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培养条件下发菜的形态建成

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摘要: 固体培养基培养的发菜(*Nostoc flagelliforme*)在黑暗与低光强(<1 μmol/m^2·s)条件下细胞发育受到抑制, 在光强 10、20、30、60 μmol/m^2·s 条件下细胞生长良好, 但在 60 μmol/m^2·s 条件下藻丝体易变黄; 液体充气培养的发菜在光强 20、60、180 μmol/m^2·s 条件下生长速率、类胡萝卜素与多糖含量均随光强升高而增加。发菜在低营养水平时形成的异形胞较多, 异形胞的发生位置也多样, 有端生、间生和连生。当琼脂浓度为 0.5%—4%时发菜具有相同的形态发育特征, 从藻殖段发育至丝状聚集体状态, 再从聚集体中释放藻丝; 当琼脂浓度为 6%—8%时发菜发育至丝状聚集体状态, 藻丝包裹在厚胶鞘中, 观察不到藻丝和藻殖段的释放。以上结果表明光照和培养基的含水量对发菜细胞发育具有重要影响。

关键词: 蓝藻; 发菜; 异形胞; 藻殖段
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发菜(*Nostoc flagelliforme*)是分布在我国西北干旱和半干旱地区的一种陆生固氮蓝藻, 具有重要的经济价值和药用价值。野生发菜的形态结构早已作过专门的研究[1], 但发菜的具体生长发育过程仍然不清楚。近年来, 随着室内培养发菜技术的突破, 发菜的形态发育研究开始受到重视[2,3], 主要集中在温度与光照的组合、氮源对液体培养发菜形态发育的影响。但仍有很多环境因素对室内培养发菜形态发育的影响未见报道。本文观察描述了不同的光照强度、营养水平、琼脂浓度对发菜形态发育的影响, 以期对发菜的人工培养提供参考依据。

1 材料与方法

发菜采自内蒙古四子王旗。试验藻株为本实验室分离得到。固体培养: 取对数生长期的藻种置于无菌玻璃匀浆器中, 加入适量的无菌蒸馏水匀浆, 无菌水离心洗涤两次后再用培养液悬浮得到叶绿素浓度 1 μg/mL 的藻液。取 0.04 mL 藻液为一个点接种于培养基上, 每个平板共接 4 个点。培养条件为 25℃, 除了光照强度的实验外其余处理的光强均为 15 μmol/m^2·s, 连续光照。液体充气培养: 发菜接种叶绿素浓度为 0.5 μg/mL, 培养液 1.5 L 充气培养, 培养温度 25℃, 光强设为 20、60、180 μmol/m^2·s。

试验除了特别说明外固体培养基都为 1.5%琼脂浓度的 $BG11_0$, 试验分三组, 第一组设置不同的光照强度, 分别为 0、<1、10、20、30、60 μmol/m^2·s; 第二组设置不同的营养水平, 分别为 BG11、$BG11_0$(BG11 缺氮)、BG11 缺磷、1/10 $BG11_0$; 第三组设置不同的琼脂浓度, 分别为 0.5%、1%、2%、4%、6%、8%。

显微观察: 发菜的显微结构在配有 pH(相差)、DIC(微分干涉差)的 Leica-DM 5000B 光学显微镜下观察、记录形态特征。

叶绿素 *a* 含量测定: 用甲醇提取, 置 4℃冰箱过夜, 用日本岛津 UV-1601 紫外可见分光光度计测定 665 nm 处的吸光度并计算叶绿素 *a* 含量[4]。

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类胡萝卜素含量测定：在 60℃水浴中先用甲醇提取 30min，冷却后加入石油醚和乙醚萃取，于 453 nm 处测定醚层的吸光度并计算类胡萝卜素含量[5]。

