2P-037  Characterization and mutational approach of a psychrophilic lipase, BpL5, from an Arctic bacterium

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Key words lipase, psychrophilic, Bacillus pumilus, rational mutation

Bacterial strains possessing lipolytic activity were isolated from Arctic region (the Chukchi Sea). Among them, the strain ArcL5 was selected as the candidate strain producing a psychrophilic lipase useful for industrial application, and it was identified as Bacillus pumilus based on 16S DNA sequence analysis. The lipase coding gene was cloned with the specific primers. The lipase enzyme from the strain ArcL5, named as BpL5, belongs to the subfamily 4 of lipase family I. The recombinant enzyme BpL5 was successfully expressed as soluble protein with functional activity in the 3D-structure model. The mutants revealed improved activity against PNP-substrates and triglycerides.

2P-038  A histidine acid phytase homodimer from Klebsiella pneumoniae 9-3B that liberates myo-inositol from phytic acid

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Key words Phytase, Dimer

Phytase enzymes are used as a monogastric livestock feed additive to utilize the phosphate locked in phytic acid, a phosphate storage molecule in feed crops. Benefits include improved livestock growth and less environmental phosphate pollution. Among various classifications, microbial histidine acid phytases (HAPhy) are currently only used in agriculture due to their acidic pH optimum and high activity. HAPhy engineering has targeted improving pH optimum, thermostability, and increasing activity. Through examining the recombinant phytase from K. pneumoniae 9-3B (Phy9-3B), we are exploring dimerization as a novel phytase engineering approach for increasing activity and specificity. The Phy9-3B sequence encodes a 43.4kDa mature enzyme with only 9 amino acid residue difference to a published HAPhy called PhyK. This allows a chance to examine their sequence-function relationship. Phy9-3B was purified by affinity and size exclusion chromatography (SEC) and confirmed by SDS-PAGE. Biochemical differences include Phy9-3B having a 10 fold increase in specific activity and the ability to liberate myo-inositol from phytic acid. PhyK cannot hydrolyze phosphate C2 due to its axial conformation, along with all current agricultural HAPhy. The PhyK structural model reveals all mutations are on the outer surface of the enzyme. Molecular weight estimation by SEC suggests Phy9-3B is a homodimer, whereas PhyK is a monomer. We hypothesize these outer surface mutations allow for homodimerization, which then causes the biochemical differences. Mutagenesis will pinpoint the essential residues, and solving the crystal structure can reveal the novel dimer-substrate complex.

2P-039  Biochemical characterization of formate dehydrogenase isolated from Citrobacter sp. S-77

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Key words Formate dehydrogenase, Citrobacter sp. S-77, formate oxidation

Formate hydrogenase (FDH) is one of the most attractive metalloenzyme, catalyzing the reversible oxidation of formate to two-electrons and CO2. FDH has a great potential for use as a useful biocatalyst in biotechnological application. However, FDH is very sensitive to O2, which can easily lose its catalytic activity by air oxidation. We have purified a highly O2-stable FDH from a facultative anaerobic bacterium Citrobacter sp. S-77. The FDH is a monomer of molecular mass of approximately 150 kDa, consisting of two subunits of 95 kDa and 32 kDa polypeptides. The catalytic activity of the FDH for formate oxidation was estimated to be 30.4 U/mg in benzyll viologen as an electron acceptor. The purified FDH in the studies did not contain hem b subunit. EPR and ICP-MS spectra indicate that the FDH is a molybdenum-containing enzyme, displaying a remarkable O2-stability along with thermostability and pH resistance.

2P-040  アメフラシ消化液由来β-グルコシダーゼのラミナラン
の完全分解

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[背景と目的]近年、バイオエタノール生産の有望な原料として海藻が注目されている。海藻の中でも褐藻類に含まれる褐藻酸は主にセレウスやアガロース、アガロゲルなどであり、陸上植物と類似しているため、植物バイオマスに用いられる酸素酸化処理が適応できると考えられる。しかし、褐藻類の褐藻酸は、ラミナランやマンノートール、アルギュリン酸などを、複数の褐藻類を含んでいる。褐藻酸の酸素酸化処理には多様な多糖分解酵素が必要であると考えられる。当研究室では、褐藻を主食とするアメフラシ消化液を用いて、褐藻類に含まれる主要な貯蔵多糖であるラミナランの分解システムについて解析している。

[方法]アメフラシ消化液を各種クロマトグラフィーにかけ、基質としてラミナラン、ラミナラン、カルボン化を用いて褐藻酸を固定化した。褐藻酸性は反応液の褐藻酸および変異体の褐藻酸を測定し、算出した。

[結果と考察]ラミナラン、ラミナラン、カルボン化を分解する酵素として110Daと210Daの2種類のβグルコシダーゼ（BGL）、90Daのカルボン化分解酵素、110Daβ-マンノシダーゼが分離された。2種類のBGLはラミナランから直接的にグルコースを遊離させることができた。さらに、各酵素の相乗効果について検討をおこなった。特にラミナランの分解においては、110kDaと210kDa BGLを反応した場合、各BGLを単独で作用した時のグルコース遊離量の和より2倍以上のグルコースが遊離し、β-1,3−グルカナーゼがなくとも完全にラミナランをグルコースへ分解することがわかった。]