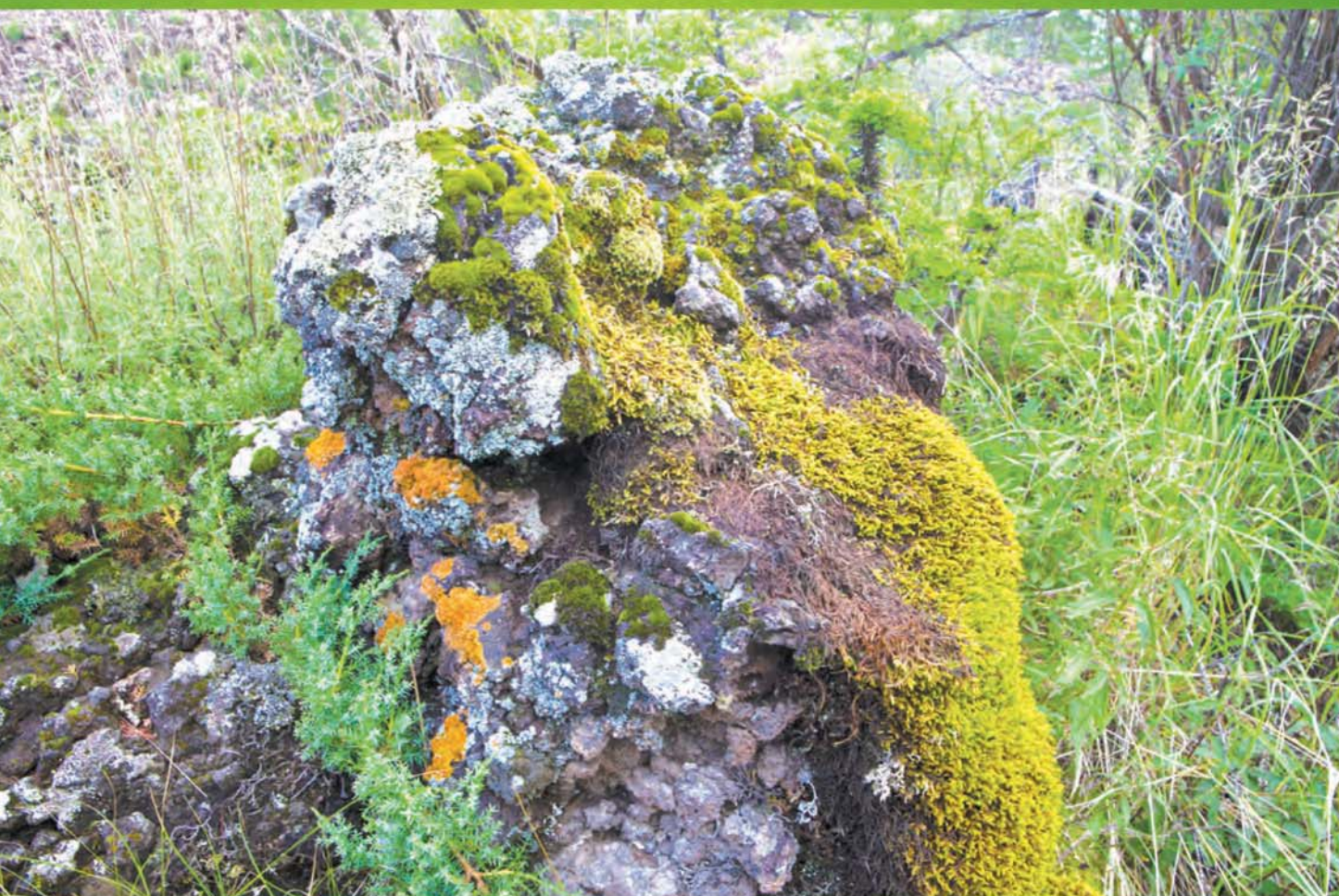


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封面图说: 植物生命演进石——这不是一块普通的火山岩,而是一块集中展示植物“原生演替”过程最有价值的石头。火山熔岩冷却后的玄武岩是无生命无土壤的真正“裸石”,风力使地衣的孢子传入,在一定温湿度环境下,开始出现了壳状地衣,壳状地衣尸体混合了自然风化的岩石碎屑提供的条件使叶状、枝状地衣能够侵入,接着苔藓侵入,是它们启动了土壤的形成,保持了土壤的湿度,并使营养物质反复循环。于是蕨类定居,草丛长了起来,小灌木出现,直到树木生长,最终形成森林。

