

豆类丝核菌发酵代谢产物中苦马豆素的测定^{*}

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[摘 要] 利用气相色谱法, 对实验室分离保存的 6 株豆类丝核菌发酵代谢产物中的苦马豆素(swainsonine, SW)进行了定性、定量分析。结果表明, 豆类丝核菌发酵代谢产物中含有大量 SW, 其中 02-6B 与 02-3A 2 株菌代谢产物中苦马豆素含量分别达到 1 093.17 与 1 199.64 mg/L, 说明这 2 株菌可用于 SW 生物合成的研究, 并有利于降低 SW 生物合成的成本。

[关键词] 豆类丝核菌; 苦马豆素; 发酵; 代谢产物

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苦马豆素(swainsonine, SW)首先由澳大利亚学者 Colegate 等^[1]分离于植物灰苦马豆(*Swainsonia canescens*), 后有美国学者 Molyneux 等^[2], 中国学者曹光荣等^[3]也分别从疯草中分离到纯品 SW, 并认为苦马豆素是动物疯草中毒的主要成分^[1~5]。Broquist^[6]和 Croom 等^[7]从美国标准菌库保存的豆类丝核菌(*Rhizoctonia legumicola*)的菌丝体中分离提取到纯品 SW, 国内杨鸣琦等^[8,9]也从自行分离并拥有独立自主知识产权的豆类丝核菌中分离提取到纯品 SW; 童德文等^[10]虽从杨鸣琦提供的豆类丝核菌菌丝体中检测到 SW, 但未分离到纯品。近年来的研究^[4,5,11~14]表明, SW 具有很好的抗肿瘤作用, 其不仅能明显抑制实体瘤的生长与转移, 而且具有明显的免疫刺激功能, 从而促进机体的抗肿瘤免疫。本试验目的是从豆类丝核菌代谢产物中直接测定 SW 含量, 从而寻找一条大批获得 SW 的途径, 以提高 SW 的产量, 为拟采用发酵动力学等原理研究 SW 的生物合成选育优秀菌株, 为 SW 药用价值的实现及抗肿瘤临床应用成本的降低提供理论依据。

1 材料与方法

1.1 材 料

1.1.1 豆类丝核菌 6 株豆类丝核菌由本实验室分别于 1994 年和 2002 年从陕西杨陵周边地区 10 种豆科植物中分离保存, 编号分别为 94-1A, 94-2A, 94-2B, 02-3A, 02-5B, 02-6B。

1.1.2 主要试剂 苦马豆素标准品, 由西北农林科技大学动物科技学院杨鸣琦提供, 1996 年分离于豆类丝核菌, 质量浓度为 15 942.0 mg/L^[8]。

1.1.3 仪 器 HITACHI 663-30 型气相色谱仪, 日本日立公司生产; XSZ-4G 型光学显微镜, 中国重庆光学仪器厂生产; 40~200 μ L 芬兰数字显示移液器, 上海雷勃分析仪器有限公司生产。

1.2 方 法

1.2.1 豆类丝核菌的培养 将改良的 Czapek's 培养基分装于 500 mL 三角瓶, 每瓶装液 250 mL, 121 $^{\circ}$ C 灭菌 15 min。将 6 株菌分别接种于含 250 mL 灭菌培养液的三角瓶中, 每株菌接种 8 瓶, 置 25 $^{\circ}$ C 温箱中培养 14 d。

1.2.2 代谢产物的收集 经 25 $^{\circ}$ C 14 d 培养后, 捞出菌丝, 将每株菌的 8 瓶含代谢产物的培养液混合, 抽滤去除不溶性杂质, 分装于生理盐水瓶中, 流通蒸汽灭菌 45 min, 即为待测的豆类丝核菌发酵代谢产物。

1.2.3 菌丝及代谢产物的观察 肉眼观察并记录代谢产物颜色; 将菌丝压片后用显微镜观察菌丝形态。

1.2.4 发酵代谢产物中 SW 的测定 将 SW 标准液(15 942.0 mg/L)及待测 SW 发酵代谢产物分别取出 100 μ L 于 50 $^{\circ}$ C 挥干, 在残留物中加入 100 μ L 吡啶溶解, 再加入 100 μ L 双三氟乙酰胺(三甲基硅)混合, 室温静置 30 min 后, 取样测定。色谱条件^[18]:

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检验器 FID, 色谱柱 DEGS 2 m, 柱温 200 °C, 进样口温度 250 °C, 进样量 1 μL, 衰减度 10×4, H₂ 40 mL /m in, N₂ 30 mL /m in, A ir 40 mL /m in, 纸速 10 mm /m in。

