

## 监测分析

## 原噬菌体诱导法在环境诱变剂研究中的应用

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**摘要** 应用原噬菌体诱导法检测 35 种不同化学物质的潜在遗传毒性,  $S_9$  作代谢激活系统。试验结果有 13 个样品具有诱变性(占 37.1%), 其中有 10 个样品在  $S_9$  激活下和 3 个样品无论有无  $S_9$  活化均能诱导溶源性大肠杆菌释放  $\lambda$  噬菌体。检出的阳性样品中有些为已知的诱变剂和致癌剂(黄曲霉毒素  $B_1$ )或抗癌药(丝裂霉素 C)。提示诱导检测法作为环境遗传毒物的预警系统初筛潜在致癌危险物质是一种极为有用的手段。

自然环境里存在许多致突变和致癌物, 这些遗传毒物极易引起 DNA 损伤而发生遗传疾病, 畸胎和癌症。为防止环境污染和对人体的危害, 对人类接触和将可能接触的化合物必须鉴定其潜在遗传毒性。目前国内外采用多种短期生物测试系统对许多化学物质进行检测, 为环境诱变剂研究提供可靠手段, 其中原噬菌体诱导法(简称诱导检测法)已引起众多研究者的注目和研究<sup>[1]</sup>。由于绝大多数致癌物质均能诱导溶源性大肠杆菌释放  $\lambda$  噬菌体, 因此原噬菌体诱导法作为环境遗传毒物的预警系统初筛潜在致癌危险的物质, 具有经济、快速、灵敏、准确等特点。本文报道应用诱导检测法对 35 种化学物质进行检测的结果。

## 一、材料与方法

## (一) 试验菌株

GY5027, 为大肠杆菌溶源性菌株, 对  $\lambda$  噬菌体敏感的指示菌为 GY4015。其遗传标记是: GY5027: *ProA his lac envA amp<sup>r</sup> AluvrB<sub>34</sub> strA* ( $\lambda$ pap<sub>A</sub>); GY4015:  $C_{600}$  的 *amp<sup>r</sup> A 601* 衍生株。上述两个菌株为上海第二军医大学蒋左庶教授赠送, 其生物学性状经鉴定和使用符合试验要求<sup>[1]</sup>。

## (二) 受试物及其提取液的制备

2-氨基芴 (2-AF)、叠氮钠 ( $NaN_3$ )、黄曲霉毒素  $B_1$  ( $AF_{B_1}$ ) 均为 Sigma 精品; 15 种真菌分离物(杂色曲霉 3 株、棕曲霉 2 株、构巢曲霉 2 株、冰岛青霉 3 株、串珠镰刀菌 2 株、交链孢霉 3 株)的制备见前文<sup>[2]</sup>。5 种抗癌药物(顺铂、丝裂霉素 C、表阿霉素、长春新碱、5-氟脲嘧啶)为我院化疗科提供; 化妆品田七粉刺露、防晒霜、北京牌冷烫精、上海牌冷烫精、海飞丝洗发精、祛斑露、电发定型水和农药灭蟑灵等均为南宁市售品; 4 份尿抽提物为肝癌高发区扶绥县人尿的氯仿提取液, 每  $10\mu\text{L}$  相当于原尿样 1mL, 由本室涂文升同志提供。以上各种样品均配制成一定浓度置  $4^\circ\text{C}$  冰箱保存备用。

## (三) 试验方法

实验按 Moreau, P. 等方法步骤和要求进行<sup>[1]</sup>。实验中, 除受试样品外, 另设阳性对照、阴性对照和平行对照。重复二次实验。所用  $S_9$  按 Ames 试验以及我室常规方法制备, 选自 Aroclor 1254 诱导 Wistar 雄性大鼠肝匀浆 ( $9000 \times g$  离心上清液), 其活性经鉴定符合要求<sup>[3,4,5,6]</sup>。

试验结果判定: 凡能诱导溶源性大肠杆菌释放  $\lambda$  噬菌体的受试物均可在纸片周围观察到密集的噬菌斑环, 说明具有诱变阳性, 反之则为阴性。一些对细菌有毒性或高浓度的

