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Research Article 研究报告

蓝藻挥发物 α-紫罗酮诱导莱茵衣藻细胞程序性死亡

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摘要：【目的】蓝藻挥发性有机化合物(VOCs)对其他藻类的化感作用可促进蓝藻成为富营养化水体优势种群，本研究旨在以VOCs主要成分α-紫罗酮为例揭示其化感致死机制。【方法】采用α-紫罗酮处理莱茵衣藻，测定藻细胞生长以及致死浓度下藻细胞光合性能、caspase-likes活性和DNA ladders。【结果】采用0.05和0.1 mmol/L α-紫罗酮处理24 h后，莱茵衣藻细胞生长均受到明显抑制，其中0.1 mmol/L处理时部分藻细胞发生死亡，死亡率为38.3%。采用0.2 mmol/L α-紫罗酮处理时，藻细胞全部死亡，同时光合色素逐渐降解、Fv/Fm逐渐降低并消失，这表明藻细胞死亡并非坏死。在藻细胞死亡过程中，caspase-9-like和caspase-3-like活性明显增强；DNA在处理1 h时出现ladders，并逐渐降解为100–250 bp片段。【结论】这表明蓝藻VOCs可通过诱导细胞程序性死亡以发挥化感作用。

关键词：蓝藻，莱茵衣藻，α-紫罗酮，细胞程序性死亡，化感机制

蓝藻通过次生代谢途径释放大量的挥发性有机化合物(volatile organic compounds，VOCs)，主要包括呋喃类、含硫类、烷烃类、萜烯类、苯类、醇类、醛类、酮类和酯类化合物，这些 VOCs 溶于水后不仅会降低水质并造成饮用水危机[1–2]，还会抑制其他藻类生长以促使蓝藻成为富营养化水体优势种群[3–5]。

水华微囊藻(*Microcystis flos-aquae*)和铜绿微囊藻(*M. aeruginosa*)是形成蓝藻水华的两种典型藻种，无氮和无磷条件可促进其 VOCs 产生与释放，同时这些 VOCs 可抑制其他藻类生长[6–7]。在蓝藻 VOCs 主要成分中，桉树脑和柠檬烯可通过抑制细胞生长、诱导光合色素降解和降低光合性以抑制甚至杀死莱茵衣藻(*Chlamydomonas*

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reinhardtii)和小球藻(*Chlorella vulgaris*)[6–8]；α-紫罗酮、β-紫罗酮、β-环柠檬醛和香叶基丙酮可抑制蛋白核小球藻(*C. pyrenoidosa*)生长[3]；0.1–0.5 mg/mL β-环柠檬醛会使谷皮菱形藻(*Nitzschia palea*)细胞破裂[9]。由此可见，蓝藻VOCs在蓝藻成为富营养化水体优势种群中具有化感作用，然而其化感机制尚不清楚。

细胞程序性死亡(programmed cell death，PCD)是细胞感受到某种信号或受到某些因素刺激后，为维持内环境稳定而发生的一种主动性消亡过程。此过程不仅在高等植物和动物生长发育以及响应生物和非生物胁迫过程中发生[10–11]，还在单细胞生物中发生，例如酸[12–13]、高温[14]、盐[15]、紫外线[15]、H_2O_2[16]、除藻剂[17]等胁迫条件可诱导单细胞藻类和酵母发生PCD。在此过程中，细胞会发生一系列变化，主要包括：细胞收缩与空泡化、细胞器退化、caspase (-like)活化、细胞核先浓缩后破裂、DNA降解成ladders等[14,16–17]。

在蓝藻VOCs中，α-紫罗酮、β-紫罗酮和β-环柠檬醛主要通过类胡萝卜素降解形成，是富营养化水体中的主要嗅味物质[4,18]，并且在2–5 mg/mL (约10–26 mmol/L)时对蛋白核小球藻产生化感作用[3]。衣藻隶属于绿藻门，广泛存在于河流、湖泊、海洋等水体中。在富营养化水体藻种演替过程中，蓝藻大量繁殖生长后，衣藻等绿藻大量减少[19–20]。因此，本研究通过测定α-紫罗酮对莱茵衣藻细胞生长的影响以及致死浓度下藻细胞光合性能、caspase-likes活性和DNA ladders等变化，以期从PCD角度揭示蓝藻VOCs对其他藻类的化感致死机制，进而提升对蓝藻成为富营养化水体优势种群的认识以利于其有效防治。

