

论著

曲霉生物膜的形成过程与结构特征

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摘要: 目的 研究曲霉生物膜的形成过程和结构特征。方法 我们利用一个曲霉生物膜体外模型研究其形成过程和结构特征。将200μL浓度为1×10⁵孢子/mL的受试曲霉(烟曲霉AF293,黄曲霉BMU03940,土曲霉BMU00802,黑曲霉BMU04689)的孢子悬液加到24孔组织培养板中的无菌塑料细胞培养盖玻片上,37℃孵育不同时间(0、2、4、8、10、12、16、18、24、48、72h),加入25μmol/L的FUN-1室温避光染色后,用波长488nm激光激发,通过共聚焦激光扫描显微镜观察曲霉生物膜的形成过程;再用波长为488nm和633nm激光同时激发,将两个波长下的图像叠加后观察曲霉生物膜的活力;利用XYZ轴成像观察其结构特征。在上述不同的时间点用钙荧光白染色后,用波长为405nm的紫外光激发,观察曲霉生物膜细胞外基质的产生。结果 烟曲霉AF293在第4h即开始有散在的孢子黏附于盖玻片上;8h时孢子开始萌芽,10-12h菌丝延长形成单细胞层;16-20h菌丝缠绕形成多层立体结构;24h形成一个具有复杂的三维立体结构特征的多细胞菌落,菌丝有序排列,细胞外基质弥散分布在菌丝的周围;48-72h生物膜逐渐成熟。成熟的烟曲霉生物膜是由细胞外基质包裹的有序排列的菌丝形成的复杂立体结构。黄曲霉BMU03940、土曲霉BMU00802、黑曲霉BMU04689与烟曲霉AF293有类似的生物膜发育阶段,包括黏附、孢子萌芽、菌丝延长、菌丝有序排列形成三维立体结构。结论 烟曲霉、黄曲霉、土曲霉和黑曲霉在体外都能形成典型的生物膜,它的形成过程和结构特征与其他真菌生物膜类似。

关键词: 曲霉 生物膜 结构特征

Development and architecture characteristics of Aspergillus biofilm

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Abstract: Objective To investigate the development and architecture characteristics of Aspergillus biofilm.Methods In vitro biofilms of Aspergillus fumigatus(AF293),Aspergillus flavus(BMU03940),Aspergillus terreus(BMU00802) and Aspergillus niger(BMU04689) formed on the surface of sterile plastic cell culture coverslips in 24-well tissue culture plates by adding 200 μL of 1×10⁵ spores/mL cell suspension to each well.After incubation at 37℃ for various time periods (2,4,8,10,12,16,18,24,48 and 72h),the biofilms were stained with FUN-1 in dark for 45 minutes.The forming process and vitality were examined under confocal microscope by 488 or combination with 633 argon ion laser stimulation.Calcofluor White stain was applied to observe cytoplasm production with 405 argon ion laser triggering.Results A.fumigatus conidial adhesion occurred at 4h and conidia began to swell and germinate at 8h.Hyphae intertwined and amonolayer formed at 10 to 12h,followed by network structure forming at 16 to 20h.Then the complex three-dimensional architecture with extracellular matrix aroundhyphae was set up at 24h,with gradual matureation at 48 to 72h.Similar to A.fumigatus,the biofilms of A.flavus,A.terreus and A.nigerhad distinct developmental phases including adhesion,germination,filamentation,monolayer development,proliferation and maturation.Conclusions A.fumigatus,A.flavus,A.terreus and A.niger are able to form typical biofilm with distinct developmental phases and architecture characteristics.

Keywords: Aspergillus spp biofilm architecture characteristic

收稿日期 2010-09-17 修回日期 网络版发布日期

DOI:

基金项目:

国家自然科学基金(30970131);北京市自然科学基金(7102149)

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