中国真菌学杂志 2010, 5(2) 101-104 DOI: ISSN: 1673-3827 CN: 31-1960/R

论著

新生隐球菌的AFLP分析

邹先彪[1], 温海[2], 廖万清[2], 吴建华[3], 仇芸[2], 杨金水[4]

1. 北京市解放军总医院304临床部皮肤科, 北京, 100048;
2. 第二军医大学长征医院皮肤科, 上海, 200003;
3. 第二军医大学长海医院皮肤科, 上海, 200433;
4. 复旦大学遗传所, 上海, 200433

摘要： 目的 评估AFLP-DNA指纹技术在新生隐球菌分类中应用情况。方法 新生隐球菌基因组DNA用双酶酶切,双链接头连于其酶切末端,用与接头和酶切位点互补的引物扩增DNA片段,其产物在高分辨的变性聚丙酰胺凝胶上电泳分离,然后进行银染。结果 分析来自5种血清型和临床分离株的18株新生隐球菌,可见有30多条大小在30～500bp的DNA-AFLP指纹,相同的血清型有不同的指纹图谱,来自同一患者不同病期的两株分离株和来自同一患者患者的不同部位的两株分离株都显示出相同的带型。结论 显示了AFLP的高分辨率,是适用于新生隐球菌流行病学调查的有力工具。

关键词： 新生隐球菌 DNA 扩增片段长度多态性

Amplified fragment length polymorphism analysis of Cryptococcus neoformans

ZOU Xian-biao[1], WEN Hai[2], LIAO Wan-qing[2], WU Jian-hua[3], QIU Yun[2], YANG Jin-shui [4]

1. Department of Dermatology, 304th Hospitol Affiliated to PLA General Hospital, Beijing 100048, China;
2. Department of Dermatology, Changzheng Hospitol, The Second Military Medical University, Shanghai 200003, China;
3. Department of Dermatology, Changhai Hospitol, The Second Military Mcrlical Universtiy, Shanghai 200433, China;
4. Insititute Genetic of Fudan Universtiy,Shanghai 200433, China;

Abstract: Objective To evaluate AFLP-DNA fingerprinting method in the discrimination of C.neoformans.Methods Genomic DNA from C.neoformans was digested simultaneously with two restriction enzymes.Double-stranded adapters were ligated to the ends of restricted fragments and DNA fragments were amplified by PCR using primers complementary to the adapters and the restriction site sequences.AFLP products were separated using high resolution denaturing polyacrylamide gel electrophoresis and visualized by silver staining.Results DNA fingerprint patterns of 18 C.neoformans isolates from five serotypes and clinical isolates were analyzed by AFLP.AFLP fingerprints comprised over 30 bands detected in size range 30-500 bp.Different AFLP patterns were detected in isolates of the same serotype and identical banding patterns were observed from in isolates from the same patient at different time interals and different sources from one patiant.Conclusions AFLP is highly discriminatory,and powerful epidemiological tool for improving our understanding of C.neoformans.

Keywords: *Cryptococcus neoformans* DNA AFLP

收稿日期 2010-02-11 修回日期 网络版发布日期

DOI:

基金项目:

国家自然科学基金(39770002)

通讯作者:

作者简介: 邹先彪,男(汉族),博士,副主任医师.E-mail:xbzou@126.com

作者Email:

参考文献：

[1] Luciana T,Laz ra MS,Wanke B,et al.Regional pattern of the molecular types of Cryptococcus neoformans and Cryptococcus gattii in Brazil[J].Mem Inst Oswaldo Cruz,2008,103 (5):455 -462.
[2] Reins E,Tanaka k,Burger G,et al,Molecular diagnosis and epidemiology of fungal infections[J].Med Mycol,1998,36(Suppl1):249-257.

