



### 深黄被孢霉 $\Delta^{12}$ -脂肪酸脱氢酶基因RNAi表达载体的构建

丁丽娜, 胡彬彬, 林连兵, 季秀玲, 魏云林, 张琦

昆明理工大学 生命科学与技术学院 生物工程技术研究中心, 云南 昆明 650500

### Construction of an RNAi plasmid targeting *Mortierella isabellina* $\Delta^{12}$ -fatty acid desaturase gene

DING Li-na, HU Bin-bin, LIN Lian-bing, JI Xiu-ling, WEI Yun-lin, ZHANG Qi

Biotechnology Research Center, Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650224, China

- 摘要
- 参考文献
- 相关文章

全文: PDF (1286 KB) HTML ( KB) 输出: BibTeX | EndNote (RIS) 背景资料

**摘要** 深黄被孢霉是国内研究生产 $\gamma$ -亚麻酸( $\gamma$ -linolenic acid, GLA)和花生四烯酸(Arachidonic acid, AA)等多不饱和脂肪酸(Polyunsaturated fatty acid, PUFA)的主要产油丝状真菌。前期的实验结果表明,深黄被孢霉M6-22具有潮霉素抗性,而且目前也没有关于深黄被孢霉营养缺陷型菌株的报道,限制了一些基于深黄被孢霉菌株进行遗传操作的研究。研究以红色荧光蛋白 $DsRED$ 基因作为报告基因,构建能同时用于丝状真菌外源基因和RNAi表达载体pS-DsRED。通过PEG/CaCl<sub>2</sub>原生质体转化法将pS-DsRED导入深黄被孢霉M6-22中进行表达,成功获得产粉红色的阳性菌落,并在此基础上构建了深黄被孢霉 $\Delta^{12}$ -脂肪酸脱氢酶基因RNAi表达质粒pSREDMID12RNAi,为下一步目的基因的敲除和基因功能分析奠定了基础。

**关键词:** 深黄被孢霉  $DsRED$ 筛选标记 RNAi表达载体  $\Delta^{12}$ -脂肪酸脱氢酶基因

**Abstract:** *Mortierella isabellina* is an oil-producing fungal species which has been widely studied for producing polyunsaturated fatty acids including  $\gamma$ -Linolenic acid and arachidonic acid in China. Our previous research showed that *Mortierella isabellina* M6-22 is a hygromycin resistant fungal strain, and no auxotrophic mutant strains from *M. isabellina* were constructed so far, which limits its applications of genetic manipulation. In this study, *DsRed* gene which encodes a red-fluorescent protein was used as a report gene to construct a vector for both heterologous filamentous fungal gene and RNAi expression. The resultant vector pS-DsRED was further delivered into *M. isabellina* M6-22 by PEG/CaCl<sub>2</sub>-mediated protoplast transformation for expression, and acquired pink positive colonies. Based on the pS-DsRED, an RNAi expression plasmid pSREDMID12RNAi targeting *Monierella isabellina* delta-12-fatty acid desaturase gene was constructed, which provided a basis for the subsequent target gene knockdown and gene functional analysis.

**Key words:** *Monierella isabellina*  $DsRED$  selectable marker RNAi plasmid  $\Delta^{12}$ -fatty acid desaturase gene

收稿日期: 2011-08-30;

基金资助: 国家自然科学基金资助项目(31160016); 云南省应用基础研究基金资助项目(KKSA201126005).

通讯作者: 张琦(1975-), 男, 云南人, 博士, 主要从事微生物分子生物学方面的研究. E-mail: qzhang37@gmail.com. E-mail: qzhang37@gmail.com

引用本文:

丁丽娜, 胡彬彬, 林连兵等. 深黄被孢霉 $\Delta^{12}$ -脂肪酸脱氢酶基因RNAi表达载体的构建[J]. 云南大学学报(自然科学版), 2012, (2): 242-248.

DING Li-na, HU Bin-bin, LIN Lian-bing et al. Construction of an RNAi plasmid targeting *Mortierella isabellina* $\Delta^{12}$ -fatty acid desaturase gene[J]. , 2012, (2): 242-248.

[1] STUMPF P K. The biosynthesis of saturated and unsaturated fatty acids[M]//STUMPF PK, CONN E E. The Biochemistry of Plants, New York: Academic Press, 1980, 4: 177-204.

[2] PEREIRA S L, LEONARD A E, MUKERJI P. Recent advances in the study of fatty acid desaturases from animals and lower eukaryotes

#### 服务

- ▶ 把本文推荐给朋友
- ▶ 加入我的书架
- ▶ 加入引用管理器
- ▶ E-mail Alert
- ▶ RSS

#### 作者相关文章

- ▶ 丁丽娜
- ▶ 胡彬彬
- ▶ 林连兵
- ▶ 季秀玲
- ▶ 魏云林
- ▶ 张琦

[J].Prostaglandins Leukot Essent Fatty Acids,2003,68(2):97-106. 

