E325 Active Defensin Derived from Insect Is Efficiently Secreted by Glucoamylase Signal Sequence in Yeast

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Many insects are known to produce inducible antibacterial peptides in response to invading pathogenic microorganisms to kill them. PhormicinA, one of insect defensin families, which has antibacterial activity against Gram-positive bacteria is composed of 40 amino acids and contains 6 Cys residues. The further study on defensin such as the elucidation of action mechanism and structure-function relationship or the evaluation of utility needs large amounts of peptide. But the limit of immunized host and low yield of peptide necessitates the large scale of heterologous expression system. We chose a yeast to establish an efficient system for secretory production of biologically active PhormicinA. To express PhormicinA in *Saccharomyces cerevisiae*, PhormicinA gene was chemically synthesized in 3 fragments and assembled *in vitro*. Two recombinant plasmids, pSGD11(glucoamylase signal sequence) and pGMD18(mating factor α 1 preprosequence), were constructed and transformed into *S. cerevisiae*. Yeast cells secreting active PhormicinA were selected through growth inhibition zone assay using *Micrococcus luteus* as a test organism. Boiling for 30 min of culture supernatant had no effect on antibacterial activity but protease K treatment abolished it.

E326 The Determination of Chitin synthases by varying pH and Divalent Cations in *Candida albicans*

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The Chs1, Chs2 and Chs3 activities of a pathogenic fungus, *Candida albicans*, perform the same biochemical reactions, but exert different functions. Therefore, determination of each enzyme activity is important. The three chitin synthases differ in their optimal pH and effect of divalent cations as either stimulatory or an inhibitory factor. The CAChs1, CAChs2 and CAChs3 activities are optimal at pH 7.5, 6.5, and 8.5 respectively. Co^{+2} stimulates CAChs1 and CAChs3, but inhibits CAChs2. Ni^{+2} inhibits CAChs1 and CAChs2 with little effect on CAChs3. Mg^{+2} stimulates CAChs2 and CAChs3, but hardly affects CAChs1. These characteristics are similar to those of the *Saccharomyces cerevisiae* enzymes excepts in degree. The sensitivity against Ni^{+2} of CAChs1 is higher than that of CAChs2, whereas the reverse is true in *S.cerevisiae*. Metal dependence of chitin synthases in *C. albicans* is less marked than that in *S. cerevisiae*, except for CAChs2. These results could provide new criteria for screening systems of antifungal agents.