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专论与综述

黄曲霉毒素 B_1 生物脱毒的研究进展

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摘 要：黄曲霉毒素是一组由黄曲霉、寄生曲霉等多种真菌产生的次级代谢产物，具有强烈的毒性，可以引起动物肝脏肿大、病变甚至癌变，对人和家畜的健康产生极大的威胁。本文简介了黄曲霉毒素 B_1 的分子结构、理化性质、污染现状，综述了黄曲霉毒素 B_1 生物脱毒方面及其应用的研究进展，重点讲述通过微生物降解黄曲霉毒素 B_1 的研究近况。

关键词：黄曲霉毒素 B_1，生物脱毒，应用，微生物降解

Progress in biological detoxification of Aflatoxin B_1

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Abstract: Aflatoxin, with strong toxicity, is a group of secondary metabolites produced by the *Aspergillus flavus*, *Aspergillus parasitieus* and some other fungies. Aflatoxin can lead to liver enlargement, diseases and even cancer, showing a great threat to humans and animals health. This article introduces the molecular structure, physical and chemical properties, pollution situations of aflatoxin B_1. Research progress about the biological detoxication especially microbiological degradation of aflatoxin B_1 and application are discussed.

Keywords: Aflatoxin B_1, Biological detoxification, Application, Microbiological degradation

黄曲霉毒素 B_1 (Aflatoxin B_1，AFB_1)是二呋喃香豆素的衍生物，其分子结构如图 1 所示，AFB_1 被世界卫生组织(WHO)列为毒物之首，2002 年被国际肿瘤研究机构(IARC)列为Ⅰ级强致癌物[1-3]，在食品、饲料等行业中，AFB_1 的脱毒显得尤为重要。AFB_1 毒素在 268−269 °C 时才分解，在强碱环境下被完全破坏，酸性条件下稳定，在 pH 1.0−3.0 的强酸性溶液中才有极少量的分解，但在 pH 9.0−10.0 碱性溶液中会迅速分解。AFB_1 易溶于氯仿、丙酮、甲醇、乙醇等多种有机溶剂中，但不溶于乙醚、石油醚、己烷和水。所以，简单的物理化学方法难适用于 AFB_1 脱毒。

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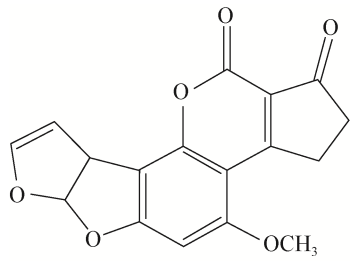


图 1 黄曲霉毒素 B_1 分子结构
Figure 1 The molecular structure of aflatoxin B_1

2013 年 3 月，据荷兰乳业组织透露，在两家荷兰牛奶农场的牛奶中发现了过量的黄曲霉毒素，被污染的牛奶立即从生产链上撤下销毁。随后美国一家公司发布召回公告，将对几款狗粮实施召回，还有很多乳制品也存在毒素污染情况。有许多检验报告表明，我国的饲料、坚果、牛奶等食品中仍可检测出 AFB_1，甚至含量存在超标的情况[4-8]。AFB_1 的污染仍是在食品、饲料等行业中亟待解决的重要问题。

AFB_1 的脱毒方法分物理、化学以及生物法(表 1)。根据联合国粮农组织(FAO)规定，黄曲霉毒素的脱毒工艺必须满足以下条件：(1) 去除黄曲霉毒素或者使之失去活性；(2) 不产生任何有毒的副产物；(3) 破坏产毒的真菌孢子或菌丝体；(4) 脱毒后食品或饲料应保持它原有的营养价值和适口性。

