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睾丸酮丛毛单胞菌*PhaR*基因敲除菌株的构建及其3, 17*β*-HSD对甾族化合物的响应特性

Construction of *PhaR* gene knock-out mutant of *Comamonas testosteroni* and response of its 3, 17*β*-HSD to steroid compounds

关键词：睾丸酮丛毛单胞菌 3,17*β*-羟基类固醇脱氢酶 *PhaR*基因敲除突变株 甾族化合物

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摘要：基于同源重组原理构建了*PhaR*基因敲除的睾丸酮丛毛单胞菌基因工程菌,并对不同诱导条件下菌体细胞的3,17*β*-羟基类固醇脱氢酶(3,17*β*-HSD)的表达情况进行了研究.结果表明,利用电穿孔法获得了一株*PhaR*基因插入失活的睾丸酮丛毛单胞菌突变体PK-4,该突变菌株具有较高的遗传稳定性.建立的ELISA法对3,17*β*-HSD表达的定量分析表明,睾丸酮丛毛单胞菌的野生株和突变株细胞内的3,17*β*-HSD蛋白都可被睾丸酮诱导,但不能被雌二醇和胆固醇诱导.在诱导条件下,突变菌株PK-4产生的3,17*β*-HSD蛋白量是野生型菌株产生量的2.6倍,并且在无诱导条件下,前者的量也明显高于后者,表明*PhaR*为3,17*β*-HSD基因表达的抑制子.雌二醇和胆固醇可明显抑制睾丸酮对睾丸酮丛毛单胞菌3,17*β*-HSD的诱导效应,但在相同条件下,睾丸酮丛毛单胞菌*PhaR*基因敲除的突变菌株PK-4产生的3,17*β*-HSD蛋白量仍然明显高于野生型菌株.说明,*PhaR*基因在睾丸酮丛毛单胞菌的3,17*β*-HSD表达调控方面具有重要意义,并且*PhaR*基因敲除的睾丸酮丛毛单胞菌基因工程菌PK-4在雄性激素污染物的环境污染治理方面还具有潜在的应用价值.

Abstract：*PhaR* gene knock-out mutants of *Comamonas testosterone*, with high genetic stability, were constructed based on homologous integration. The expression of 3,17*β*-HSD in the cells cultured under different conditions was also determined. The results show that one mutant strain PK-4 was obtained by the electroporation method. ELISA method was established for the determination of 3,17*β*-HSD. The ELISA results indicated that the expression of 3,17*β*-HSD was induced by testosterone in cells of wild type and mutant PK-4 of *Comamonas testosteroni*, but estradiol and cholesterol showed no induction effect. Under the condition of testosterone induction, the amount of 3,17*β*-HSD in the cells of mutant PK-4 was 2.6 times the amount in the cells of wide type *Comamonas testosteroni*. Moreover, the expression of 3,17*β*-HSD in the cells of mutant PK-4 was also higher than that in the cells of wide type *Comamonas testosteroni*. This indicates that *PhaR* might be a repressor for the expression of 3,17*β*-HSD in *Comamonas testosteroni*. The addition of estradiol and cholesterol significantly inhibited induction effect of testosterone on the 3,17*β*-HSD expression. However, in the same condition, the expression level of 3,17*β*-HSD in mutant PK-4 cells was still higher than that in wild type *Comamonas testosteroni* cells. The above results show that *PhaR* gene has important significance in the regulation of 3,17*β*-HSD in *Comamonas testosteronei*. The *PhaR* gene knock-out mutant PK-4 has potential application value in the treatment of polluted environment with androgenic hormones.

Key words：*Comamonas testosteroni* 3,17*β*-HSD gene expression *PhaR* gene knock-out mutant steroid compounds