彩图提供: 陈建伟教授 北京林业大学 E-mail: cites.chenjq@163.com

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强壮前沟藻化感物质分析

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摘要:微藻化感作用是一种极其复杂的生理、生态学现象。选取强壮前沟藻指数生长初期 I 和平台生长初期 II 两个阶段的滤液对中肋骨条藻、海洋原甲藻、锥状斯氏藻及球等鞭金藻生长的影响进行了研究,并萃取了阶段 II 的粗提物,抑藻检测表明其具有“杀藻”效应,通过 GC/MS 分析该粗提物中具有潜在化感作用的物质种类。研究发现强壮前沟藻两个生长阶段的滤液对中肋骨条藻均产生强烈致死效应(phase I: $F=15.18475$, $P=0.00298<0.05$; phase II: $F=6.24559$, $P=0.03149<0.05$);锥状斯氏藻在强壮前沟藻滤液中生长,实验结束时两个阶段中的细胞密度分别是对照组的 79.3% 和 68.9%;海洋原甲藻在强壮前沟藻生长阶段 II 滤液实验的最后 3d,其生长受到显著抑制($F=4.84438$, $P=0.04925<0.05$);而等鞭金藻在强壮前沟藻两个生长阶段滤液中被抑制现象不明显($P>0.05$)。强壮前沟藻滤液实验表明,强壮前沟藻能够向微环境中分泌代谢产物来抑制中肋骨条藻和海洋原甲藻的生长,并且这种抑制效应具有种类特殊对应性。上述实验结果还表明,强壮前沟藻生长阶段 II 的滤液具有的生长抑制作用较为明显。采用乙酸乙酯萃取强壮前沟藻生长阶段 II 滤液中的代谢产物,检测发现其代谢粗提物具有溶藻效应,GC/MS 分析结果表明粗提物中存在 4 种可能产生化感抑制作用的物质,其中二丁基羟基甲苯(Butylated Hydroxytoluene BHT)被认为具有抗滤过性病原体 and 抗微生物活性。

关键词:强壮前沟藻去藻滤液;粗提物;化感物质;溶藻效应;GC/MS 分析

Analysis on allelochemicals in the cell-free Filtrates of *Amphidinium carterae*

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Abstract: Algal allelopathy is an ecological or physiological phenomenon that has focused attention on the interactions among algae and the production of algal toxins. A species of marine bloom-forming microalga, *Amphidinium carterae* was selected in the present study to estimate its potentially allelopathic effects on other species of microalgae. The cell-free filtrates of *A. carterae* in two growth phases: exponential growth phase (abbreviated as growth phase I) and stationary growth phase (abbreviated as growth phase II) were prepared, and its inhibitory effects on growth of other species of marine microalgae were studied under stable environmental conditions (temperature, light and illuminating periodicity, etc.). The cell-free culture filtrates were prepared firstly when *A. carterae* were cultured phase I and phase II, which was about the the growth 7th and 17th respectively, and enriched with f/2 medium. The above prepared culture filtrates were used to culture 4 species of marine microalgae: *Skeletonema costatum*, *Prorocentrum micans*, *Scrippsiella trochoidea* and *Isochrysis galbana*. Moreover, the crude extractions in the cell-free filtrate of *A. carterae* of growth phase II were extracted by GC/

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MS, and the major components were analyzed. Results showed that: in the enriched cell-free filtrates, *S. costatum* were obviously inhibited in either filtrates of phase I ($F = 15.18475$, $P = 0.00298 < 0.05$) or phase II ($F = 6.24559$, $P = 0.03149 < 0.05$) as compared to that in the control which in f/2 enriched medium, but the cell numbers of *S. trochoidea* were only about 79.3% and 68.9% in filtrates prepared from phase I and phase II as compared to the control by the end of experiment. As to *P. micans*, its growth was obviously suppressed ($F = 4.84438$, $P = 0.04925 < 0.05$) in the filtrate prepared from phase II during the last 3 days. Little inhibition was observed in *I. golbana* in either filtrates prepared from either phases ($P > 0.05$). Cell-free filtrate cultures experiment of *A. carterae* is one of the basic approaches on allelopathy research. Results in the present study suggested that *A. carterae* secreted or released some substances into the culture medium, which inhibited the co-cultured *S. costatum* and *P. micans*, and these inhibitory effects could not caused by light, nutrients or cell contact to gain a competitive advantage. Many algae could release allelochemicals into the environment which may alter phytoplankton growth to enhance its predominance. Besides, growth of these two species was more inhibited in enriched cell-free filtrate prespred from growth phase II than that from phase I. It also proved that the allelochemical effects on algae were species-specific because growth of *S. costatum* and *P. micans* was inhibited, but exerted little effects on *S. trochoidea* and *I. golbana*. Another experiment was performed to further prove the allelopathic effect of *A. carterae*. The crude extractions from cell-free filtrates of *A. carterae* at different concentrations were added into *S. costatum* and *P. micans* culture media, found that cells dissolved in higher concentration after many hours. It is noted that the crude extractions had the function of dissolving some algal cells. Then the crude extractions were analyzed by GC/MS. Four components in the crude exactions were suspected to have the allelopathic effects, they were Butylated Hydroxytoluene, Butyl citrate, Benzene, 1-Chlorodifluoromethoxy-4-nitro-, and Benzyl butyl phthalate, among with Butylated Hydroxytoluene (BHT) was reported with oxidative characteristic and was used as an antioxidant additive. Results in the present study brought light on the hypothesis that *A. carterae* could realease allelochemical(s); however, deep research should carry out to explain the possible inhibiting mechanism.