样品,在纸片和噬菌斑环之间出现抑菌圈。

## 二、试验结果

35 种化学物质诱导溶源性大肠杆菌释放  $\lambda$  噬菌体能力的阳性结果见表 1。作为阴性对照的生理盐水无诱导作用,而阳性对照物 MMC 则有很强的诱导作用,说明本实验系统结果可靠。在受试的 35 种样品中有 13 种能诱导溶源性大肠杆菌释放  $\lambda$  噬菌体 (占 37.1%),具有诱变性,其中 8 种样品诱导能力较强,5 种具有一般诱导效果,其余均为阴性结果。

在  $S_0$  激活下,受试物则有 10 种样品能诱导溶源性大肠杆菌释放  $\lambda$  噬菌体,其中 6 种诱导能力较强,其余 4 种为一般的诱导效果,抗癌药顺铂和 MMC 无论有或无  $S_0$  激活均有很强的诱导能力,说明两者为强直接

诱变剂。

为检测某些样品诱导  $\lambda$  噬菌体释放的剂量效应,取不同浓度的化合物 0.1ml 直接和加有  $S_0$  混合液的含菌半固体琼脂混和,倒在铺有 LB 固体培养基平板上,凝固后 37℃ 培养 10 小时,计数平板上出现的噬菌斑数目。图 1 是 MMC 诱导  $\lambda$  噬菌体释放的剂量效应曲线,由图可见,MMC 无论加与不加  $S_0$  其诱导能力最强的浓度为 100r/ml,高于或低于此浓度其诱导能力较弱,提示 MMC 的遗传毒性表现的剂量范围。AFB<sub>1</sub> 和真菌代谢产物诱导  $\lambda$  噬菌体释放也有类似的剂量效应。

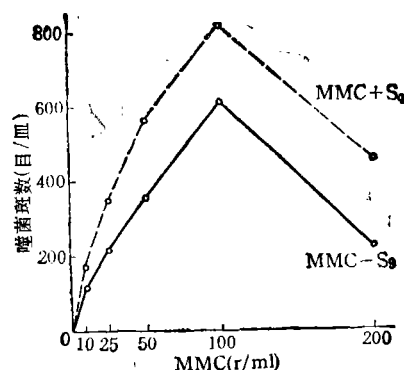


图 1 丝裂霉素 C 诱导  $\lambda$  噬菌体释放的剂量效应曲线

## 三、讨 论

研究表明,一切遗传毒物均可引起 DNA 损伤而导致细菌细胞中 RecA 蛋白的激活,被激活的 RecA 蛋白能分解  $\lambda$  噬菌体的 CI 阻遏蛋白和 SOS 系统中的 LexA 阻遏蛋白,从而使溶源性大肠杆菌释放  $\lambda$  噬菌体并通过 SOS 系统诱发突变。因此可通过原噬菌体诱导法作为环境遗传毒物的预警系统初筛潜在致癌危险的物质。本实验应用该法对 35 种不同类型的化学物质进行潜在遗传毒性测定,结果有 13 种样品具有诱变性,检出的阳性样品中有些为已知致癌物或诱变剂,这与文献报道相符<sup>[2,6,7]</sup>。

表 1 各种化学物质诱导溶源性大肠杆菌释放  $\lambda$  噬菌体的能力

受试物	剂量/纸片*	$S_0$ **	诱导能力***	诱变性
黄曲霉毒素 B <sub>1</sub>	15ng	+	++	+
顺铂	1 $\mu$ g	±	+++	+
丝裂霉素 C	250ng	±	+++	+
重铬酸钾	50 $\mu$ g	-	+	+
杂色曲霉 0	100mg	+	+++	+
杂色曲霉 22	100mg	+	+++	+
杂色曲霉 44	100mg	+	+++	+
棕曲霉 5	100mg	+	++	+
棕曲霉 7	100mg	+	++	+
构巢曲霉 8	100mg	+	+	+
构巢曲霉 20	100mg	+	+	+
串珠镰刀菌 3	100mg	+	+	+
串珠镰刀菌 112	100mg	+	+	+
生理盐水 (阴性对照)	0.85%10 $\mu$ L	-	-	-
丝裂霉素 C (阳性对照)	50ng	-	++	+
链霉素	1 $\mu$ g	-	抑菌	-