多糖含量的测定：取不同培养时间的发菜培养液 40 mL 分别提取胞外多糖和热水溶性多糖[6]。

2 结 果

2.1 光强对发菜生长发育的影响

固体培养条件下黑暗与低光强(<1 μmol/m^2·s)抑制细胞生长发育，发菜停留在单根藻丝状态(图版Ⅰ-A)，无法完成发育过程；发菜在暗处放置 16d 后再置于 30 μmol/m^2·s 的光强下连续培养 6d，生长得以恢复，并且同步发育形成具异形胞的丝状体，部分藻丝在异形胞处断裂，释放出藻殖段和游离的异形胞(图版Ⅰ-B)，继续光照培养发菜生长的同步性消失，藻殖段发育为藻丝体(图版Ⅰ-C)；在光强为 10、20、30、60 μmol/m^2·s 时发菜生长良好(图版Ⅰ-D)，在 60 μmol/m^2·s 条件下发菜藻丝容易变黄(图版Ⅰ-E)。液体充气培养发菜在 20、60 μmol/m^2·s 时生长良好，180 μmol/m^2·s 条件下藻丝才变黄(图版Ⅰ-F-H)。与固体培养的发菜相比，液体充气培养的发菜更能耐受高光照。液体充气培养发菜的生长速率、类胡萝卜素与多糖含量均随光强升高而增加(图 1、2)。

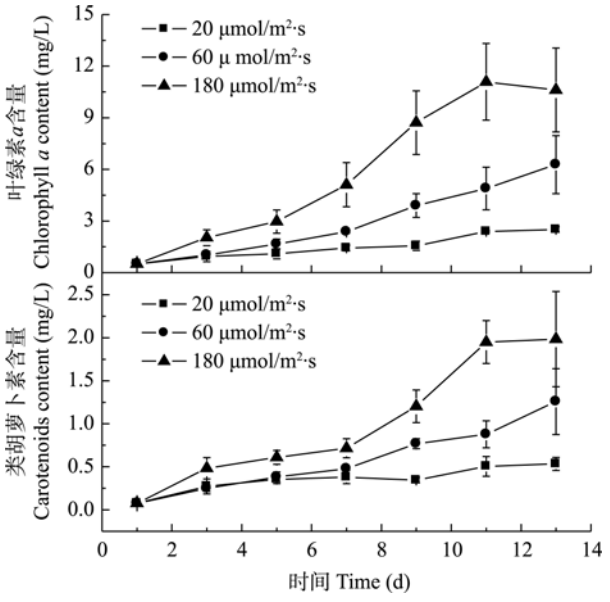

图 1 光强对发菜生长速率(以叶绿素 *a* 浓度表示)和类胡萝卜素含量的影响

Fig. 1 The effects of illumination on the growth rate (measured as chlorophyll *a* concentration) and carotenoids contents of *N. Flagelliforme*

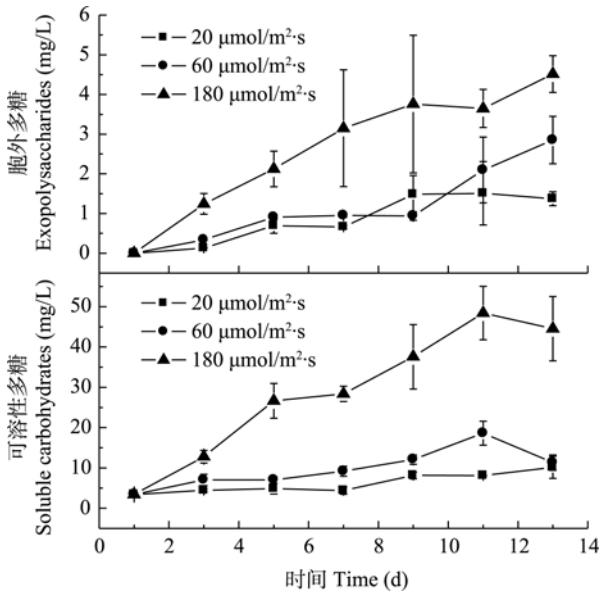

图 2 光强对发菜胞外多糖和可溶性多糖含量的影响

Fig. 2 The effects of illumination on the extracellular polysaccharides and soluble carbohydrate contents of *N. flagelliforme*

2.2 营养水平对发菜生长发育的影响

发菜在低营养水平条件下(1/10 $BG11_0$)也能发育形成聚集体，但藻丝上的异形胞发生频率较高，发生的位置有端生、间生还有连生(图版Ⅱ-A)；氮缺乏($BG11_0$)诱导产生异形胞(图版Ⅱ-B)；在含氮培养基(BG11)中则无异形胞分化(图版Ⅱ-C)。磷缺乏导致了一类特殊的小细胞出现(图版Ⅱ-D)，小细胞的形状和大小变化较大，这些小细胞的产生可能是对不良环境的一种响应，它们的生物功能还有待于进一步研究。

