学校编码：10384　　分类号_____密级_____
学号：200226084　　UDC _____

厦　门　大　学

硕　士　学　位　论　文

大蒜种质资源遗传多样性及大蒜素抑制肺癌细胞 A-549 的研究

Studies on Genetic Diversity of *Allium sativum* L. and Suppressive Action of Allicin on Growth of Human Lung Cancer Cell Line A-549

陈　昕

指导教师姓名：林　鹏　教授

专　业　名　称：生态学

论文提交日期：2005 年 6 月 31 日

论文答辩时间：2005 年 7 月 31 日

学位授予日期：

答辩委员会主席：郑文教　教授

评　　阅　　人：_____

2005 年 8 月

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摘 要

大蒜(*Allium sativum* L.），又名胡蒜、葫、独蒜等,是百合科葱属多年生草本植物的地下球型鳞茎。自古以来就是世界各国人民广为食用的调味品。我国是大蒜的主要生产国，产量占世界总产量的 1/4。国际上对大蒜的研究一直很活跃,已证明其有卓越的抗菌、抗病毒、降血脂、抗肿瘤、提高机体免疫力、降血糖、解毒等功效,并已开发出多种制剂应用于临床。如果将大蒜深加工，制成蒜油、蒜蓉、蒜粉等便可耐贮藏，方便运输，特别是大蒜素的研究开发必然会大大提高出口创汇能力。本论文对十个选自中国不同地区大蒜品种的种植；生物学聚类；分子标记聚类；蒜头中大蒜素的提取以及含量测定等方面进行了研究，旨在通过植物学，生理学，分子遗传学三个方面对大蒜种质资源遗传多样性进行较全面的探讨和研究，同时，研究了大蒜素对人肺癌细胞 A-549 的抑制作用，为大蒜及其制品的进一步规模化生产提供科学依据。

1）本论文在生命科学学院的温室中，种植了 10 个选至中国不同地区的大蒜品种。种植期持续了一个完整的生长季节（2003.9.-2004.7）。在大蒜的生长期间根据数值分类学的性状选择原则，剔除逻辑相关性和不变性状，采集各种相关的形态学和生物学数据，进行聚类分析。

2）利用 RAPD 和 ISSR 两种分子标记技术，对这 10 个不同地区的大蒜品种进行了种质资源遗传多样性研究。从 30 个 RAPD 引物当中，筛选出 11 个具有多态性的引物，共扩增出多态性带 224 条，多态性条带比率为 41.18%，从 12 个 ISSR 引物中筛选出 5 个具多态性的 ISSR 引物，共扩增出多态性带 121 条，多态性条带比率为 50.21%。对 RAPD 和 ISSR 两种标记

分别运用 Nei 指数法，计算出 10 个大蒜品种的平均遗传距离为 0.28 和 0.32。根据这两种标记的结果，采用 UPGMA 进行聚类分析，得到与形态学和生物学分类地位不完全相同但有相似之处的结果：按照 10 个大蒜品种原产地的纬度和省份将它们划分成三个生态品种群。结果表明，在实验稳定性上，ISSR 优于 RAPD，且 ISSR 比 RAPD 能检测到更多的遗传变异。同时，RAPD 和 ISSR 都能将 10 个不同的大蒜品种区分开，说明这两种标记可以有效地应用于大蒜这一遗传多态性低的物种的遗传多样性研究中。

3）选择其中的 6 个大蒜品种（分别属于三个生态品种类群），各自提取大蒜素。利用 HPLC 测大蒜素标准品，以浓度为横坐标，峰面积积分值为纵坐标，绘制标准曲线。HPLC 分析 6 个大蒜品种的大蒜素，根据 HPLC 峰面积积分值，在标准曲线上计算出待测样品各自的大蒜素含量。结果表明：白皮蒜的大蒜素含量高于红皮蒜；相同栽培时期，不同产地的大蒜品种，大蒜素的含量不同；相同产地，不同品种的大蒜，大蒜素的含量也不相同；相对于大蒜的不同生态型，白皮与红皮特性对大蒜素含量的影响更大。

4）本研究采用 MTT 比色试验,研究了大蒜素对人肺癌 A-549 细胞的剂量毒性情况。实验结果表明：大蒜素在 10～160μg/mL 的浓度范围内可显著抑制人肺癌 A-549 细胞的生长,当大蒜素浓度范围在 10-80μg/mL 的时候，随着大蒜素浓度的提高，其对肺癌细胞 A-549 的抑制作用也逐渐增高，且随着作用时间的延长，抑制作用愈明显。当大蒜素浓度范围在 80-160μg/mL 的时候，对人肺癌 A-549 细胞的抑制率没有明显的变化（分别为 44.4%和 44.5%），表明在大蒜素浓度超过 80μg/mL 的时候，抑制率不依浓度的变化而改变，达到一个阈值。

综上所述，RAPD 和 ISSR 两种分子标记方法可用于大蒜种质遗传多样性的研究。同时，大蒜素具备研发成一种新的化疗药物，并对肺癌患者进

行治疗的潜力，为肺癌患者带来新的希望。

关键词：大蒜 RAPD ISSR 大蒜素 遗传多样性 肺癌细胞 A-549 抑癌作用

Studies on Genetic Diversity of *Allium sativum* L. and Suppressive Action of Allicin on Growth of Human Lung Cancer Cell Line A-549