2 结果与分析

2.1 菌丝形态

6 株菌菌丝均表现较多分枝, 具分枝不远处均有隔膜, 分枝以直角为主偶有锐角。

2.2 发酵代谢产物颜色及其 SW 含量

表 1 各菌株发酵代谢物颜色及其 SW 含量

Table 1 Amounts of SW and colors in the metabolites respectively

菌株 Strains	代谢产物颜色 Colors of metabolites	SW 含量/(mg · L ⁻¹) Amounts of SW
94-1A	黄 Yellow	272.87
94-2A	黄褐 Yellow ish-brown	980.85
94-2B	黄 Yellow	540.54
02-3A	浅褐 Light brown	1199.64
02-5B	深褐 Dark brown	922.74
02-6B	深褐 Dark brown	1093.17

从表 1 可以看出, 6 株豆类丝核菌发酵 14 d 后, 其代谢产物中均可测出 SW。其中 02-3A 中的 SW 含量最高, 为 1 199.64 mg/L, 代谢产物呈浅褐色; 94-1A 中的 SW 含量最低, 为 272.87 mg/L, 代谢产物颜色为黄色, 与原始培养液颜色基本一致。

3 讨论

豆类丝核菌(*Rhizoctonia leguminicola*) 属真菌门(*Fungi*) 半知菌类(*Fungi imperfecti*) 无孢子群(*Mycelia sterilia*) 丝核菌属(*Rhizoctonia*)^[4, 5, 8, 9, 15, 16], 国内除本实验室外, 尚无该菌保存, 虽其形态与丝核菌特征基本一致, 但分类鉴定仍需进一步研究。

国内学者^[8]已证明豆类丝核菌菌丝体中含有大量 SW, 杨鸣琦等曾于 1997 年将豆类丝核菌发酵培养产物浓缩后加赋型剂制成 1 500 枚胶囊, 赠送于台湾友人颜水泉博士, 被口服用于额部癌肿病人, 2 周后癌体积缩小了 30% 以上。目前, 尚未有对发酵液中的 SW 进行规模化测定的报道, 本试验用气相色谱法, 将代谢产物的物质出峰情况与 SW 标准品溶液的出峰时间与峰高及峰形进行对比发现, 该 6 株豆类丝核菌发酵代谢产物中均含有 SW, 且含量较高。另对代谢产物的颜色深浅与 SW 含量进行比较发现, 色素产生量大的菌株, 代谢产物中 SW 含量高, 但相关问题还有待于进一步研究。

从各株菌发酵代谢产物中 SW 含量看, 02-6B 和 02-3A 代谢产生的 SW 较高, 分别达到 1 093.17 和 1 199.64 mg/L。因此, 这 2 株菌可用于苦马豆素生物合成的发酵动力学研究。

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The study of SW in the metabolism product of *Rhizoctonia legum inicola*

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Abstract We study the metabolism products of 6 strains of *Rhizoctonia legum inicola* which are reserved in our laboratory by using gas chromatography to show the character and the amount of swainsonine (SW). And these show that metabolism products of *Rhizoctonia legum inicola* have large amount of SW. And we infer that SW may have relation with some root of energy metabolism inside *Rhizoctonia legum inicola*. Among those strains, metabolism products of 02-6B and 02-3A have the largest amount of SW, and the amounts are 1 093 17 mg/L and 1 199 64 mg/mL respectively. It shows that these two strains can be used in the study of biosynthesis of SW, and we also can use them to reduce the cost of SW biosynthesis

Key words: *Rhizoctonia legum inicola*; swainsonine; fermentation; metabolism product

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The effect of electro-acupuncture spleen point on the regulation of gastric activity and peripheral nerve mechanism of rabbit

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Abstract The paper studied the influence of electro-acupuncturing spleen point on the gastro-electrical activity and its peripheral nerve mechanism with electro-physiology method under different conditions. The result was as following: ① The amplitude and frequency of GEA of pre-post electro-acupuncture was notably different ($P < 0.05$, $P < 0.01$) from that of the center nervous system (CNS). ② The frequency of GEA of pre-post electro-acupuncture was still notably different ($P < 0.01$) while the amplitude of GEA (GEAA) had no change ($P > 0.05$) when the connection of spleen point to DRG had been cut. ③ The frequency of GEA of pre-post electro-acupuncture was different ($P < 0.05$) when the intercostal nerves were cut. ④ The varies of amplitude and frequency of GEA of pre-post electro-acupuncture had no statistic meaning ($P > 0.05$) when the celiac and anterior mesenteric was cut. ⑤ After cutting the T₈-T₁₂ spinal nerve dorsal roots and ventral roots and intercostals, the gastro-activity was regulated bidirectionally and notably by electro-acupuncturing the spleen point. The results suggest that there was a reflex arc outside the CNS though CNS was very important during the course of spleen point effecting GEA. The afferent fiber of spleen point was intercostal nerves and the efferent fibers was sympathetic nerve, the sympathetic ganglion was important in the reflex-arc outside the CNS.

Key words: electro-acupuncture; spleen point; gastro-electricity; reflex arc outside the CNS