2.3 培养基中不同琼脂浓度对发菜生长发育的影响

琼脂浓度为 0.5%—4%时发菜的形态发育过程相同(图版Ⅲ-A-E)。最初接种的藻殖段细胞膨大分裂形成不连续丝状体，进而形成非丝状体聚集体(开始有胶鞘)。随着发育的进行，细胞紧密挤压在一起的非丝状体细胞团结合形成丝状聚集体，最后有些丝状聚集体破裂释放出藻丝，藻丝在异形胞处断裂形成的藻殖段又将经历以上的发育过程。在发育过程中不同细胞间的结合现象在其他念珠藻中也有报道[7]，而且藻丝由于受到胶鞘的束缚而呈现不同程度的弯曲。发菜的发育不是完全同步的，往往以上多种发育形态并存。在 1%琼脂浓度的固体培养基表面直接观察到一束藻殖段从聚集体中的某些位点释放，成流滑出(图版Ⅲ-F)。当这些藻殖段停止运动后又通过上面描述过的顺序发育成新的丝状聚集体。

在琼脂浓度为 0.5%—4%时藻殖段的释放时间随琼脂浓度的升高而推迟, 在 0.5%、1%琼脂浓度的培养基上培养 8d 后观察到藻殖段的释放, 在 2%—4%琼脂浓度的培养基上培养 11d 才观察到藻殖段的释放。当琼脂浓度为 6%—8%时, 只观察到形成的发菜聚集体(图版Ⅲ-G), 没有观察到发菜藻殖段与藻丝的释放。培养 7 个月后, 发菜形成一层连续而致密的胶鞘(图版Ⅲ-H, I), 内部的藻丝弯曲缠绕、堆积排列, 由于胶鞘较厚并且紧密的束缚, 内部藻丝无法游离出来, 以致不能观察到藻丝和藻殖段的释放。

3　讨　论

3.1　发菜生长发育对光强的响应

在黑暗或光强小于 1 μmol/m^2· s 时发菜发育停留在单根藻丝状态, 没有其他形态的发生。这说明发菜的形态发育与光及其强度有着极大的关系。发菜生长发育受到抑制可能因为缺乏在光照条件下形成用于形态建成的物质[8]。发菜在光照较强时藻丝变黄与类胡萝卜素含量的增加有关, 在高光强条件下微藻会产生较多的类胡萝卜素来抵抗氧化损伤[9]。多糖含量随光强的升高而增加说明能量利用是影响碳水化合物合成的重要因素之一[10]。蓝藻胶鞘的主要成分是多糖, 在高光强下液体培养的发菜胶鞘会发生解体现象[2], 因此, 测定多糖与光强的关系对于理解发菜胶鞘的形成与光强的关系具有重要意义。发菜异形胞的形成受到营养条件的影响, 异形胞的分化是蓝藻对环境变化的一种响应。异形胞的数目、发生位置均受到基因调控, 当 *HetR* 或者 *HetP* 基因出现多拷贝时能够导致异形胞的连生[11]。低营养条件下异形胞的发生频率较高且有连生现象, 说明可能在控制异形胞的发生和位置的分子水平上产生了不同。

3.2　发菜生长发育中胶鞘形成的环境条件

在发菜的人工培养探索中, 胶鞘对于发菜的存活具有重要意义。发菜是胶质复合群体, 水分过多引起胶鞘的解体, 导致藻体死亡[12]。胶鞘可以保持住大量的水分[13], 一方面用来阻止水分的丢失, 抵抗胁迫, 对群体的形态起稳定作用; 另一方面胶鞘也阻碍了藻丝的移动, 降低了藻丝与外界环境的交换, 降低生长速度, 这也是发菜在野外生长缓慢的原因之一。在发菜的人工培养中胶鞘的形成是极其重要的, 但在室内液体培养形成的发菜胶鞘薄而透明[2], 不能稳定群体形态。在低琼脂浓度条件下培养形成的发菜胶鞘也较薄, 很难发挥稳定群体形态、抵抗外界胁迫的功能。高琼脂浓度条件下的发菜形成的胶鞘较厚, 水势降低可以导致发菜多糖含量的增加[14], 高琼脂浓度降低了培养基的水势[15], 在此条件下发菜容易积累多糖, 增厚胶鞘, 藻丝很难从中游离出来, 从而使胶鞘发挥了稳定群体形态的功能。但是发菜的繁殖与生长需要水分, 低水势只是发菜生存的条件, 不能满足生长需要[16]。因此, 在发菜的人工培养过程中, 需要进一步摸索发菜的生长条件, 既能使发菜形成胶鞘, 还能促进发菜快速生长。