Key Words: cell-free filtrate of *A. carterae*; crude extraction; allelochemicals; dissolving algae; GC/MS

微藻化感作用的研究在国外很早就引起了重视^[1-2]。Inderjit 和 Dakshini^[3]首次于 1994 年对微藻化感作用进行了定义。微藻的化感作用是一种极其复杂的生理、生态学现象,会对细胞分裂、离子和水分的吸收、水分子平衡、生长素的新陈代谢、呼吸作用、光合作用、酶的功能、信息传递以及基因表达等产生重要的影响^[4-6]。

微藻的化感作用是一种普遍存在的现象,Pratt^[7]早在 1940 年就发现小球藻(*Chlorella vulgaris*)在生长过程中分泌毒素抑制其他两种浮游藻类的生长;Myklestad^[8]等人也报道了产毒的定鞭藻(*Chrysochromulina polylepis*)能够抑制中肋骨条藻的生长。Granéli^[9]列出了部分能产生化感作用的有害赤潮藻:微囊藻属(*Microcystis* sp.)产生的化感物质具有抑制细胞生长、抑制光合作用和细胞致死效应^[10-11];尖刺拟菱形藻(*Pseudo-nitzschia pungens*)产生的化感物质具有抑制细胞生长的效应^[12];塔玛亚历山大藻(*Alexandrium tamarense*)产生的化感物质能够抑制微藻细胞生长及致死效应^[13-16];小定鞭金藻(*Prymnesium parvum*)产生的小定鞭素 Prymnesin 也有抑制细胞生长、导致细胞肿胀及致死效应^[17-21]。关于微藻化感物质的鉴定,Granéli^[9]只是统计了部分有害赤潮藻产生的化感物质,并且这些有害赤潮藻的化感作用机制、具体作用物质及作用对象范围等还有许多空白处,而其他微藻是否也具有化感作用,以及产生怎样的化感物质等还知之甚少,对现象的本质及化感物质了解不深,所以本文选择具有化感作用的典型赤潮藻种强壮前沟藻(*Amphidinium carterae* Hulburt)为例进行研究。强壮前沟藻是一种能产生溶血性毒素的有害赤潮藻原因种^[22-23],通过前期的共培养实验,已探知其对中肋骨条藻(*Skeletonema costatum*)、海洋原甲藻(*Prorocentrum micans*)和锥状斯氏藻(*Scrippsiella trochoidea*)都具有不同程度的生长抑制作用,而研究认为,化感作用的产生

及作用强度还与化感藻种的生长阶段(growth stage)有关^[24],亚历山大藻(*Alexandrium*)在平台期就表现出更强的化感作用^[13],因此本实验选择强壮前沟藻两个生长阶段的去藻滤液分别培养其他几株藻种,并利用乙酸乙酯萃取平台生长初期Ⅱ的去藻滤液中的代谢物,初步分析代谢粗提物中能产生化感作用的物质种类。

1 实验材料与方法

1.1 微藻及培养条件

实验用强壮前沟藻(*Amphidinium carterae*)、中肋骨条藻(*Skeletonema costatum*)、海洋原甲藻(*Prorocentrum micans*)、锥状斯氏藻(*Scrippsiella trochoidea*)及球等鞭金藻(*Isochrysis galbana*)均来自中国科学院海洋研究所海洋生态与环境科学重点实验室藻种库,f/2培养基培养,培养温度(20 ± 1)℃,光暗比12h:12h,光照强度(60 ± 2) $\mu\text{mol m}^{-2} \text{s}^{-1}$ 。实验所用海水均经0.45 μm 纤维膜过滤后高温灭菌,按Guillard^[25]添加f/2营养盐,盐度30。

1.2 强壮前沟藻去藻滤液化感作用研究

取强壮前沟藻指数生长初期Ⅰ和平台生长初期Ⅱ的去藻滤液(图1),离心取上清液经0.45 μm 膜过滤和0.22 μm 滤膜除菌得到滤液做为培养基,补充f/2营养盐,100 mL培养瓶中分别培养60 mL的中肋骨条藻、海洋原甲藻、锥状斯氏藻及球等鞭金藻4种微藻。相同条件下以f/2培养基培养的微藻作为对照。每组3个平行样。每天取1 mL藻液细胞计数。

1.3 强壮前沟藻代谢粗提物化感成分分析

1.3.1 强壮前沟藻代谢粗提物萃取

实验室条件下扩大培养强壮前沟藻,至其平台生长初期Ⅱ将藻液离心,取上清液,经0.45 μm 和0.22 μm 滤膜过滤得强壮前沟藻去藻滤液,以有机溶剂乙酸乙酯作为萃取剂,提取强壮前沟藻代谢产物。为排除干扰,实验室也以同样方法萃取f/2培养基中的物质作为对照。将获得的粗提物37℃下旋转蒸发,浓缩至3 mL,4℃冰箱内保存待用。

1.3.2 代谢粗提物的抑藻检测

中肋骨条藻和海洋原甲藻做为检测生物对代谢粗提物进行抑藻活性检测;取强壮前沟藻代谢粗提物1 mL,氮气吹干溶于100 μL 二甲亚砜中,分别取5,3,1 μL 样品加到1 mL检测生物中,每组3个平行样;将加入5 μL 二甲亚砜的中肋骨条藻和海洋原甲藻作为对照。放置1至6 h后观察溶藻现象。

1.3.3 代谢粗提物GC/MS分析

将得到的代谢粗提物脱水、有机滤膜过滤后GC/MS分析,仪器为美国Agilent7890N-975C气相质谱联用仪,色谱柱为HP-5MS,气相色谱条件为载气高纯He,柱流量0.8 mL/min,起始柱温130℃,升温速率10℃/min,升至250℃,维持20 min,进样量1.0 μL ;质谱条件为电离源用EI源,电子能量70 eV,电离源温度230℃,质量范围为30—500 amu,扫描速度0.5 s/dec。

