

## Cupriavidus metallidurans CH34中2个苯酚降解基因簇的筛选与功能鉴定

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### Screening and Characterization of Two Phenol Degrading Gene Clusters from *Cupriavidus metallidurans* CH34

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**摘要** *Cupriavidus metallidurans* (*C.metallidurans*) CH34是一种重金属耐受性细菌,能在以苯酚、甲苯酚、苯甲酸、苯胺等芳香族化合物为唯一碳源和能源的培养基中生长,其基因组中含有2个苯酚降解基因簇.以载体pIndigo BAC 5构建*C.metallidurans* CH34的细菌人工染色体(bacterial artificial chromosome, BAC)文库,获得约3万个克隆,平均插入片段大小为30 kb,插入频率为98%,推测该文库覆盖CH34基因组约1 240倍.用PCR筛选文库中的3 000个单克隆,共获得9个阳性克隆,其中5个克隆含有长基因簇,4个含有短基因簇,并从中得到含有全长苯酚降解基因簇的克隆.利用以苯酚为唯一碳源的无机盐培养基,研究2个基因簇在大肠杆菌中的表达情况.结果显示,两个基因簇均表现出了苯酚降解能力,短簇的降酚能力要优于长簇.

**关键词:** *Cupriavidus metallidurans* CH34 基因组 细菌人工染色体文库 苯酚 基因簇

**Abstract:** *Cupriavidus metallidurans* (*C.metallidurans*) CH34 is a kind of heavy metal resistant bacterium, whose genome own two phenol degrading gene clusters and can grow on the medium that has phenol, cresol, benzoic acid, aniline as sole carbon and energy source. By using vector pIndigo BAC 5, a bacterial artificial chromosome (BAC) library of *C.metallidurans* CH34 was constructed and thirty thousand clones were obtained, from which 98% of the clones had an insertion fragment with average size of 30 kb. It was deduced that the whole BAC library size is about 1 240 times of the genome size. More than 3 000 clones were screened with PCR and nine positive clones were obtained: five with longer gene clusters and four with shorter clusters. Two clones with total length of phenol degrading gene cluster were used to study its expression in *Escherichia coli* (*E.coli*) on the medium using phenol as the sole carbon source. The results indicate that both clones have the ability to degrade phenol, and the one carrying a shorter cluster shows stronger ability than that of the one with longer cluster.

**Keywords:** *Cupriavidus metallidurans* CH34, genome, bacterial artificial chromosome (BAC) library, phenol, gene cluster

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