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THE MORPHOGENESIS OF *NOSTOC FLAGELLIFORME* UNDER CULTURE CONDITIONS

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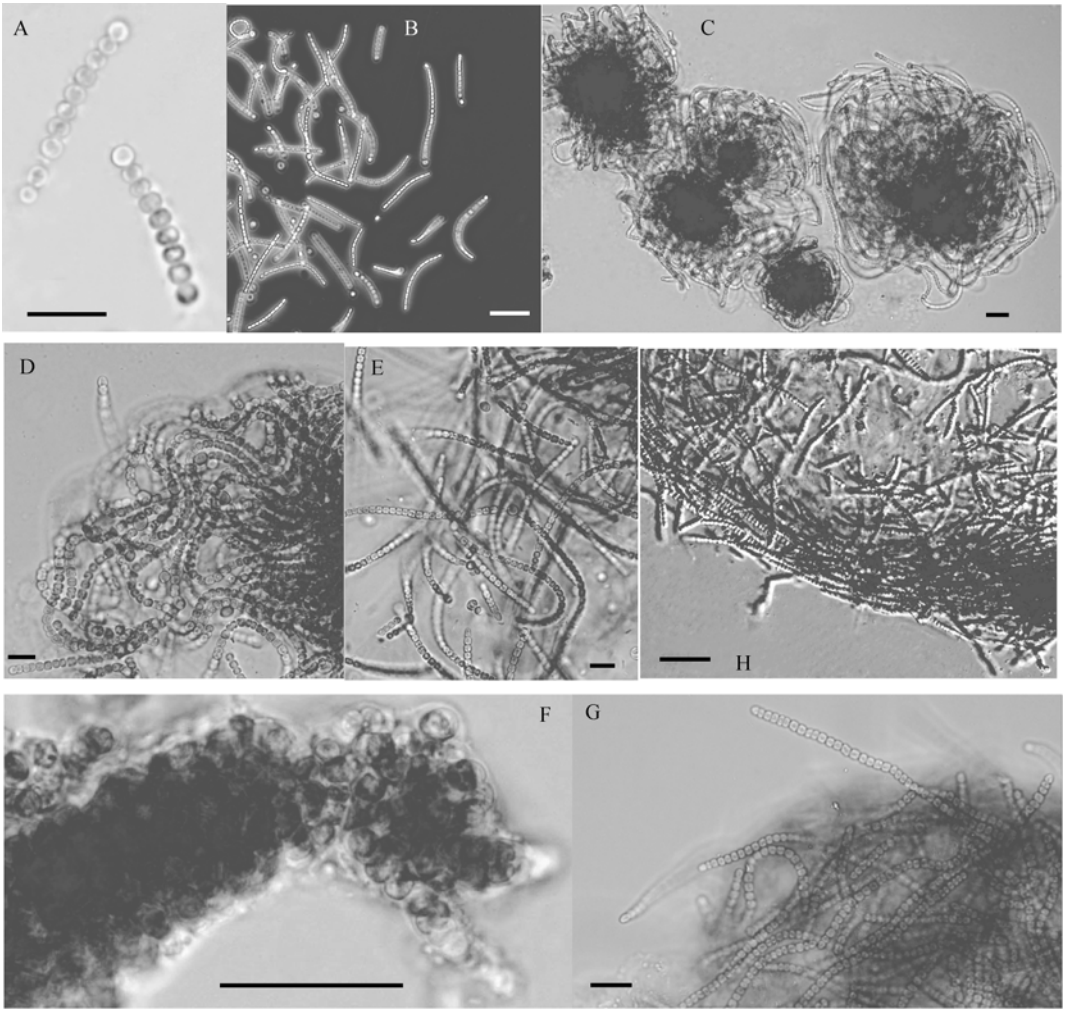
Abstract: Many studies were about the morphological development of *Nostoc flagelliforme* with the improvement of techniques of cultivation. Although the effects of the combination of temperature and illumination, nitrogen sources on development of *N. flagelliforme* have been described by many investigators, the effects of many environmental factors on developmental cycle of *N. flagelliforme* have not been studied. In the present investigation, the effects of different light intensity, nutrition and agar concentration on the morphological development of *N. flagelliforme* were studied. *N. flagelliforme* was collected at Siziwangqi, Inner Mongolia. The experimental strain was purified by our laboratory. The light intensity was designed with 0, <1, 10, 20, 30 and 60 μmol/m^2·s, nutrition levels were BG11, BG11$_0$ (nitrogen-deficiency), BG11 (phosphorous-deficiency) 1/10 BG11$_0$, and agar concentration was 0.5%, 1%, 2%, 4%, 6% and 8%, respectively. The liquid culture of *N. flagelliforme* was 20, 60 or 180 μmol/m^2·s at 25℃. The development of *N. flagelliforme* was inhibited by complete dark and low illumination of <1 μmol/m^2·s with solid media in experiments. *N. flagelliforme* was found to stay at sole filament and could not finish the developmental cycle. When *N. flagelliforme* was exposed to continuous illumination at 30 μmol/m^2·s after the dark-grown for 16 days, synchronous development took place. The part of filaments split to form hormogonia and heterocyst. If these hormogone and heterocysts were continuously kept at illumination, the synchronous development would disappear. There was quick growth of *N. flagelliforme* at 10, 20, 30 and 60 μmol/m^2·s, but its filament became yellow at 60 μmol/m^2·s. The growth rate, carotenoid contents and polysaccharide contents of *N. flagelliforme* increased with the increase of illumination with liquid bubbled culture. In contrast to solid culture, the liquid bubbled culture of *N. flagelliforme* could tolerate high illumination. *N. flagelliforme* formed more heterocysts under low nutrient conditions. The location of heterocyst formation was varied in terminal, intercalary, contiguous place. Nitrogen-deficiency induced the formation of heterocysts. No heterocysts differentiation occurred in BG11 media. Phosphorous-deficiency induced the formation of more small cells, which was varied in shape and size. *N. flagelliforme* had the same development characteristic when agar concentration from 0.5% to 4%. It developed from hormogonia to filamentous aggregation then released filaments from aggregations. The filaments in aggregations were winding and twisting. The released hormogonia would repeat the above developmental cycle. The development of *N. flagelliforme* was not in-phase. In general, the above developmental morphology of *N. flagelli-*