1 材料和方法

1.1 实验材料

莱茵衣藻CC400株系(中国科学院遗传与发育生物学研究所刘翠敏研究员惠赠)隶属于绿藻门(*Chlorophyta*)团藻目(*Volvocales*)衣藻属(*Chlamydomonas*)，采用TAP培养基[21]进行培养。培养条件为：温度25 °C、光照(16 h)/黑暗(8 h)、光强50 μmol/(m^2·s)、摇床转速100 r/min。待藻细胞达到对数生长期时，5000 r/min离心后转入新鲜培养基中，使藻细胞密度为5×10^6细胞/mL，并采用0.05、0.10、0.20 mmol/L α-紫罗酮(上海源叶生物科技有限公司)进行处理，以未处理作为对照。待α-紫罗酮处理莱茵衣藻24 h后，测定藻细胞密度；待0.2 mmol/L α-紫罗酮处理0、0.5 (caspase-likes活性)、1.0、2.0、6.0、12.0、24.0 h时测定藻细胞光合色素吸收光谱、光合性能、caspase-likes活性和DNA ladders。4次重复。

1.2 藻细胞密度与死亡率测定

采用中性红对莱茵衣藻进行染色以区分活细胞和死细胞(红色)，利用血细胞计数板(25×16)计数两种藻细胞数量，并计算活细胞密度与藻细胞死亡率[17]。

1.3 光合色素吸收光谱测定

取3 mL莱茵衣藻培养液，5000 r/min离心5 min后，用3 mL 96%乙醇重悬藻细胞并置于黑暗处静提24 h。待光合色素提取完成后，采用TU-1900 UV-Vis分光光度计在400–700 nm处进行扫描以测定吸收光谱，并计算每10^6个细胞的吸收光谱[17]。

1.4 叶绿素荧光测定

取约 1×10^7 个藻细胞，6000 r/min 离心 5 min，将藻细胞重悬于 10 μL 培养基中，用移液枪吸取后滴于 3 cm^2 滤纸上以形成大小约 0.5 cm^2 斑点，黑暗中放置 15 min。采用 YZQ-500 非调制式叶绿素荧光仪测定其叶绿素荧光，并计算光系统 II (PSII)最大光量子产量(Fv/Fm)[7]。

1.5 Caspase-9-like 和 caspase-3-like 活性测定

Caspase-9-like 和 caspase-3-like 活性分别采用 caspase-9 和 caspase-3 活性检测试剂盒进行测定。取 25 mL 莱茵衣藻培养液，5000 r/min 离心 5 min 后，加入 100 μL 裂解缓冲液冰浴裂解 10 min。在 4 °C 下离心后，取上清液并分别加入 100 μmol/L caspase-3 反应液(Ac-DEVD-pNA)和 caspase-9 反应液(IETD-pNA)。在 37 °C 孵育 2 h 后，通过测定反应产物在 405 nm 处的吸光值，从而计算 caspase-9-like 和 caspase-3-like 活性。

1.6 DNA ladders 检测

取 10 mL 莱茵衣藻培养液，5000 r/min 离心 5 min 后，加入 350 μL NET 溶液[100 mmol/L NaCl、50 mmol/L EDTA、20 mmol/L Tris-HCl (pH 8.0)]重悬藻细胞，并加入 25 μL SDS (200 g/L)和 25 μL 蛋白酶 K (10 g/L)，55 °C 水浴 2 h 后加入 200 μL 5 mol/L KAC。在 4 °C 下离心后，取上清加入等体积的酚:氯仿:异戊醇(25:24:1)抽提 2 次后，加入 2 倍体积的无水乙醇，在–70 °C 条件下沉淀 DNA。将 DNA 溶于适量 ddH_2O 中，加入 RNase A 消化 30 min 后，采用 1.5%琼脂糖凝胶电泳检测 DNA ladders[13]。

1.7 数据处理

采用 Origin 8.0 进行单因素方差分析并绘图。

2 结果和分析

2.1 α-紫罗酮对藻细胞生长的影响

采用 0.05 和 0.1 mmol/L α-紫罗酮处理莱茵衣藻 24 h 后，其藻细胞密度均极显著(P<0.01)低于对照，同时 0.1 mmol/L 处理时藻细胞死亡率为 38.3% (P<0.01)。采用 0.2 mmol/L α-紫罗酮处理 24 h 后，藻细胞全部死亡(图 1)。

2.2 α-紫罗酮对光合色素吸收光谱的影响

未采用 α-紫罗酮处理时，莱茵衣藻光合色素吸收光谱在藻细胞生长过程中未发生明显变化(图 2-A)；采用 0.2 mmol/L α-紫罗酮处理后，其光合色素吸收光谱在 413、433、457、663 nm