Chen Xin

Abstract

Garlic(*Allium sativum* L.) is the subterranean globose bulb which belonging to liliaceous herbaceous plant。It has been the food that the people of many countries ate far and wide since ancient times. Our country is the main producing country of garlic and the output account for 1/4 world of total output. The study on garlic has been very active all the time in the world. It has been proved that garlic has much remarkable efficacy such as antibiotic , antivirus , lowering fat of blood , antitumor improving organism immunity , lowering blood sugar , efficiency of detoxifying etc. People have already developed many kinds of medicament to apply to clinic. If we process the garlic into the allicin or other kinds of finished product, it could be easy to store and transport. Especially the research and development of the garlic will inevitablely improve the capacity to earn foreign exchange through exports greatly. This research includes the following content: planting samples in greenhouse; collecting biological data; application in garlic germplasm studies by RAPD and ISSR; separating allicin from garlic with ethanol; allicin content confirmed by HPLC; suppressive action of allicin on growth of human lung cancer cell

line A-549. The purpose of this research is to offer scientific basis for further large-scale production.

1) Plant 10 varieties of garlic from different regions and provinces of China in the greenhouse of Xiamen University. The growth period is from September of 2003 to July of 2004. In the growth period we collected biologic characteristic data and used UPGMA cluster analysis to analyse the data.

2) Used random amplified polymorphic DNA(RAPD) and inter-simple sequence repeat(ISSR) methods to detect genetic variation of 10 varieties of garlic from different regions and provinces of China. 11 RAPD primers generated 224 polymorphic bands. Percentage of polymorphic bands(PPB) of RAPD was 41.18%. 5 ISSR primers generated 121 polymorphic bands. PPB of ISSR was 50.21%. The result of UPGMA cluster analysis based on two molecular markers data was similar to the result based on the biologic characteristic. Comparing with the two marker systems it showed that ISSR was better than RAPD in terms of reproducibility and ability of detecting genetic polymorphism. Consequently, the results showed that RAPD and ISSR could be applied to detect genetic diversity among garlic strains.

3) Choose six varieties of garlic from our samples (belonging to three ecotype) and separate allicin from them。Allicin’s content was confirmed by HPLC. Regard density as the abscissa and acreage as the ordinate and protract the normal curve. Use the normal curve to figure out the content of the 6 garlic varieties. The results showed: the allicin’s content of white peel garlic are higher than

red peel garlic; the different variety has different allicin's content in the same growing time; the different variety has different allicin's content although they are in the same habitat; the peel characteristic impact on allicin's content is higher than ecological characteristic.

4) Cultural cell lines of lung cancer A-549 were used as the target cells to observe the effect of allicin of different concentrations on these cell lines by MTT experimentation. The results showed : when the concentrations of allicin were in the range of 10～160μg/mL, the inhibiting rates of on A-549 cells were remarkable. when the concentrations of allicin were in the range of 10～80μg/mL, the inhibiting rates of A-549 were enhanced with the concentration and time increase. But when the concentrations of allicin were in the range of 80～160μg/mL, the inhibiting rates of A-549 had no obviously change(44.4% and 44.5%, respectively). It showed that when the concentrations of allicin were over 80μg/mL, it has lost the relationship of dosage.

In conclusion, the results of this research showed that RAPD and ISSR could be applied to detect genetic diversity among garlic. The allicin can remarkably inhibit the growth of lung cancer A-549 cells. It has the potential to be a new kind of chemotherapy medicine to treat Lung cancer and give the patient a great hope.

Key word: garlic; RAPD; ISSR; allicin; lung cancer A-549 anticancer

前　　言

大蒜(*Allium sativum* L.)又名胡蒜、独蒜、葫、独头蒜,是百合科葱属一年生植物的地下鳞茎,原产于亚洲西部，汉代传入我国，现在世界各地均普遍种植。它的地下部分是由多瓣组成的膨大的地下球型鳞茎，习惯上称为“蒜头”。地上部分的茎叶称为“青蒜”，花茎称为“蒜薹”。大蒜有很好的药用价值，作为药用可追溯到古罗马、埃及时代和五千年前的中国。《旧约全书-古埃及记》记载,古埃及人为了让军队战士身体强壮,就让他们多吃大蒜。古罗马的普利民利用大蒜治疗伤风、哮喘等。我国《本草纲目》也记载,大蒜有解毒散臃肿、消毒气、除风破冷、健脾治泄等奇效[1，2]。近年来,围绕大蒜的有效成分、功效及应用的研究很多,大量科学研究进一步证实了大蒜的各种保健功能,例如，抗菌、抗病毒[3，4，5]、抗肿瘤[6]、抗动脉粥样硬化、降血脂[7，8，9]、降血糖、解毒、提高机体免疫功能等[10]，阐明了其作用机理及功能因子，并开发出多种制剂应用于临床。我国于 50 年代起即对大蒜的药理，临床及化学成分等方面进行了广泛的研究，取得了丰硕成果。

大蒜含有丰富的维生素、氨基酸、蛋白质、糖类、无机盐等营养成分[11]及磷、镁、钙等多种微量元素[12]。大蒜的独特辛辣气味可以解除鱼、肉的腥味，增进食欲，是膳食烹调中不可缺少的调味品。然而鲜蒜头休眠期短，易发芽霉变，不耐贮藏，会造成巨大浪费，并且大蒜的初级产品价格较低。我国是大蒜的主要生产国，产量占世界总产量的 1/4，如果将大蒜深加工，制成蒜油、蒜蓉、蒜粉等便可耐贮藏，方便运输，特别是大蒜素的提取及其在医疗保健方面的研究和大蒜素相关产品的开发，必定会大大提高大蒜的出口创汇能力，加快促进我们国家的经济发展和建设。