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Marine bacterial enzymes for molecular genetics and structure-function studies

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The marine environment conditions involving low temperature, high pressure and high salinity made the marine habitants to synthesize such enzymes, the characteristic features of which were the higher catalytic effectiveness and unusual specificity compared to the terrestrial counterparts due to their molecular flexibility and still poor explored biological function. The recombinant alkaline phosphatase from the marine bacterium *Cobetia amphilecti* KMM 269 (CmAP) with the higher activity (≥ 12,000 U/mg) among the known analogues was successfully applied for genetically modifying the lectins from marine invertebrates as well as the porin of pathogen *Yersinia pseudotuberculosis*, which are promise diagnostic agents in the cancer and infectious diseases, respectively [1-4].The CmAP activity of the recombinant bifunctional GalNAc/Gal-specific lectin (CGL) from the mussel *Crenomytilus grayanus* well extrapolated the results of substitution of each amino acid residues affected the mucin-binding activity on *in silico* analysis of binding mechanisms of CGL to galactose, globotriose and porcine stomach mucin.

It has been found for the first time that the CGL binding effectiveness depends on the monosaccharide composition of oligosaccharide due to the ability to form additional hydrogen bonds with both the terminal galactose and neighboring residues. The CmAP module was used to investigate the specificity of CGL toward tumor markers CA19-9, CA125, CA72-4, carcinoembryonic antigens (CEA), alpha-fetoprotein (AFP) and prostate-specific antigen (PSA). CGL genetically fused with CmAP was shown to be promise tool for the development of enzyme-linked lectin assay for identify tumor markers CEA and CA19-9 in clinical specimens [3-5].The use of alkaline phosphatase CmAP in the genetically labelled CGL and OmpF-porin

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hybrid proteins for the diagnostics eliminated the use of enzyme-labeled secondary antibodies necessary for detecting specific antibodies in the biological samples to simplify the procedure and shorten the analysis time. The CmAP/OmF-porin functionality was confirmed by the binding of antibodies to OmpF-porin in murine antisera, as well as in the sera of patients with pseudotuberculosis. Moreover, the CmAP activity was stimulated in the presence of specific antibodies to OmpF porin, improving the diagnostic relevance [2].

Apart from the structure-function studies, the marine bacterial enzymes could be used for the elucidation of some cell properties such as their growth or biofilm formation at the molecular genetic level. CmAP inhibited the growth of the MDA-MB-231 cell line (breast adenocarcinoma) by 44.9% at concentration 2.3 U/mg after 48h of their enzymatic treatment and incubation. The dose-dependent effect of CmAP was observed on both the mature and *de novo* biofilms of most known pathogens. Remarkably, the a-galactosidase from the marine bacterium *Pseudoalteromonas* sp. KMM 701 also showed an effect on the level of biofilm-regulating genes expression in the widespread pathogen *Pseudomonas aeruginosa* [1]. However, the question of which the molecular mechanisms trigger of these cell processes has yet to be investigated in detail.

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