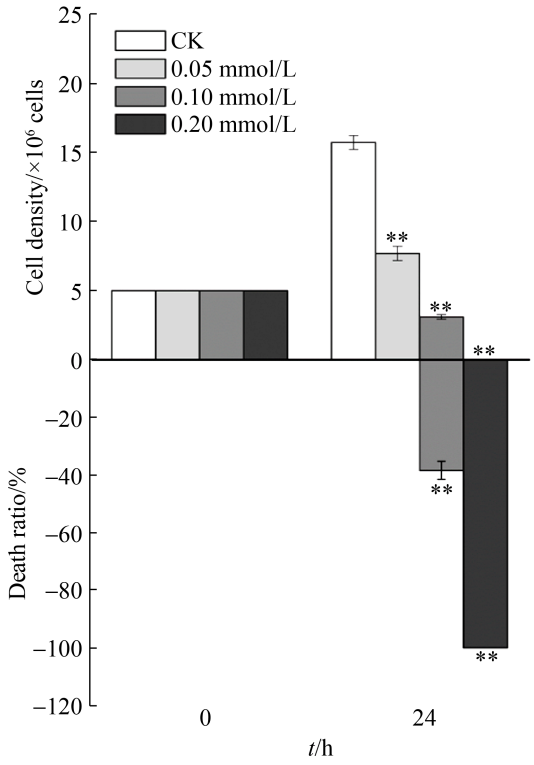

图 1. α-紫罗酮对莱茵衣藻细胞生长的影响

Figure 1. Effects of α-ionone on the growth of *C. reinhardtii*. CK: the control. **: compared to the control, the significant difference at P<0.01 level.

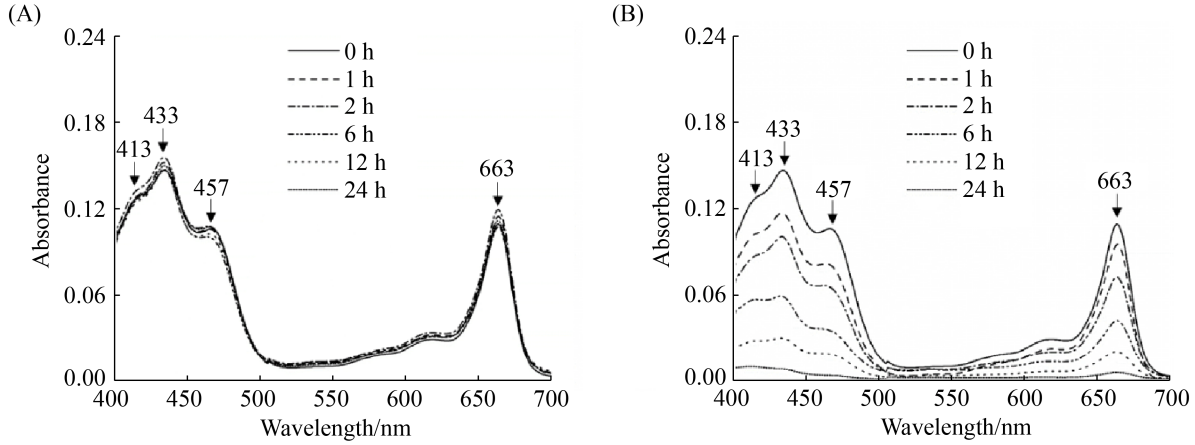

图 2. 0.2 mmol/L α-紫罗酮对莱茵衣藻光合色素吸收光谱的影响

Figure 2. Effects of 0.2 mmol/L α-ionone on absorbance spectra of photosynthetic pigments in *C. reinhardtii*. A: the control, without α-ionone treatment; B: the treatment with α-ionone at 0.2 mmol/L.

处的吸收峰均随处理时间延长而逐渐降低，这表明光合色素逐渐降解(图 2-B)。

2.3 α-紫罗酮对 Fv/Fm 的影响

采用 0.2 mmol/L α-紫罗酮处理莱茵衣藻 1 h 后，其 Fv/Fm 显著(P<0.01)低于对照。随处理时间延长，Fv/Fm 逐渐降低，并在处理 24 h 时消失(图 3)。