2 实验结果

2.1 强壮前沟藻培养滤液对其他微藻生长的影响

2.1.1 对中肋骨条藻生长的影响

中肋骨条藻在强壮前沟藻指数生长初期Ⅰ去藻滤液中的生长状况如图2(A)所示。接种密度为 10^4 个/mL的中肋骨条藻在滤液中生长被强烈抑制($F=15.18475, P=0.00298 < 0.05$),接种第2天细胞密度下降,并在前4 d细胞生长停滞,第5天密度才有所上升。中肋骨条藻在强壮前沟藻平台生长初期Ⅱ去藻滤液中的生长状况如图2(B)所示。滤液中生长的中肋骨条藻被完全抑制($F=6.24559, P=0.03149 < 0.05$),细胞密度一

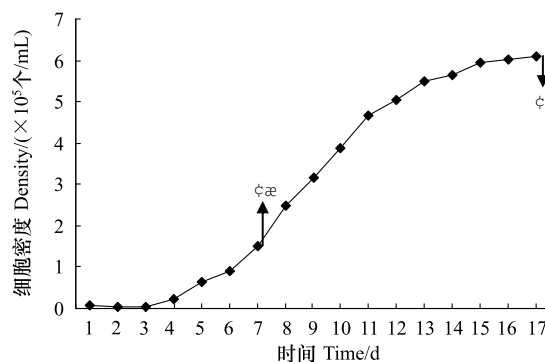


图1 强壮前沟藻生长曲线图

Fig. 1 The growth curve of *Amphidinium carterae*

直处于低水平。与对照组相比,强壮前沟藻两个生长阶段的去藻滤液都能够显著地抑制中肋骨条藻的生长,说明强壮前沟藻去藻滤液中存在具有能破坏中肋骨条藻细胞生长的物质,这种化感作用与释放到培养液中的某些物质有关。

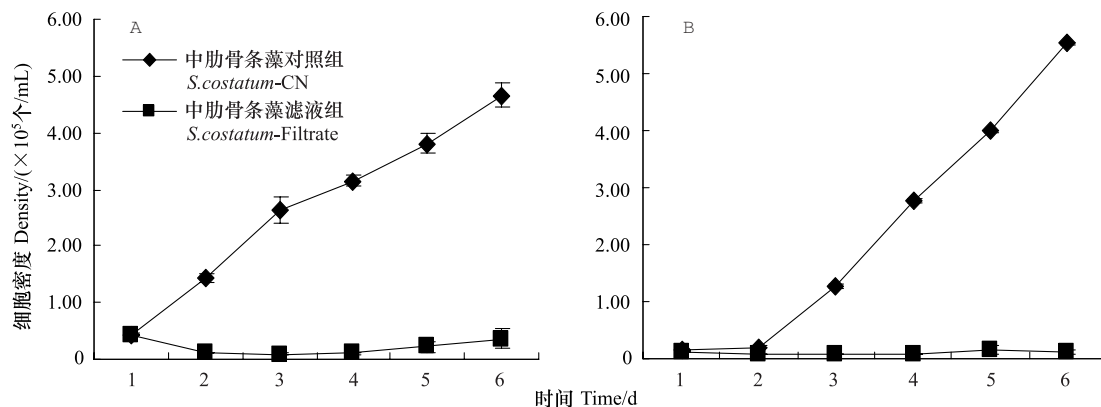


图2 中肋骨条藻在强壮前沟藻指数生长初期 I (A) 和平台生长初期 II (B) 去藻滤液中生长曲线图

Fig. 2 The growth curve of *S. costatum* in cell-free filtrate of *A. carterae* in growth phase I (A) and phase II (B)

2.1.2 对海洋原甲藻生长的影响

海洋原甲藻在强壮前沟藻指数生长初期 I 培养滤液中生长状况如图 3 (A) 所示。滤液中的海洋原甲藻生长受到轻微抑制作用 ($F=0.36086$, $P=0.56140>0.05$), 实验结束时, 对照组原甲藻密度达到 0.46×10^4 个/mL, 滤液中生长的原甲藻密度也达到了 0.35×10^4 个/mL。海洋原甲藻在强壮前沟藻平台生长初期 II 去藻滤液中生长状况如图 3 (B) 所示。实验第 3 天开始, 滤液中生长的海洋原甲藻细胞密度明显小于对照组, 并且这种差距随时间增加愈加明显, 实验结束时滤液中的海洋原甲藻细胞数仅为对照组的 45.4%, 强壮前沟藻去藻滤液对海洋原甲藻的生长抑制作用在实验后期表现显著 ($F=4.84438$, $P=0.04925<0.05$)。由图 4 也可以发现, 强壮前沟藻平台生长初期 II 的去藻滤液对海洋原甲藻生长抑制作用比强壮前沟藻指数生长初期 I 的去藻滤液对海洋原甲藻生长抑制作用明显, 可能是因为平台生长初期 II 的强壮前沟藻密度增大, 产生的具有生长抑制作用的物质也随之增多, 导致了对原甲藻的生长抑制明显。

