Microbulbifer sp. SH-1依赖褐藻胶裂解酶AlgSH6、AlgSH7和AlgSH17协同增效降解褐藻

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摘要 褐藻是多糖、多酚、萜类等天然活性化合物的重要来源。然而, 如何高效提取藻体内的活性物质是褐藻资源高值化利用的瓶颈问题。本文报道了一株高效降解褐藻的海洋细菌*Microbulbifer* sp. SH-1。该菌株在36 h内对海带、马尾藻、泡叶藻等褐藻的降解率达76.21%~96.31%。全基因组测序及基因注释结果发现, *Microbulbifer* sp. SH-1的基因组大小为4.68 Mbp, 共编码3788个基因, 包含3个对褐藻降解起关键作用的褐藻胶裂解酶基因*algsh6*、*algsh7*和*algsh17*。异源表达结果显示, 重组酶AlgSH6、AlgSH7和AlgSH17对褐藻胶的降解活性分别为1577.29、15608.53和1709.18 U/mg。分子对接、薄层色谱(thin layer chromatography, TLC)及电喷雾质谱(electrospray ionization mass spectrometry, ESI-MS)分析表明, AlgSH7为内切型褐藻胶裂解酶, 降解产物为聚合度(degree of polymerization, DP) 2~5的不饱和寡糖; AlgSH6与AlgSH17兼具内切活性和外切活性, 二者单一降解产物均包含褐藻胶单糖及DP 2~5的寡糖。值得注意的是, AlgSH6与AlgSH17酶组合能够完全降解褐藻胶为单糖。此外, 3种酶两两组合的酶活性比单酶活性提高了20.51%~61.55%, 三酶组合的活性提高了136.41%。以上结果表明, 菌株*Microbulbifer* sp. SH-1通过褐藻胶裂解酶AlgSH6、AlgSH7及AlgSH17共同作用实现褐藻的高效降解, 这为褐藻资源的开发利用提供了科学依据。

关键词 褐藻降解菌, *Microbulbifer* sp. SH-1, 褐藻胶裂解酶, 内外切活性, 协同作用

海洋大型藻类主要分为褐藻、红藻、绿藻等三大类, 广泛生长分布于世界各大洋的近海水域^[1]。海藻是海洋生态系统中重要的初级生产者, 在海洋碳循环中发挥着重要的作用^[2,3]。海藻在长期的进化过程中形成了独特的次生代谢系统和防御机制来应对高盐、高压、低氧、寡照以及温度迅速变化的海洋环境, 并在体内累积了大量的具有独特结构和功能的天然生理活性物质, 如海藻多糖、多酚、多肽、多不饱和脂肪酸、生物碱、萜类化合物等^[4,5]。大量研究表明, 海藻源生理活性物质具有抗氧化、抗肿瘤、免疫调节、抑

菌、保鲜、促进植物生长等作用, 目前已被广泛应用于食品、生物医药、饲料、化妆品、保健品、生物肥料、生物刺激素等产业中^[5,6]。

褐藻在海藻中属于比较高级的类群, 与红藻和绿藻相比, 褐藻的生物量及活性物质含量也较高^[5,7]。海带、裙带菜、羊栖菜、马尾藻等是我国褐藻的主要栽培品种。据联合国粮食及农业组织(Food and Agriculture Organization of the United Nations, FAO)报道, 中国是世界上海藻产量最高、养殖面积最大的国家, 中国海藻产量约占世界产量的2/3, 其中海带约占世界海藻

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产量的1/2^[8]。由此可见, 高效开发利用褐藻资源将对促进我国海洋经济的发展具有重要意义。然而, 由于缺乏绿色高效的褐藻加工和活性物质提取工艺, 褐藻资源仍然被认为是尚未被完全利用的生物资源。传统的褐藻加工工艺往往采用苛刻的处理条件, 如使用化学溶剂、高温、高压处理, 这可能导致化学废弃物产生、大量的能源消耗以及活性物质的损失^[9,10]。目前, 微生物酶解提取工艺作为一种生物处理方法, 因其降解效率高、专一性强、条件温和可控、副产物少等优势, 具有广阔的应用前景和良好的经济效益^[10]。褐藻胶裂解酶已经从广泛的生物中分离出来, 包括藻类、海洋无脊椎动物和海洋微生物等^[11]。然而, 目前大多数褐藻胶裂解酶底物适应性差、活性较低, 难以满足工业生产的条件, 这些缺陷限制了酶解工艺的商业化应用。

前期工作中, 我们从福建漳州滨海土壤中分离得到一株褐藻高效降解菌株*Microbulbifer* sp. SH-1(以下简称SH-1), 该菌株可以降解多种褐藻, 然而其降解机理还有待进一步阐释^[12]。本研究采用PacBio三代测序技术对SH-1菌株进行了全基因组测序, 对其基因组序列进行生物信息学分析, 并对基因组中3个褐藻胶裂解酶进行了克隆和异源表达, 以及对重组酶的协同作用关系进行了研究, 以期阐明SH-1菌株高效降解褐藻的调节机制。