- [3] 李明春,刘莉,胡国武,等.深黄被孢霉 $\Delta^6$ -脂肪酸脱氢酶基因在转基因烟草中的表达[J].生物工程学报,2003,19(2):178-183.
- [4] 卜云萍,王广科,胡国武,等.深黄被孢霉 $\Delta^6$ -脂肪酸脱氢酶基因导入大豆[J].生物技术,2003,13(3):6-8. 
- [5] 刘莉,李明春,胡国武,等.深黄被孢霉M6-22  $\Delta^6$ -脂肪酸脱氢酶基因在酿酒酵母中的表达[J].微生物学报,2001,41(4):397-401.
- [6] 李明春,李航,张琦,等. $\Delta^{12}$ -脂肪酸脱氢酶基因在大肠杆菌中的表达[J].微生物学通报,2004,31(4):43-48. 
- [7] 张学炜,王笑梅,李明春,等.深黄被孢霉中 $\Delta^6$ -脂肪酸脱氢酶基因敲除及鉴定[J].南开大学学报:自然科学版,2007,40(2):67-72.
- [8] 李明春,刑来君. $\gamma$ -亚麻酸生产菌深黄被孢霉原生质体的形成和再生[J].菌物系统,1997,16(1):24-29.
- [9] 徐新丽,谢必峰.深黄被孢霉3.3410原生质体的制备和再生研究[J].生物技术,2009,12(2):46-48.
- [10] 张学炜,王笑梅,李明春,等.以潮霉素B抗性为选择标记的深黄被孢霉原生质体转化[J].生物工程学报,2007,23(3):462-466. 
- [11] COOLEY R N,SHAW R K,FRANKLIN F C H,et al.Transformation of the phytopathogenic fungus *Septoria nodorum* to hygromycin B resistance [J].Current Genetics,1988,13(5):383-389. 
- [12] ZHOU X,WEI Y,ZHU H,et al.Protoplast formation,regeneration and transformation from the taxol-producing fungus *Ozonium* sp.[J].African Journal of Biotechnology,2008,7(12):2 017-2 024.
- [13] 张志光.真菌原生质体技术[M].长沙:湖南科学技术出版社,2003.
- [14] 陈赞娟,韦建福.丝状真菌遗传转化的研究进展[J].云南农业大学学报,2009,24(3):448-454.
- [15] 黄亚丽,叶婧,蒋细良,等.真菌转化系统的研究进展[J].微生物学通报,2007,34(6):1 213-1 217.
- [16] MIZUNO H,SAWANNO A,ELI P,et al.Red fluorescent protein from *Discosoma* as a fusion tag and a partner for fluorescence resonance energy transfer[J].Biochemistry,2001,40(8):2 502-2 510. 
- [17] RODRIGUES F,VAN HEMERT M,STEENSMA H Y,et al.Red fluorescent protein (DsRed) as a reporter in *Saccharomyces cerevisiae*[J].J Bacteriol,2001,183:3 791-3 794.
- [18] 郝丽梅,李唐棣,梅兴国.红色荧光蛋白的研究进展[J].国外医学药学分册,2006,33(2):131-133.
- [19] 陈坚,薛绪潮,方国恩,等.携带红色荧光蛋白的RU486可诱导真核表达载体的构建及其表达[J].生物工程学报,2008,24(8):1 458-1 463.
- [20] MIKKELSEN L,SARROCCO S,LVBECK M,et al.Expression of the red fluorescent protein DsRed-Express in filamentous *Ascomycete* fungi [J].FEMS Microbiol Lett,2003,223(1):135-139. 
- [21] JANUS D,HOFF B,HOFMANN E,et al.An efficient fungal RNA-silencing system using the DsRed reporter gene[J].Appl Environ Microbiol,2007,73 (3):962-970. 
- [22] ATKAINS S D,MAUCLINE T H,KERRY B R,et al.Development of a transformation system for the *Nematophagous* fungus *Pochonia chlamydosporia*[J].Mycol Res,2004,108(6):654-661. 
- [23] FITZGERALD A M,MUDGE A M,GLEAVE A P,et al.Agrobacterium and PEG-mediated transformation of the phytopathogen *Venturia inaequalis* [J].Mycol Res,2003,107(7):803-810. 

没有找到本文相关文献

版权所有 © 《云南大学学报(自然科学版)》编辑部

编辑出版: 云南大学学报编辑部 (昆明市翠湖北路2号, 650091)

电话: 0871-5033829(传真) 5031498 5031662 E-mail: yndxxb@ynu.edu.cn yndxxb@163.com