, AG-10, was used for further purification of enzyme. AG-10 showed highly induced lipase production at 48-60 hrs cultivation with an addition of olive oil as a carbon source. The enzyme was purified by one step adsorption/desorption method with a high homogeneity. Yield of the purification step was about 40%. The purified enzyme showed optimum temperature at 60℃ and optimum pH at 8.5. The most effective substrate was pNP caproate (C6) among tested C4-C18 pNP substrate. The enzyme was relatively stable for 30 minutes treatment at 50℃ and half of the activity was remained at 60℃ however, inactivated at 70℃. Several protease inhibitors and detergents did not affect enzyme activity at 1 mM or 0.1-0.2% concentration however, strongly inhibited by SDS and sodium deoxy cholate. Cloned gene of AG-10 lipase represents an open reading frame as well as a putative ribosome binding site within 12 bp upstream of translation initiation signal.

Table 1. Lipase producing strains selected in secondary screening.

	Agar plate assay		Microplate assay(ABS_{405nm})				16S rDNA sequence identirication
	24℃	37℃	24℃		37℃		
			Nutrient	M9	Nutrient	M9	
AJ-5	+++	++++	0.57	0.22	0.22	0.18	*Pseudomonas* sp.
V-3	++	++	0.44	0.26	0.47	0.20	*Burkholderia* sp.
V-6	++	++	0.61	0.24	0.47	0.21	*Burkholderia* sp.
AT-3	+++	++	0.35	0.77	0.31	0.56	*Serratia* sp.
AL-1	++	+++	0.39	0.2	0.19	0.15	*Pseudomonas* sp.
AL-2	+++	++	0.46	0.23	0.74	0.24	*Endophyte* sp.
AL-3	++	++	0.35	0.65	0.51	0.71	*Serratia* sp.
X-6	++	++	0.85	0.19	0.66	0.24	*Burkholderia* sp.
AA-4	++++	++++	1.08	0.62	0.2	0.12	*Pseudomonas* sp.
AQ-1	++	++	0.4	0.14	0.2	0.14	*Acinetobacter* sp.
AG-10	+++	++	0.42	0.51	0.72	0.19	*Burkholderia* sp.
control	++	+++	0.28	0.65	0.14	0.13	*P. fluorescens*

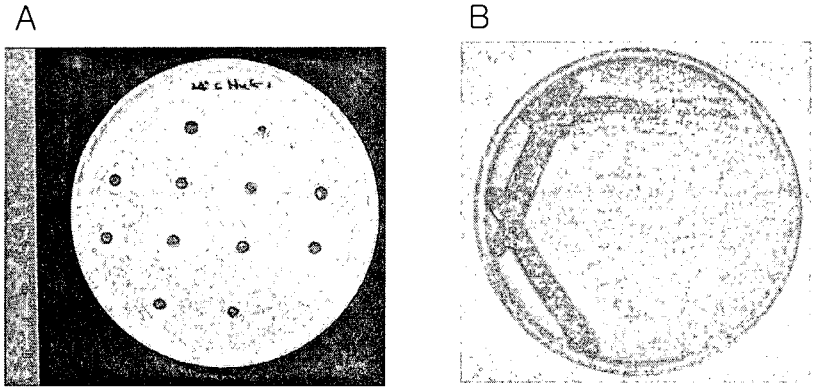

Fig.1. Identification of lipase activity in rhodamin B agar (A) and tributyrin agar (B)