注: \* 每纸片点样 10 $\mu$ L。

\*\*  $S_0$  活化剂量 30 $\mu$ L/皿。

\*\*\* “-”表示无诱导能力;“+”有一般诱导能力;“++”有明显的诱导效果;“+++”有很强的诱导能力。

文献[7]中指出:肝癌的发生可能与人摄入一些致癌性真菌毒素或其代谢产物的作用有关。本实验受试的 15 株真菌分离物有 9 株具有诱变性,进一步表明肝癌高发的扶绥县粮食真菌分离物中含有诱变性物质。这些 DNA 损伤剂不仅导致 DNA 结构的改变而致癌且可发生遗传性疾病,说明摄入霉变食物在肝癌发生上可能起协同作用<sup>[2,7,8]</sup>。本实验以 S<sub>9</sub> 活化真菌分离物其诱导原噬菌体释放的能力明显增强,从而估计这些物质对动物或人体的潜在危险是有一定实际意义的。

近十多年,多种细胞诱变试验已广泛应用于环境诱变剂的监测和研究中。原噬菌体诱导法和 Ames 试验一样都有经济、快速、灵敏等特点,但 Ames 试验作为初筛致癌物质假阳性太多,诸如用 Ames 法检测为阳性物的 2-氨基嘌呤、苯(e)芘已知并无致癌作用,这些没有致癌作用的诱变剂(如上述二者)用诱导检测法测得的结果均为阴性<sup>[1]</sup>,从而排

除 Ames 试验的一些假阳性结果。此外,诱导检测法只用一组菌株就可检测许多不同类型的化合物,尤其成份复杂的混合物潜在致癌活性。由于大多数致癌物质均能诱导溶源性大肠杆菌释放  $\lambda$  噬菌体,所以诱导检测法作为遗传毒物的预警系统初筛潜在致癌危险的物质,具有经济、快速、灵敏、准确等特点,如能配合其它测试系统,这对环境诱变剂的研究无疑是一种极为有用的手段。

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## 大气中甲酸和乙酸测定方法研究

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**摘要** 本文研究了一种用红色担体经强碱(KOH)处理作为吸附的载体,去离子水超声浸取,离子色谱法分析测定大气中甲酸和乙酸的方法。对固体吸附管的特性进行了实验室内模拟及野外研究。在采样体积为 2.4m<sup>3</sup> 时,该法对大气中甲、乙酸的最低检出限分别为 0.2 $\mu$ g/m<sup>3</sup> 和 0.4 $\mu$ g/m<sup>3</sup>。利用此法,测出北京冬季大气中甲、乙酸浓度分别为 0.5—2.0 $\mu$ g/m<sup>3</sup> 和 0.6—8.7 $\mu$ g/m<sup>3</sup>,广州白云山春季大气中甲、乙酸浓度分别为 0.2—1.0 $\mu$ g/m<sup>3</sup> 和 <0.4—2.0 $\mu$ g/m<sup>3</sup>,桂林市大气中甲、乙酸浓度分别为 0.37—0.4 $\mu$ g/m<sup>3</sup> 和 0.5—1.1 $\mu$ g/m<sup>3</sup>。

自从 Galloway 等<sup>[1]</sup>在纽约雨水中首次测到挥发性的有机酸(C<sub>1</sub>—C<sub>10</sub>)以来,许多研究<sup>[2,3]</sup>表明有机酸是对流层大气中的重要化学组份。Keene 等<sup>[2]</sup>在研究边远地区的湿沉降时,得出有机酸(主要是甲酸和乙酸)对降水自由酸的最大贡献为 25% 到 98%;Chapman 等<sup>[3]</sup>在美国威士康星春季降雨中测出甲、乙酸浓度范围分别为 1.2—56 $\mu$ eq/l

和 1.9—33 $\mu$ eq/l,其对自由酸的最大贡献为 2.4—68.1%,他们认为有机酸是边远陆地地区降水的重要致酸因子。最近俞绍才等<sup>[4]</sup>的研究初步得出树木尤其是针叶松林伴随着树叶的蒸腾作用可向大气排放甲、乙酸,这对陆地地区的降水有一定的酸化作用。总之,随

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Shanghai): *Chin. J. Environ. Sci.*, 11(3), 1990, pp. 24—27

It has been proved that there is a root microecosystem in the process of dyeing wastewater treatment by the hyacinth. With four days' retention, the percentage of COD removal in the oxidation tank, where the hyacinth was planted and its root sterilized with chloride, was 13%. However the percentage of COD removal in ordinary biological oxidation pond without the hyacinth was 15%. In comparison with this, a water hyacinth oxidation tank without sterilization showed higher COD removal percentage of 35% due to existence of a root microecosystem. Similar results were obtained when wastewater containing PVC, detergent and some dyes were treated with the three methods mentioned above. Some organic compounds that could scarcely be absorbed by the hyacinth, for example, those easy to be coagulated or flocules, could be stuck and fixed on the root surface, and then degraded by the root microecosystem.