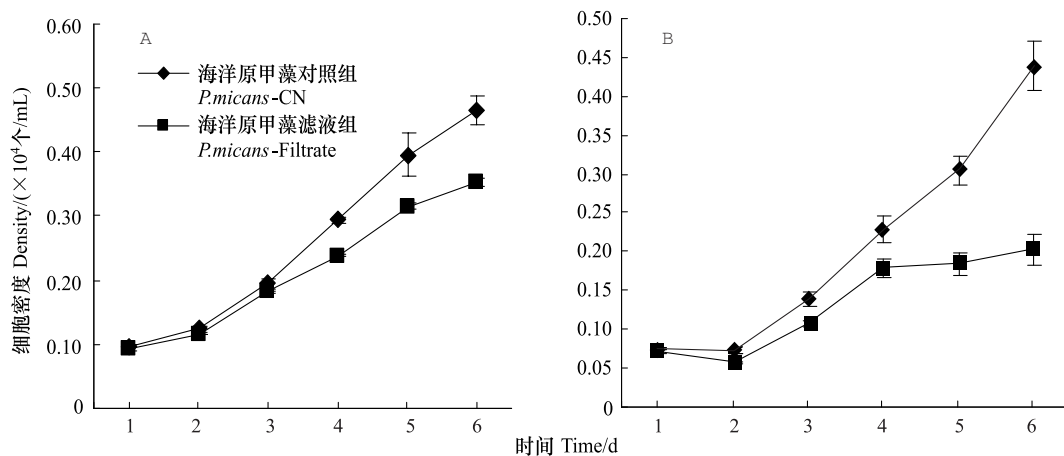


图3 海洋原甲藻在强壮前沟藻指数生长初期 I (A) 及平台生长初期 II (B) 去藻滤液中生长曲线图

Fig. 3 The growth curve of *P. micans* in cell-free filtrate of *A. carterae* in growth phase I (A) and phase II (B)

2.1.3 对锥状斯氏藻生长的影响

锥状斯氏藻在强壮前沟藻指数生长初期 I 去藻滤液中生长状况如图 4 (A) 所示。可以看出, 滤液中生长

的锥状斯氏藻前 4 天基本与对照组一致;4 d 后,锥状斯氏藻受到较强的抑制作用($F=0.10461$, $P=0.75303>0.05$)。锥状斯氏藻在强壮前沟藻平台生长初期 II 去藻滤液中生长状况如图 4(B) 所示。经过 2 d 的生长迟滞,滤液中生长的锥状斯氏藻与对照组一起进入指数生长期,第 4 天开始,滤液中的锥状斯氏藻受到轻微抑制($F=0.44200$, $P=0.52119>0.05$)。第 6 天,两个生长期的去藻滤液中生长的锥状斯氏藻细胞密度分别是对照组的 79.3% 和 68.9%。强壮前沟藻平台生长初期 II 的去藻滤液对锥状斯氏藻的抑制作用比指数生长阶段初期 I 的明显,说明其释放的具有生长抑制作用的物质浓度较大,抑制作用也较为显著。

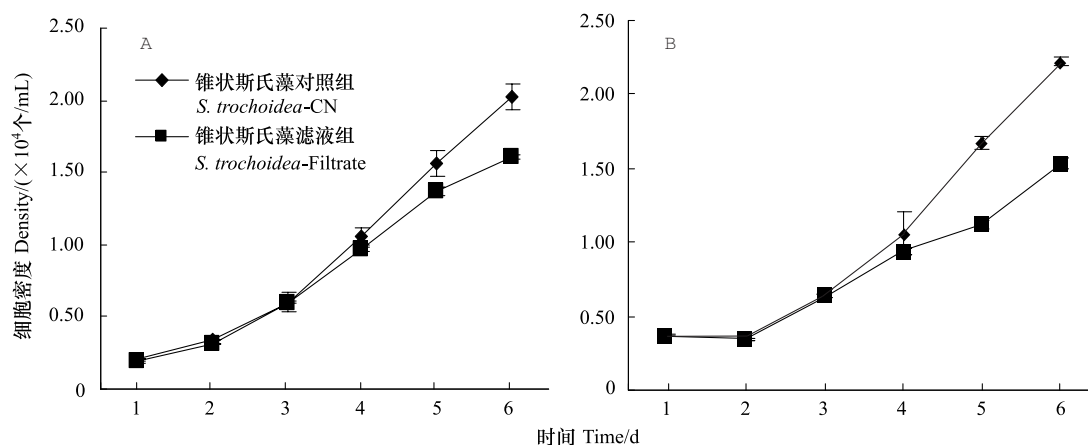


图 4 锥状斯氏藻在强壮前沟藻指数生长初期 I (A) 及平台生长初期 II (B) 去藻滤液中生长曲线图

Fig. 4 The growth curve of *S. trochoidea* in cell-free filtrate of *A. carterae* in growth phase I (A) and phase II (B)

2.1.4 对球等鞭金藻生长的影响

球等鞭金藻在强壮前沟藻指数生长初期 I 去藻滤液中生长状况如图 5(A) 所示。实验过程中,滤液中的球等鞭金藻生长一直被轻微抑制($F=0.31194$, $P=0.58878>0.05$),实验结束时密度达到了 2.25×10^5 个/mL,对照组密度为 2.77×10^5 个/mL。球等鞭金藻在强壮前沟藻平台生长初期 II 去藻滤液中生长状况如图 5(B) 所示。初始密度为 3×10^4 个/mL 的球等鞭金藻在滤液中生长受到轻微抑制作用($F=0.07146$, $P=0.79465>0.05$),第 6 天密度达到 2.02×10^5 个/mL,是对照组的 90.1%。强壮前沟藻去藻滤液对球等鞭金藻的生长抑制现象并不显著。