表 1 黄曲霉毒素 B_1 的脱毒方法
Table 1 The detoxification methods of AFB_1

项目 Item	物理法 Physical method	化学法 Chemical method	生物法 Biological method	
			吸附法 Adsorption	降解法 Degradation
具体措施 Specific measures	加热、辐照、溶剂萃取、吸附剂吸附等[9-14] Heating, radiation, solvent extraction, adsorbent, etc[9-14]	碱处理、氧化法(臭氧、过氧化氢处理)等[15-19] Alkali treatment, oxidation (ozone, hydrogen peroxide), etc[15-19]	干酪乳杆菌、酿酒酵母、鼠李糖乳杆菌等，利用其细胞壁吸附毒素[20-21] *Lactobacillus casei*, *Saccharomyces cerevisiae*, *Lactobacillus rhamnosus*, etc. Using their cell walls to adsorb toxins[20-21]	黑曲霉、枯草芽孢杆菌等通过发酵或产酶降解毒素[22-24] Fermentation or enzyme degrade toxin by *Aspergillus niger*, *Bacillus subtilis*, etc[22-24]
脱毒效果及评估 Detoxication effect and evaluation	去毒效果好，固态样品中脱毒不彻底。存在试剂回收问题；仪器成本太高；可能改变物质的性质；可应用的领域小 The detoxication effect is better for liquid sample than solid. There are some problem such as reagent recycle, cost too much, may change the nature of the substance, small application field	脱毒效果良好。易导致副产物的产生以及处理剂的残留，且容易造成二次污染；对产品的成分有影响 Detoxification effect is good. It is easy to lead to the generation of by-products, the residual of treatment agent and secondary pollution. The compositions of the products could be impacted	同物理吸附，对于液态样品脱毒效果更好。存在毒性的可逆性恢复，导致去毒不彻底；去毒周期长 As well as physical adsorption, the detoxification effect for liquid samples is better. Some problems are as follows: the toxin reversibly recover, resulting in that detoxification is not complete, long detoxification period	脱毒效果彻底。高效性，无污染，无危害；所需的条件相对温和；还有增加产品营养价值的可能性 Detoxification effect is complete. There are many advantages such as efficient, non-polluting, non-hazardous, conditions are relatively mild. There is the possibility of increasing the nutritional value of products

1 黄曲霉毒素 B_1 的生物脱毒

AFB_1 的生物脱毒主要是利用微生物或其产生的酶及其制剂通过生物催化的方法脱毒，与物理、化学方法相比生物脱毒条件相对温和，产品的品质得以保证，甚至有些益生菌还能提升产品的营养价值。微生物对 AFB_1 的作用主要有两方面：(1) 微生物细胞对 AFB_1 的吸附作用；(2) 微生物发酵产生的酶对 AFB_1 的降解作用。

1.1 微生物吸附黄曲霉毒素 B_1

自然界中有些微生物可以吸附 AFB_1，如：乳酸菌、酵母菌等，形成菌体-毒素复合体。这些微生物吸附 AFB_1 后，其吸附能力下降，就会同 AFB_1 一起被排出体外，从而降低毒素对畜禽的伤害。

乳酸菌的脱毒机理主要是细胞壁中 N-乙酰胞壁酸及 N-乙酰葡萄糖胺为主要成分的肽聚糖对 AFB_1 进行物理吸附。Hernandez-Mendoza 等[20-21]筛选得到对 AFB_1 有高吸附率的干酪乳杆菌 *Lactobacillus casei* L30；并发现毒素-菌体复合物的稳定性良好，其吸附过程是一个有限制的可逆过程，反复水洗后只有极少的毒素解吸附，证明了细胞中胞壁酸成分对吸附 AFB_1 有重要作用。

酵母细胞因其细胞壁中含有葡聚糖、甘露聚糖、几丁质等特殊成分使其具有较好吸附 AFB_1 的能力。Shetty 等[25]筛选出的酿酒酵母 A18 和 26.1.11，对 PBS 中 AFB_1 的吸附能力分别为 38.7% 和 36.1%；并提出了酵母吸附 AFB_1 的原理是其细胞壁上的碳水化合物或甘露聚糖参与了毒素的吸附。在国内，刘畅等[26]首次筛选出了吸附效果最佳的酿酒酵母 Y1，其对 AFB_1 的吸附率高达 81.16%。所以，从酵母细胞中提取的葡聚糖、甘露聚糖等成分可广泛应用于畜禽饲养过程中，降低 AFB_1 对畜禽的伤害。

虽然有较多的微生物对 AFB_1 具有吸附作用，但这种吸附不能从根本上去除 AFB_1，因此，研究 AFB_1 的微生物降解具有十分重要的意义。

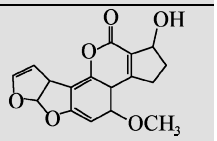
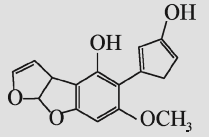
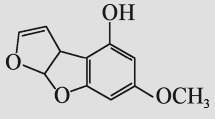
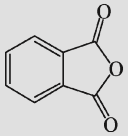
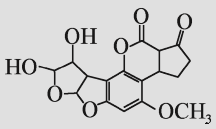
1.2 微生物降解黄曲霉毒素 B_1