**The Toxicological Effect of Cr (VI) on Chlorophyll and Iron Contents and Activities of Some Enzymes in the Leaves of Pepper (*Capsicum annuum*).** Zhou Yiyong, Liu Tongchou, Deng Boer (Dept. of Soil and Agrochemistry, Huazhong Agricultural University, Wuhan): *Chin. J. Environ. Sci.*, 11(3), 1990, pp.28—29

The toxicological effect of Cr(VI) on some biochemical parameters in pepper were studied both in soil culture and in nutrient cultural experiments. The treatments of the heavy metals decreased fresh weight and promoted senescence of the pepper plant by decreasing chlorophyll and activities of superoxide dismutase and catalase as well as increasing iron content and peroxidase activity over control values.

**Study on the Pretreatment of Coke-Plant Wastewater by Anaerobic Acidification.** Zhao Jianfu, Qian yi, Gu Xiasheng (Dept. of Environmental Engineering, Tsinghua University, Beijing): *Chin. J. Environ. Sci.*, 11(3), 1990, pp.30—34

According to analysis of the constituents and concentrations of organic pollutants in wastewater at Beijing Coke Plant using combined gas chromatography and mass spectrometry (GC/MS), the effect of anaerobic acidification on the bio-treatability of coke-plant wastewater has been studied and the possibility of using anaerobic acidification as pretreatment of aerobic treatment has also been explored. The results described that aerobic biotreatability of coke-plant effluent could obviously increase through 2—6 hours' anaerobic acidification. After 6 hours and 12 hours, anaerobic acidification, COD in the effluent could be removed by 91%, i.e. removal rate increased about

40% more than that without applying anaerobic acidification. As the influent COD of the wastewater was 1780 mg/L, the effluent COD removed to 158 mg/L.

**Preparation of Polyaluminum Chloride with Sulfate Ion and Study on Its Properties.**

Gao Baoyu et al. (Environmental Science Center, Shandong University, Jinan): *Chin. J. Environ. Sci.*, 11(3), 1990, pp. 34—37

Polyaluminum chloride with sulfate ion (PACS) has been prepared by using aluminum hydroxide, hydrochloric acid, sulfuric acid and sodium carbonate as raw materials, and the properties of PACS have also been studied. The factors affecting the flocculating effect of PACS have been investigated. The experimental results show that the flocculating effect of PACS is influenced by the amount of sulfate ion in PACS, basicity of PACS and pH of water solution. When the molar ratio of  $Al^{3+}$  to  $SO_4^{2-}$  is in the range of 15 to 17, the flocculating effect of PACS is best.

**Problems on Yellow-Colouring of the Wastewater Treated with the Coagulant, Ferrous Salt.** Guan Xijun, Wang Fei (Dept. of Environmental Engineering, Qingdao Institute of Architectural Engineering): *Chin. J. Environ. Sci.*, 11(3), 1990, pp.38—40

When ferrous salt is used as a coagulant to treat wastewater, if there exists superfluity in the process of coagulation to sedimentation, purged water will be clear. However, When the purged water is laid aside, it becomes turbid and turns to yellow-colouring. The reason is that oxygen in the air has dissolved in it as time goes on. The authors have proposed a measure to control the phenomenon that a higher pH or an optimal quantity of the mixed coagulant paralleled with tests can avoid color changing.

**Application of Inductest in Research of Environmental Mutagens.** Ruan Cuicai et al. (Guangxi Cancer Institute, Nanning): *Chin. J. Environ. Sci.*, 11(3), 1990, pp.41—43

The possible mutagenic activity of 35 different chemicals has been tested with inductest, in which  $S_9$  mixture was used as a metabolizing system. The results showed that 13(37%) chemicals had mutagenic activity, 10 of these chemicals gave positive reaction in inductest in the presence of  $S_9$  mixture, three chemicals gave positive reaction in inductest in the presence of  $S_9$  mixture or without it. Some of the chemicals are known as potent mutagens and carcinogens (aflatoxin  $B_1$ ) or anticancer drugs (mitomycin C). It is considered that inductest is an effective method in research of environmental mutagens.

**A Study on Determination of Formic and Acetic Acids in the Atmosphere.** Yu Shaocai,