forme coexisted. When agar concentration was at 0.5%—4%, the time of hormogonia released delayed with the increase of agar concentration. The release of hormogonia was investigated at 0.5%—1% agar concentration media for 8 days, and 2%—4% agar concentration media for 11 days. In solid culture with 1% agar concentration, stream of hormogonia migrating on the surface of agar was investigated. *N. flagelliforme* developed from hormogonia to filamentous aggregation when agar concentration was from 6% to 8%. Filaments were enveloped by thick sheath so that they were winding and could not be released. The results indicated that illumination and water content of culture medium were important for the development of *N. flagelliforme*.

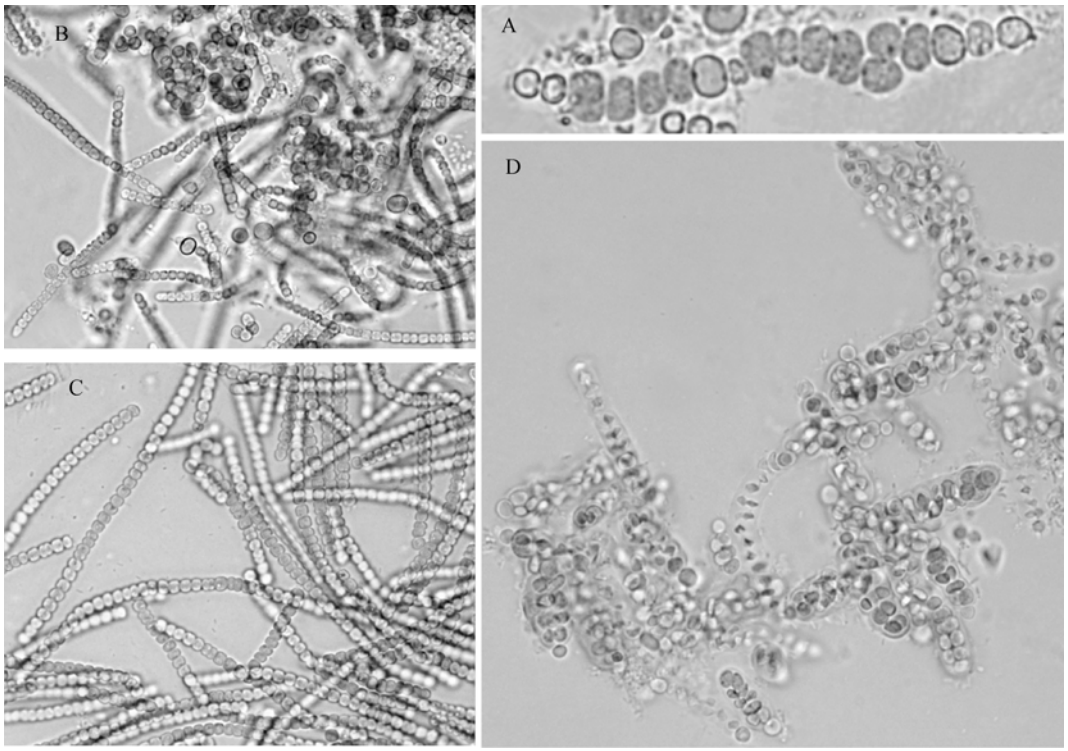
Key words: Cyanobacterium; *Nostoc flagelliforme*; Heterocyst; Hormogonia