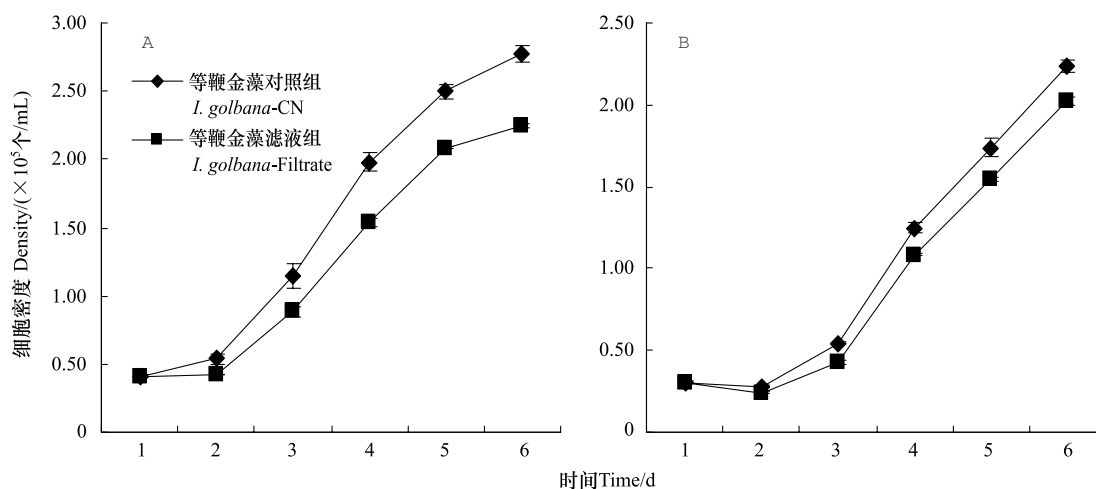


图 5 球等鞭金藻在强壮前沟藻指数生长初期 I (A) 及平台生长初期 II (B) 去藻滤液中生长曲线图

Fig. 5 The growth curve of *I. golbana* in cell-free filtrate of *A. carterae* in growth phase I (A) and phase II (B)

2.2 强壮前沟藻代谢粗提物抑藻检测

2.2.1 强壮前沟藻代谢粗提物对中肋骨条藻生长的影响

强壮前沟藻代谢粗提物加入到中肋骨条藻 1 h 后观察,发现中肋骨条藻藻液颜色明显发白,且加入的代谢物量越多,颜色变化越大。镜检发现中肋骨条藻细胞破碎,加入代谢物量为 1 μL 时(图 6B),骨条藻链条断裂,细胞碎裂,已经没有完整的个体;加入的代谢物量为 5 μL 时(图 6D),中肋骨条藻细胞已经完全被“溶解”,只剩下空壳的链条骨架。

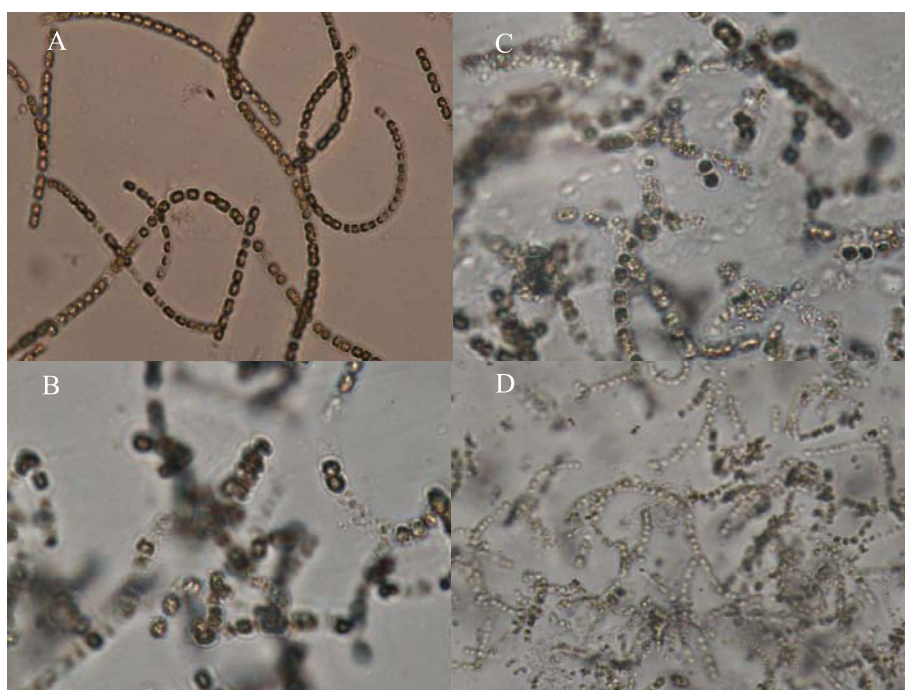


图 6 1h 后强壮前沟藻代谢粗提物对中肋骨条藻的影响

Fig. 6 A. *carterae* crude extraction affect growth of *S. costatum*

A: 加入二甲亚砷的中肋骨条藻对照; B: 1 μL ; C: 3 μL ; D: 5 μL 粗提物对中肋骨条藻的影响

2.2.2 强壮前沟藻代谢粗提物对海洋原甲藻生长的影响

强壮前沟藻代谢粗提物加入到海洋原甲藻 1, 2, 4, 6 h 后观察,发现海洋原甲藻的生长逐渐被抑制;原甲藻细胞基本停止增殖,加入的强壮前沟藻代谢粗提物量较少时,与对照相比,原甲藻细胞运动停止,内容物分布结构改变,细胞质壁分离,细胞质萎缩(图 7B),海洋原甲藻细胞受到伤害而衰老凋亡;加入的代谢粗提物量较多时,藻细胞发生破裂,细胞内容物释放出来,溶藻现象显著(图 7C);加入的代谢粗提物量处于上两者之间时,海洋原甲藻细胞由萎缩现象向溶藻现象转变。