AFB_1 的微生物降解是指微生物所分泌的胞内、胞外酶可以将 AFB_1 分子破坏，同时产生无毒的降解产物的过程。

1.2.1 黄曲霉毒素 B_1 降解产物的结构：毒性的减弱是 AFB_1 脱毒的必要条件。目前已知的一些无毒或低毒降解产物结构见表 2。

1.2.2 微生物酶降解黄曲霉毒素 B_1：目前，已发现有很多微生物可以产生能降解 AFB_1 的酶。在国内，Liu 等[29]和 Li 等[30]最早研究生物降解 AFB_1，发现假蜜环菌(*Armillariella tabescens*)及其胞内多酶复合体可以作用于 AFB_1 双呋喃环的烯醚键生成 AFB_1 的环氧化物，再经水解生成 AFB_1-二氢二醇达到去除毒素的目的；并得到黄曲霉毒素氧化酶(Aflatoxin-oxidase)。通过酶处理 AFB_1，可以发现 AFB_1 的特征性蓝色荧光明显减弱，说明了毒素的内酯环被破坏，这将有助于 AFB_1 降解机制的深入探究。该酶的基因已经获得，并成功进行了重组表达，将会逐步应用于 AFB_1 的检测和去除。

表 2 黄曲霉毒素 B_1 降解产物结构
Table 2 The structure of production from AFB_1 degradation

名称 Name	分子式 Molecular formula	结构 Structure
Aflatoxinal	$C_{17}H_{14}O_6$ (314)	
AFD_1[27]	$C_{16}H_{14}O_5$ (286)	
AFD_2[27]	$C_{11}H_{10}O_4$ (206)	
AFD_3[27]	$C_8H_4O_3$ (149)	
AFB_1-8,9-dihydrodiol[28]	$C_{17}H_{14}O_8$ (346)	

漆酶是目前已知的一种广泛存在的 AFB_1 降解酶。王会娟等[31]和周露等[32]筛得了糙皮侧耳(*Pleurotus ostreatus*) P_1，P_1 的产酶能力高，培养条件优化后，降解率可达 82.43%。Alberts 等[33]和 Zeinvand-Lorestani 等[34]还利用糙皮侧耳(*Pleurotus ostreatus*)、隔孢伏革菌(*Peniophora*)、变色栓菌(*Trametes versicolor*)和黑曲霉(*Aspergillus niger*)产生的漆酶及其重组酶使 AFB_1 的致突变性完全消失，AFB_1 的荧光减弱，降解产物的诱变性显著降低。漆酶在 AFB_1 分子上的作用靶点是双呋喃环结构并将其氧化，证明了漆酶可以作为一种有效生物降解真菌毒素的生物大分子广泛运用于食品生产，而且糙皮侧耳作为食用菌，以其作为材料的食品工业产品将对 AFB_1 的防治和去毒有良好的应用前景。

最新发现的过氧化物酶 MnP 为酶降解毒素提供了新思路。Wang 等[28]发现白腐真菌(*Phanerochaete sordida* YK-624)产生的能降解木质素的锰过氧化物酶 MnP 具有 AFB_1 降解功能，MnP 与 AFB_1 降解率高达 86.0%，加入 Tween 80 后促进了 AFB_1 的降解。Umu 试验检测处理后的 AFB_1 诱变活性降低了 69.2%。分析其降解机制是 AFB_1 首先被 MnP 氧化为 AFB_1-8,9-环氧化物，然后水解转化成 AFB_1-8,9-二氢二醇(图 2)。这也是首次关于 MnP 可以将 AFB_1 转化为 AFB_1-8,9-二氢二醇来降低 AFB_1 诱变性的报道。根据这种酶的作用机制，用氧化酶类进行 AFB_1 的脱毒也是一种合理的思路。