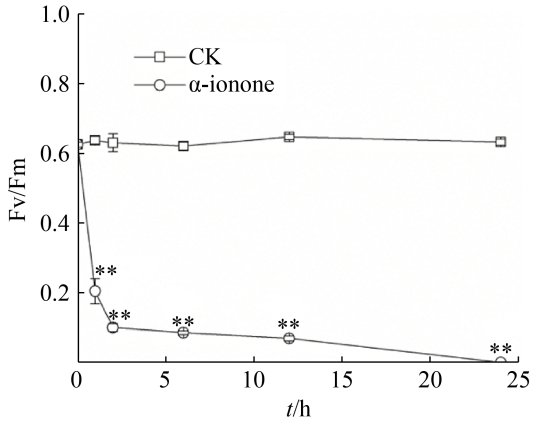

图 3. 0.2 mmol/L α-紫罗酮对莱茵衣藻 Fv/Fm 的影响

Figure 3. Effects of 0.2 mmol/L α-ionone on Fv/Fm in *C. reinhardtii*. **: compared to the control, significant difference at P<0.01 level.

2.4 α-紫罗酮对 caspase-likes 活性的影响

0.2 mmol/L α-紫罗酮处理莱茵衣藻 1 h 后，caspase-9-like 和 caspase-3-like 活性均增至最高，与对照相比分别增加了 4.5 (P<0.01)和 5.1 (P<0.01)倍。随处理时间延长，caspase-9-like 和 caspase-3-like 活性呈现逐渐降低趋势，但均显著(P<0.01)高于对照(图 4)。

2.5 α-紫罗酮对 DNA ladders 的影响

采用 0.2 mmol/L α-紫罗酮处理莱茵衣藻 1 h 后，其 DNA 出现 ladders。随着处理时间延长，DNA 降解逐渐增强，在处理 24 h 时降解为 100–250 bp 片段(图 5)。

3 讨论

细胞死亡可分为坏死和 PCD，其中发生 PCD 时细胞生理活性逐渐消失，例如乙酸、甲萘醌、除藻化合物(芳樟醇和 α-松油醇)诱导莱茵衣藻 PCD[13,17,22]；H_2O_2 诱导杜氏藻(*Dunaliella tertiolecta*) PCD[23]；以及 H_2O_2、NaCl 和 KCl 诱

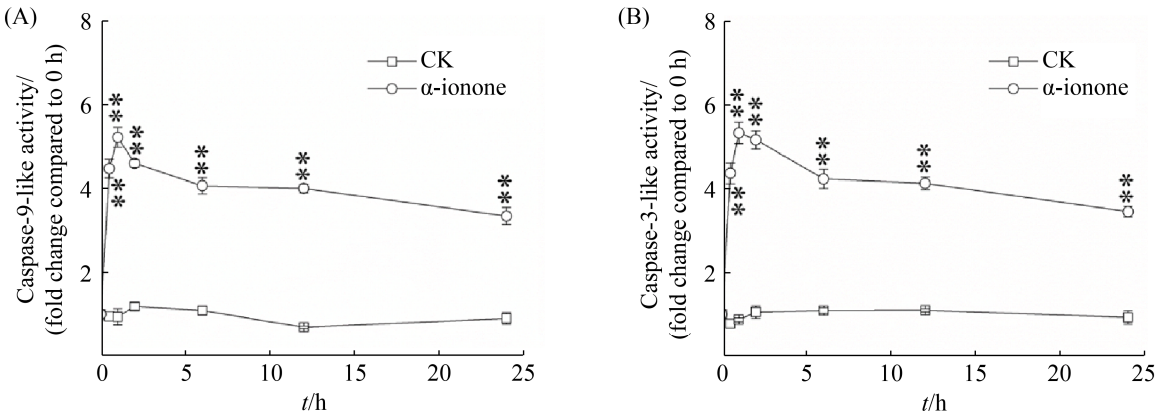

图 4. 0.2 mmol/L α-紫罗酮对莱茵衣藻 caspase-likes 活性的影响

Figure 4. Effects of 0.2 mmol/L α-ionone on the activities of caspase-likes in *C. reinhardtii*. A: Caspase-9-like; B: Caspase-3-like. **: compared to the control, significant difference at $P<0.01$ level.

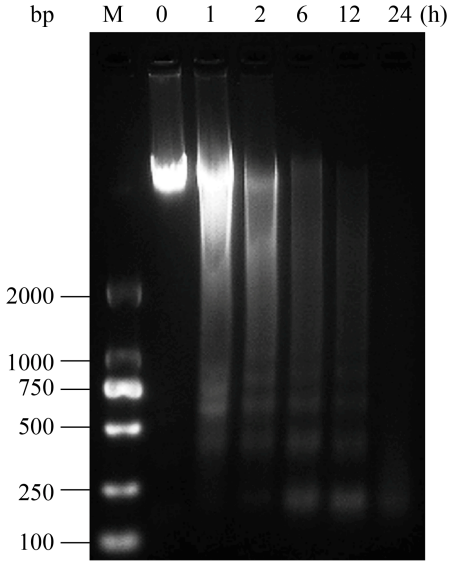

图 5. 0.2 mmol/L α-紫罗酮对 DNA ladders 的影响

Figure 5. Effect of 0.2 mmol/L α-ionone on DNA ladders. M: DNA marker.