2.3 强壮前沟藻代谢粗提物 GC/MS 分析

GC/MS 分析强壮前沟藻平台生长初期 II 的代谢粗提物及 f/2 培养基的乙酸乙酯相萃取物,经化学检索及标准质谱图库,结合相关文献人工谱图解析强壮前沟藻滤液粗提物的各化学成分,以及除去对照组后,判断出 4 种可能的化感物质是二丁基羟基甲苯、二氟一氯甲烷-4-氮苯、柠檬酸丁酯、邻苯二甲酸丁苄酯(表 1),其化学结构式如图 8 所示。

3 讨论

Rice^[27]曾指出化感作用的有无以及强弱不仅取决于化感物质的浓度,还取决于化感物质所作用的对象,具有种类特殊对应性。本文中肋骨条藻、强壮前沟藻、锥状斯氏藻以及等鞭金藻在强壮前沟藻两个生长阶段去藻滤液中不同响应的实验又进一步证实了这种现象。

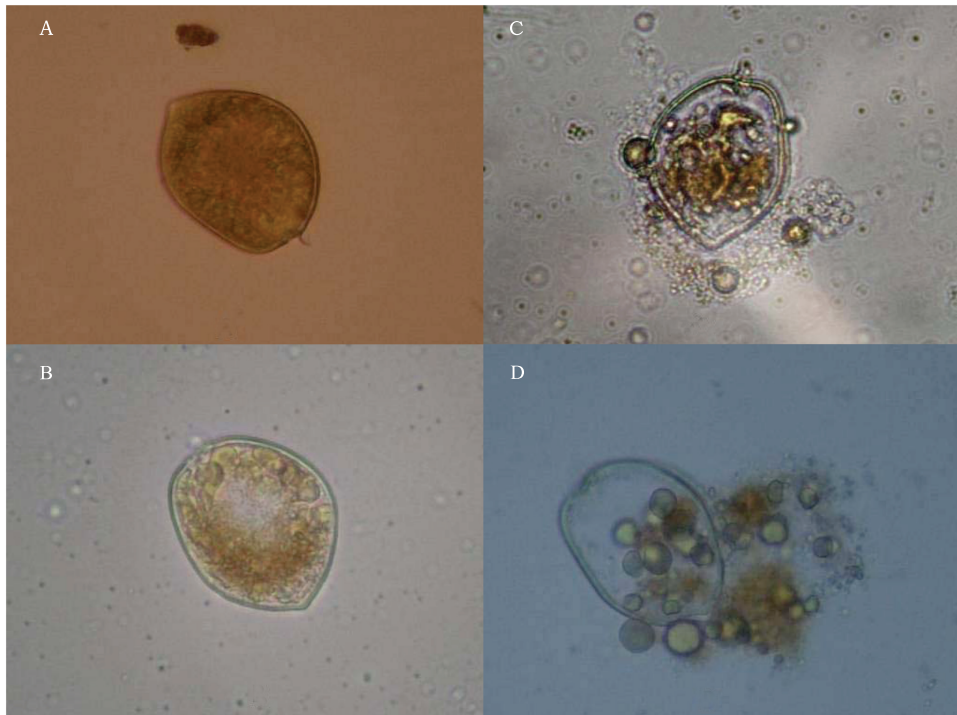


图7 6h后强壮前沟藻代谢粗提物对海洋原甲藻的影响

Fig.7 A. carterae crude extraction affected growth of P. micans

A: 加入二甲亚砆的海洋原甲藻对照;B: 1μL;C: 3μL; D: 5μL 粗提物对海洋原甲藻的影响

表1 强壮前沟藻代谢粗提物中可能的化感物质

Table 1 Major potential allelochemicals in A. carterae crude extraction

序号 Number	检出成分及对应物名称 Major potential allelochemicals	分子式 Molecular formula	相似度/% Similarity	文献 Literature
1	二丁基羟基甲苯	C ₁₅ H ₂₄ O	87	[26]
2	二氟一氯甲烷-4-氮苯	C ₇ H ₄ O ₃ F ₂ NCl	93	
3	柠檬酸丁酯	C ₁₈ H ₃₂ O ₇	90	
4	邻苯二甲酸丁苄酯	C ₁₉ H ₂₀ O ₄	95	

由表1可以看出,强壮前沟藻去藻滤液中具有生长抑制作用的可能的化感物质主要是一些酯、苯类等化合物。邻苯二甲酸丁苄酯(Benzyl butyl phthalate-BBP)属于邻苯二甲酸酯类化合物,是一种环境内分泌干扰物,已被美国国家环保局(EPA)列为6种优先控制污染物之一。二丁基羟基甲苯(BHT)是一种酚类抗氧化剂,H. Tuner等^[26]认为BHT具有抗滤过性病原体 and 抗微生物活性。也有人提出BHT并没有在水生生物体内累积的倾向,但此种化合物在对照组的乙酸乙酯萃取物的GC/MS分析中没有出现,因此,BHT可能是强壮前沟藻释放到滤液中的一种代谢物质。