图版 I Plate I

A. 完全黑暗固体培养 15d 的发菜藻丝; B. 暗处放置 16d, 然后置于 30 μmol/m^2· s 光强下连续培养 6d 发菜同步发育为具异形胞的丝状体(相差状态下拍摄); C. 继续光照培养发菜同步性发育消失, 形成的藻丝堆积缠绕排列; D. 光强 10、20、30 μmol/m^2· s 培养 15d 的发菜藻丝; E. 光强 60 μmol/m^2· s 培养 15d 后发菜藻丝变黄; F. 光强 20 μmol/m^2· s 液体充气培养的发菜聚集体; G、H. 光强 60、180 μmol/m^2· s 液体充气培养的发菜藻丝(比例尺=20 μm)

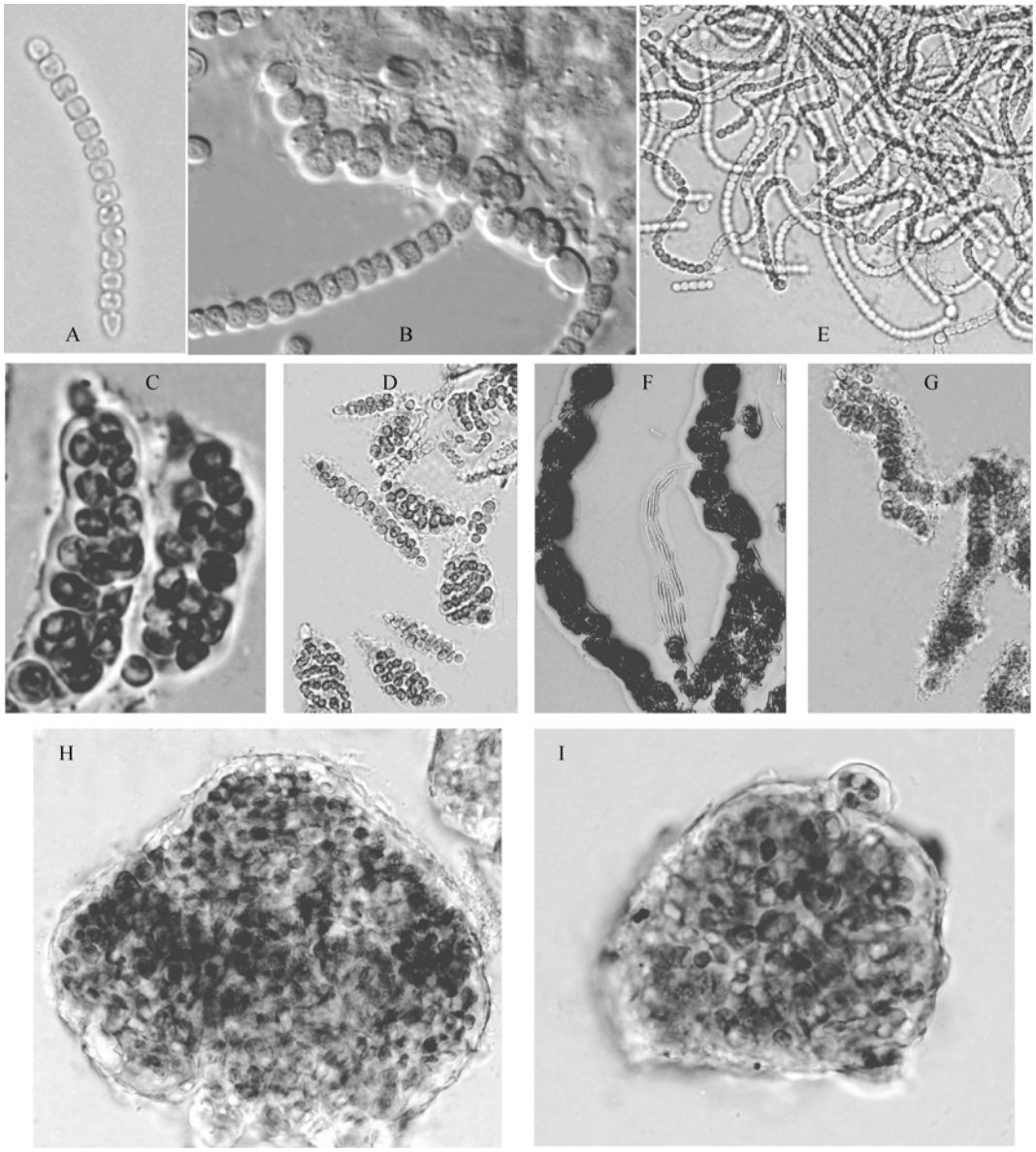
A. Filaments of *N. flagelliforme* cultivation in complete darkness in solid medium after 15 days; B. 16 days dark growth followed by 6 days in the light of 30 μmol/m^2· s. *N. flagelliforme* synchronously developed to filaments with heterocysts (shoot under pH stage); C. The synchronous development disappeared after continuous illumination. The filaments aggregated and twisted; D. Filaments growth at illumination of 10, 20 and 30 μmol/m^2· s after 15 days; E. Yellow filaments growth at illumination of 60 μmol/m^2· s after 15 days; F. Aggregations of *N. flagelliforme* in liquid bubbled culture at 20 μmol/m^2· s; G, H. Filaments of *N. flagelliforme* in liquid bubbled culture at 60 and 180 μmol/m^2· s (Bar =20 μm)



图版 II Plate II

A. 1/10 $BG11_0$ 培养 10d 的发菜藻丝异形胞较多且有连生; B. BG11 缺氮培养的发菜藻丝具有异形胞; C. BG11 培养的发菜藻丝无异形胞; D. BG11 缺磷培养的发菜形成形状大小不一的小细胞(比例尺=20 μm)

