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论文

不同碳源下毕赤酵母GS115蛋白组学分析

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摘要:

毕赤酵母(*Pichia pastoris*)表达系统是目前最为成功的外源蛋白表达系统之一。该表达系统不存在原核表达系统的内毒素难以除去的问题,也不存在哺乳动物细胞表达系统的病毒和支原体污染问题;并能够对目的蛋白进行类似于高等真核生物的信号肽剪切、二硫键形成、糖基化等蛋白翻译后加工。但是不论是胞内表达或是分泌表达,大多数外源蛋白均面临着被降解的问题,这也是影响目的蛋白表达量的一个重要因素,同时还增加了纯化目的蛋白的难度。目的:研究毕赤酵母GS115在不同碳源培养过程中胞内外蛋白质组学的差异,指导毕赤酵母表达系统的优化。方法:利用LC-ESI-MS/MS方法分析了不同碳源的四种培养基中毕赤酵母GS115的胞内和胞外蛋白种类,利用Griffin等的计算方法计算各个蛋白的含量。结果:利用LC-ESI-MS/MS结合Griffin等的归一化非标定量法SI_N得到GS115胞内胞外详尽的蛋白质种类及准确百分比含量。结论:分析不同培养基之间蛋白质组成的差异,从而为以后构建新的毕赤酵母表达体系,为外源蛋白表达系统的优化提供一定的指导意义。

关键词: 毕赤酵母(*Pichia pastoris*)GS115 不同碳源培养基 LC-ESI-MS/MS 蛋白质组 表达优化

Proteomic Analysis of *Pichia pastoris* GS115 Cultured in Different Carbon Source Mediums

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Abstract:

Pichia Pastoris is one of the most successful heterologous protein expression systems until now. It doesn't have the endotoxic problem which always existed in the prokaryotic expression system nor the viral contamination of recombinant proteins produced in mammalian cells culture. Furthermore, unlike bacteria, yeasts can carry out the similar post-translational protein processing as higher eukaryotes, such as signal peptide cleavage, disulfide bonds formation and the glycosylation. However, it is not clear which protease may present in the culture media and degrade heterologous protein which leads poor yield of protein of interest. Objective:Comparing the GS115 proteomics differences cultured in different carbon source media to optimize the heterologous protien expression system.Methods:By using LC-ESI- MS/MS combined with Griffin's new calculation method, intracellular and supernatant protein profiling and protein abundance of GS115 in 4 different carbon source media were identified and compared.Results:Intracellular and supernatant protein profiling and related protein abundance of GS115 in 4 different carbon source media were obtained with LC-ESI-MS/MS analysis and Label-free normalized quantification method. Conclusions:The findings will be helpful to develop novel strains which may optimize the *Pichia pastoris* heterologous protein expression system.

Keywords: *Pichia pastoris* GS115 Carbon source mediums LC-ESI-MS/MS Proteomics Expression optimization

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