导 *Micrasterias denticulata* PCD[24]时，藻细胞光合性能均逐渐消失。采用 0.2 mmol/L α-紫罗酮处理莱茵衣藻后，其光合色素逐渐降解(图 2)，Fv/Fm 逐渐减弱至 24 h 时完全消失(图 3)，这表明藻细胞死亡并非坏死，而可能是 PCD。

Caspases 活化与细胞 PCD 过程密切相关，其最早在哺乳动物细胞中发现，而植物和藻类细胞中与其功能相似的酶则被称为 caspase-likes。在发生 PCD 时，细胞色素 c 通过线粒体膜上的渗透转移孔进入细胞质后与 Apaf-1 和 caspase-9 相结合，从而激活 PCD 途径中的 caspase 级联反应[25]。Caspase-9 是内源途径启动分子，可激活多种下游 caspases，包括引发 DNA 降解的 caspase-3[26]。此外，外源途径的 caspase-8 也可激活 caspase-3[27]。在藻细胞中，caspase-like 活化是其 PCD 的关键特征[22,28]，抑制其活化可阻止细胞死亡[22]。α-紫罗酮处理可激活莱茵衣藻 caspase-9-like 和 caspase-3-like，且两者活性变化趋势相一致，这表明 caspase-3-like 应该是由 caspase-9-like 通过内源途径激活(图 4)，进而引发 DNA 降解(图 5)。

当细胞发生 PCD 时，DNA 快速降解并呈现 ladders 是其典型特征[24]，然而并非所有 PCD 过程都能产生 ladders[13,17]。例如，UV、H_2O_2 和甲萘醌诱导莱茵衣藻 PCD 时会出现 DNA ladders[22,29–30]，

而乙酸、蜂毒素、芳樟醇和 α-松油醇诱导则不出现 DNA ladders[13,17,31]，这可能是由于藻细胞株系和诱导因子不同所致。在本研究中，0.2 mmol/L α-紫罗酮处理莱茵衣藻 1 h 时出现 PCD 典型特征——DNA ladders，随着处理时间延长 DNA 降解逐渐增强，在 24 h 时降解为 100–250 bp 片段(图 5)。

蓝藻 VOCs 对其他藻类生长具有明显的化感作用[3–4,6–7]。α-紫罗酮是蓝藻 VOCs 的主要化感成分之一[3]，其可通过诱导 PCD 以杀死莱茵衣藻，这表明蓝藻 VOCs 可通过诱导 PCD 以发挥化感作用，从而促进蓝藻成为富营养化水体优势种群。

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Programmed cell death in *Chlamydomonas reinhardtii* induced by cyanobacterial volatile α-ionone

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Abstract: [Objective] Allelopathic effects of cyanobacterial volatile organic compounds (VOCs) on other algae promote cyanobacteria becoming the dominant species in eutrophicated waters. The aim of the present study is to uncover the allelopathic lethal mechanism of cyanobacterial VOCs by using α-ionone. **[Methods]** In the treatment with α-ionone, the cell growth of *Chlamydomonas reinhardtii* was investigated, and the photosynthetic abilities, caspase-like activities and DNA ladders were investigated at lethal concentration. **[Results]** When *C. reinhardtii* cells were treated with α-ionone at 0.05 and 0.1 mmol/L, the cell growth was significantly inhibited, and 38.3% of the cells were killed by 0.1 mmol/L α-ionone. However, all the cells were killed by 0.2 mmol/L α-ionone. During the cell death, the photosynthetic pigments gradually degraded with prolonging the treatment time, and Fv/Fm gradually declined and even disappeared, indicating that the cell death is not a necrosis. Meanwhile, the activities of caspase-9-like and caspase-3-like increased remarkably. In the treatment with 0.2 mmol/L α-ionone for 1 h, DNA showed ladders and then gradually degraded to the fragments of 100–250 bp. **[Conclusion]** This suggests that cyanobacterial VOCs play the allelopathic role by inducing programmed cell death.

Keywords: cyanobacteria, *Chlamydomonas reinhardtii*, α-ionone, programmed cell death, allelopathic mechanism

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