A. Filament with more and contiguous heterocysts in 1/10 $BG11_0$ medium after 10 days growth; B. Heterocystous filament in BG11 medium without nitrogen; C. There were no heterocystous filaments in BG11 medium; D. Small cells with varied shape and size in BG11 medium without phosphorus (Bar =20 μm)



图版III　Plate III

A. 藻殖段, 顶端细胞尖细; B. 藻殖段中间细胞膨大后分裂, 顶端细胞分化为异形胞(微分干涉差状态下拍摄); C. 非丝状体聚集体阶段; D. 丝状聚集体; E. 丝状聚集体破裂释放出藻丝; F. 在 1%琼脂浓度的固体培养基表面藻殖段从聚集体中释放, 成流滑出; G. 6%—8%琼脂浓度培养形成的发菜聚集体; H-I. 6%—8%琼脂浓度培养 7 个月后的发菜藻丝聚集体具有厚胶鞘(比例尺=20 μm)

A. A hormogonia with a tapered appearance of the terminal cell; B. The differentiation of intercalary cells of hormogonia was coincident with filament expansion, the terminal cell differentiates to heterocyst (shoot under DIC stage); C. Aseriate stage cells; D. Aggregation of filaments; E. Aggregation of filaments broke and formed new filaments; F. Streams of hormogonia released from aggregates migrating on the surface of 1% agar solid medium; G. Aggregations of filaments in BG11 medium with 6%—8% agar concentration; H-I. Aggregations of filaments in BG11 medium with 6%—8% agar concentration had thick sheath after 7 months growth (Bar =20 μm)