随着化学分析技术的不断进步,有关化感物质种类鉴定的研究正在逐渐展开。目前,对高等植物^[28-29]及大型浮游植物^[30-31]的化感物质报道较为详细。关于微藻化感物质的分离和鉴定,国内外学者证明了“不同种类的微藻会产生不同的化感物质”^[32-36],因而同一株微藻会对不同的化感藻种产生“因藻而异”的响应,即上述的种类特殊对应性。例如,赤潮异弯藻(*Heterosigma akashiwo*)的化感物质能对特定藻种产生生长抑制作用,而对另一些藻种则有促进生长的效应^[37-38]。颜天等^[39]研究认为赤潮异弯藻释放毒素类物质,其能抑制其他硅藻的生长^[40],而对其本身的生长则有促进作用^[41]。本文的实验结果也证实,强壮前沟藻去藻滤液对某些微藻(中肋骨条藻等)产生生长抑制作用,对其他微藻(球等鞭藻)则没有显著的生长抑制作用。这种化感抑制作用在化感藻种的生长快到达平台期时表现更强烈,化感藻种的生长阶段在一定程度上决定了化感现

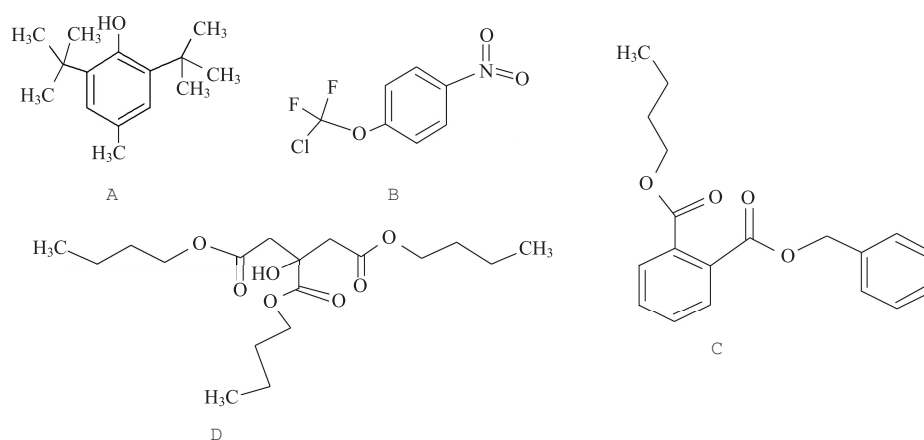


图8 4种可能产生化感作用的物质结构式

Fig. 8 Structure of major potential allelochemicals in *A. carterae*

A: 二丁基羟基甲苯; B: 二氟一氯甲烷-4-氮苯; C: 柠檬酸丁酯; D: 邻苯二甲酸丁苄酯

象产生的强弱。

Kajiwar^[42]等发现长崎裸甲藻释放的挥发性物质能加速某些藻细胞的破裂,而强壮前沟藻代谢粗提物的抑藻检测实验也证实,这些抑藻物质具有“溶藻效应”。Echigoya 等^[43]从强壮前沟藻细胞中萃取得到的 5 种具有抗真菌和还原血红蛋白类似物的物质,Rhodes 等^[44]研究认为强壮前沟藻萃取得到的西加鱼毒对小鼠具有致死效应。本实验得出强壮前沟藻去藻滤液粗提物中含有 4 种主要的化感物质,并且本实验设置了对照组,可以有效地除去底液带来的影响,获得的结果将更为可靠。

目前研究化感物质的一种有效方法是扩大培养化感目标藻,提取滤液中的化感物质。Tameishi^[45]使用丙酮对微型原甲藻滤液萃取进行多糖与脂肪酸的 GC 分析。也有学者调节滤液的 pH 值,按照酸碱性对化感物质进行提取。但是,由于分离方法的局限性,分离出的抑藻物质比较复杂,多数无法确定具体结构,因此必须寻找一套可靠的方法对粗提物做进一步提纯分析。采用高效液相色谱(HPLC)、色谱质谱(GC/MS)以及薄层色谱(TLC)联用技术等方法相结合当是将来研究化感物质的有效手段。

4 结论及展望

本文以强壮前沟藻去藻滤液为研究对象,围绕强壮前沟藻在生长过程中释放的化感物质进行一系列的分析,结果表明:

(1)强壮前沟藻去藻滤液培养中肋骨条藻、海洋原甲藻、锥状斯氏藻和球等鞭金藻等 4 种微藻,发现去藻滤液中强壮前沟藻分泌的代谢产物对中肋骨条藻强烈抑制,对球等鞭金藻的生长没有显著影响,化感作用具有种类特殊对应性;平台生长初期Ⅱ的去藻滤液比指数生长初期Ⅰ的去藻滤液对 4 种微藻生长抑制作用明显,化感作用与生长阶段有关;

(2)提取强壮前沟藻平台生长初期Ⅱ去藻滤液中产生生长抑制作用的物质,以中肋骨条藻和海洋原甲藻检测强壮前沟藻代谢粗提物的抑藻效应,观测到强壮前沟藻代谢粗提物对中肋骨条藻和海洋原甲藻细胞具有溶藻、杀藻作用,并且加入的代谢粗提物量越大,溶藻现象越明显;

(3)对强壮前沟藻代谢粗提物 GC/MS 分析,除去对照后得到 4 种可能的化感物质,分别为二丁基羟基甲苯、柠檬酸丁酯、二氟一氯甲烷-4-氮苯和邻苯二甲酸丁苄酯。

(4)本文只是研究了强壮前沟藻 2 个生长阶段滤液的化感作用,可以再进行细化研究。

(5)有必要购买标准品来验证这 4 种可能的化感物质的化感效应。

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