[3] Yang CD,Zhang JW,XU Q,et al.Establishment of AFLP technique and assessment of primer combinations for mei flower[J].Plant Molecular Biology Reporter,2005,23 (3):79 a-791.
[4] Day JN.Cryptococcal meningitis[J].Practical Neurology,2004,4(5):274-285.
[5] Sato Y,Osabe S,Kuno H,et al.Rapid dignosis of cryptococcal meningitis by microscopic examination of centrifuged cerebrospinal fluid sediment[J].J Neurol Science,1999,164(1):72-75.
[6] Meyer W,Mitchell TG.Polymerase chain reaction fingerprinting in fungi using single primers specfic to minisatellites and simple repetivive DNA sequences:strain variation in Cryptococcus neoformans [J].Electrophoresis,1995,16(9):1648-1656.
[7] 温海,廖万清,姚志荣,等.新生隐球菌随机扩增DNA指纹分型研究[J].中华皮肤科杂志,1998,31(5):295-297.
[8] 顾菊林,廖万清,柴建华.新生隐球菌的PCR指纹分析[J].中华皮肤科杂志,1998,31(5):313-314.
[9] Currie BP,Freundlich LF,Cassadevall A,et al.Restriction fragement length polymorphism analysis of Cryptococcus neoformans isolates from environmental(pigeon excreta) and clinical sources in the New York City[J].J Clin Microbiol,1994,32 (5):1188-1192.
[10] Eudtz HP,Ang CW,Braak N,et al.Molecular characterization of Campylobacter jejuni from patients with Guillan-Barre and Miller Fisher Syndromes[J].J Clin Microbiol,2000,38 (6):2297-2301.
[11] Restrepo S,Duque M,Tohme J,et al.AFLP fingerprint:an efficient technique for detecting genetic variation of Xanthomonas anonopodis pv.manihotis[J].Microbiol,1999,145 (Pt 1):107-114.
[12] Wendy WJ,Gorkink R,Simons G,et al.Genotyping of Madurella mycetomatis by selective amplification of restriction fragments (AFLP (R)) and subtype-correlation with geographical origin and lesion size[J].J Clin Microbiol,2005,43 (9):4349-4356.

本刊中的类似文章

1. 李平, 温海, 朱红梅, 徐红, 胡婵, 谭宏月.隐球菌免疫相关的甘露糖受体MR重组腺病毒载体的构建及鉴定[J]. 中国真菌学杂志, 2012,(2): 70-72
2. 李秀丽, 田媛, 史玉玲, 顾俊瑛, 李晓建, 刘至昱, 马越娥, 高飞, 王葆葆.新生隐球菌MIS1基因的siRNA表达载体的构建及鉴定[J]. 中国真菌学杂志, 2012,7(1): 17-19,23
3. 李秀丽, 田媛, 史玉玲, 顾俊瑛, 刘至昱, 李晓建, 高飞.蒺藜中甾体皂苷对新生隐球菌生物膜形成的抑制作用[J]. 中国真菌学杂志, 2011,6(6): 341-343
4. 周南, 黄晨, 潘炜华, 廖万清.舍曲林抗新生隐球菌的体外及动物实验研究[J]. 中国真菌学杂志, 2011,6(5): 267-270
5. 王晓慧, 刘伟, 李若瑜.简并PCR结合RACE技术克隆申克孢子丝菌未知过氧化氢酶基因[J]. 中国真菌学杂志, 0,(): 271-275
6. 潘炜华, 廖万清, 温海, 赵瑾, Ferry Hagen, Teun Boekhout.利用微卫星标记研究新生隐球菌分子流行病学[J]. 中国真菌学杂志, 0,(): 281-284
7. 赵正娟, 田伟, 赵敬军.DNA序列分析用于常见致病真菌鉴定和分型[J]. 中国真菌学杂志, 2011,6(5): 316-320
8. 周南, 黄晨, 潘炜华, 廖万清.舍曲林抗新生隐球菌的体外及动物实验研究[J]. 中国真菌学杂志, 0,(): 267-270
9. 王晓慧, 刘伟, 李若瑜.简并PCR结合RACE技术克隆申克孢子丝菌未知过氧化氢酶基因[J]. 中国真菌学杂志, 2011,6(5): 271-275
10. 潘炜华, 廖万清, 温海, 赵瑾, Ferry Hagen, Teun Boekhout.利用微卫星标记研究新生隐球菌分子流行病学[J]. 中国真菌学杂志, 2011,6(5): 281-284
11. 赵正娟, 田伟, 赵敬军.DNA序列分析用于常见致病真菌鉴定和分型[J]. 中国真菌学杂志, 0,(): 316-320
12. 冉梦龙, 鲁巧云, 涂平, 万喆, 杨淑霞, 吴艳, 李若瑜, 王爱平.播散性隐球菌病1例及其实验研究[J]. 中国真菌学杂志, 2011,6(4): 207-211
13. 张晓利, 吕雪莲, 沈永年, 吕桂霞, 王淼淼, 葛一平, 刘维达.4种黑曲霉基因组DNA提取方法的比较[J]. 中国真菌学杂志, 2011,6(3): 145-148
14. 孙继梅, 王艳玲, 周秀珍, 郑伟, 张智洁, 刘勇.新生隐球菌感染12例临床特点及实验室检测的回顾性分析[J]. 中国真菌学杂志, 2011,6(3): 154-157
15. 胡小平, 万喆, 王晓红, 李若瑜.我国代表地区须癣毛癣菌复合体的分子鉴定与分型研究[J]. 中国真菌学杂志, 2011,6(2): 70-76