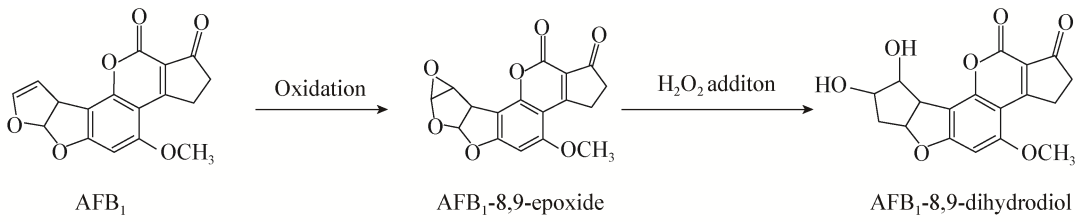
具有 AFB_1 降解能力的菌株还有很多，如 Sangare 等[35]选育出的假单胞菌(*Pseudomonas*)、Shcherbakova 等[36]筛选的茎点霉菌(*Phoma glomerata*) PG41、Adebo 等[37]选育的沃氏葡萄球菌(*Staphylococcus warneri*)、*Lysinibacillus fusiformis*，蔡俊等[38-40]筛选出了可以降解 AFB_1 的菌体 *Sinornonas* sp. 和夏孢生枝孢(*Cladosporium uredinicola*)，夏孢生枝孢降解 AFB_1 后可以检测到在紫外光下呈蓝色荧光的产物。利用加热、蛋白酶 K、SDS 处理后毒素的降解率明显降低，由此推断这些菌体也可以产降解 AFB_1 的酶。

2 黄曲霉毒素 B_1 生物脱毒应用

饲料、食品生产中 AFB_1 的脱毒是保证畜禽健康养殖和食品安全的重要环节，也是研究的热点。AFB_1 生物脱毒技术的应用研究主要体现在以下几个方面：

一方面，改善发酵工艺方法。Chen 等[41]利用嗜热链球菌(*Streptococcus thermophilus*)和保加利亚德氏乳杆菌(*Lactobacillus delbrueckii* subsp. *bulgaricus*)将花生粕加热 10 min 后再与菌混合进行厌氧发酵，3 d 检测花生粕中 AFB_1 的转化率达到 100%，细胞毒性试验检测花生粕中毒性已经几乎完全丧失，而且加热和厌氧发酵并未改变花生粕中氨基酸浓度等其他性质。通过发酵工艺的改善从而优化毒素降解工艺，这对以花生为原料的产品的安全性提供了保障。以微生物发酵为基础，对发酵条件等加工方式的改良从而达到去毒目的，这是一种在生产实际中适当的 AFB_1 生物脱毒方法。

另一方面，研究和生产可降解 AFB_1 的酶制剂产品。曾凡正等[42]将酶法脱胶(采用磷脂酶 A_1)与去毒反应相结合，应用于花生油的精炼工艺，AFB_1 降解率高达 81%，反应后花生油也达到精炼要求



AFB_1 AFB_1-8,9-epoxide AFB_1-8,9-dihydrodiol

图 2 Mn 过氧化物酶降解 AFB_1 过程

Figure 2 The process of degradation of AFB_1 by MnP

(磷含量降至 13 mg/kg)。汤海欧等[43]在饲粮中添加葡萄糖氧化酶(GOD)后，肉鸭各项生长性能有显著改善。胡常英等[44]研究表明，在小鸡饲粮或饮用水中加入 GOD，小鸡的存活率明显升高，没有表现其他不良现象。程伟等[45]和 Zuo 等[46]在饲粮中添加了益生菌和 AFB_1 降解酶明显改善了肉鸡的生长、代谢及体内益生菌的积累。由此可见，益生菌、AFB_1 降解酶及他们的组合应用，可以为食品安全和家禽健康提供有效保障。酶制剂在脱毒方面已经有了初步的应用，并且有了明显的成效，但还存在一些问题如：酶的需求量过大，酶与产品混合使用时的合理配比，保证酶在贮藏过程中的活性等。目前，AFB_1 的生物脱毒方面应用显得贫乏、简单，一些酶的功能性机制还没有研究彻底，对这些酶进行固定化、修饰用于生产以及合适的生物反应器的制作等工艺还需要更进一步的研究。

3 总结与展望

近年来，对 AFB_1 降解方法的研究从物理、化学、生物各方面的脱毒技术都取得了一些成果，相较之下生物法应是最优的选择，却也存在各种各样的弊端，如需求温和的脱毒环境难以保证，没有合适的反应器等。所以，具有吸附作用的微生物要分析并提取出其吸附毒素的有效成分，结合益生菌制备脱毒剂更有应用价值；微生物降解 AFB_1，能够从根本上将其去除，对于那些能够降解 AFB_1 的微生物，应提取、分离、纯化 AFB_1 降解酶，在明确其降解原理的基础上找到安全、有效的固定化载体，解决酶在脱毒反应过程中容易失活的问题，而且要确保 AFB_1 降解产物的食用安全性，还要根据酶的性质和应用方法设计出合适的生物反应器，用于各类产品生产。

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