1 材料与方法

(i) 材料与试剂。SH-1菌株由本实验室分离纯化得到^[12]; *E. coli* TOP10和*E. coli* BL21(DE3)购于上海唯地生物技术有限公司; 质粒载体pET28a(+)、限制性内切酶*Nco* I和*Xho* I购于宝生物工程(大连)有限公司; 细菌基因组DNA抽提试剂盒购自天根生化科技(北京)有限公司; Ni-NTA蛋白纯化试剂盒购自上海生工生物工程有限公司; Silica gel 60 F254铝板购自德国默克公司; 海藻酸钠(M/G: 2/1)购自上海麦克林生化科技有限公司; 其他药品均为国产分析纯。海带、马尾藻、泡叶藻、面条藻、树皮藻、F藻和N藻由广州申晶雅农业科技有限公司馈赠。

(ii) SH-1菌株形态观察。将生长对数期(12 h)和稳定期(24 h)的菌体涂布于载玻片上, 用酒精灯外焰加热固定后按照革兰氏染色试剂盒(环凯, 广州)的使用说明对菌体进行染色, 然后用光学显微镜(BX43, OLYMPUS)进行观察。收集生长对数期(12 h)和稳定期(24 h)的菌体, 参照已报道的方法^[13], 将样品依次进行固定、

脱水、干燥喷金后, 用场发射扫描电子显微镜(EVO MA15, ZEISS)进行观察。

(iii) SH-1菌株对褐藻降解效果分析。SH-1菌株以1%的接种量接种于海带、马尾藻、泡叶藻和面条藻为底物的培养基中, 培养基组分为: 褐藻片段(1 cm×1 cm) 10 g/L、NaCl 15 g/L、(NH₄)₂SO₄ 5 g/L、K₂HPO₄ 1 g/L、MgSO₄·7H₂O 1 g/L、FeSO₄·7H₂O 0.01 g/L, pH 7.5。在32℃条件下培养36 h, 每12 h对培养基中褐藻状态进行观察, 并拍照记录, 同时采用称重法计算菌株对褐藻的降解率:

$$\text{褐藻降解率(\%)} = \frac{\text{褐藻原始重量} - \text{降解残渣重量}}{\text{褐藻原始重量}} \times 100\% \quad (1)$$

(iv) 菌株基因组DNA提取、测序与注释。将SH-1菌株接种于发酵培养基, 32℃培养24 h, 菌液在10000×g下离心10 min, 收集菌体。按照细菌基因组DNA提取试剂盒(天根, 北京)说明书提取该菌株的基因组DNA。使用Qubit(Thermo Fisher Scientific, 美国)和Nanodrop(Thermo Fisher Scientific, 美国)检测DNA质量, 同时采用1%琼脂糖凝胶电泳, 检测基因组DNA样品的完整性。检测合格的DNA样品送广州基迪奥生物科技有限公司进行测序分析。采用NCBI ORF Finder对菌株基因组中编码基因进行预测, 用于后续基因功能的注释。

(v) 褐藻胶裂解酶AlgSH6、AlgSH7及AlgSH17三维结构预测及分子对接。利用modeller 9.19以来源于*Glaciecola chathamensis* S18K6的AlyGC(PDB ID: 5GKQ)、*Vibrio splendidus* OU02的AlyB(PDB ID: 5ZU5)以及来源于*Saccharophagus degradans*的Alg17C(PDB ID: 4OJZ)为模板分别对AlgSH6、AlgSH7和AlgSH17进行同源建模^[14-16]。采用LeDock进行分子对接, 并利用PyMol进行可视化分析^[17]。

(vi) 重组酶AlgSH6、AlgSH7、AlgSH17的克隆表达、纯化及活性检测。*algsh6*、*algsh7*和*algsh17*的基因去除信号肽后, 利用primer premier 5.0软件设计带有*Nco* I和*Xho* I酶切位点的引物(表S1), 以SH-1基因组DNA为模板, 利用表S1中的引物进行PCR扩增, 获得目的基因。限制性*Nco* I和*Xho* I分别酶切目的基因与pET28a(+)质粒, 连接酶连接构建重组表达载体pET28a(+)-*algsh6*、pET28a(+)-*algsh7*和pET28a(+)-*algsh17*, 并转化至*E. coli* TOP10, 提取质粒DNA进行双酶切验证, 并进行测序。最后将测序正确的重组质粒转化至*E. coli*

BL21(DE3)中进行表达. 重组菌株按照1%的接种量转接到50 mL含有50 $\mu\text{g/L}$ 卡那霉素的LB培养基中, 在37°C、180 r/min条件下培养至 A_{600} 为0.6~0.8时, 向溶液中添加终浓度为0.1 mmol/L的异丙基- β -D-硫代半乳糖苷(isopropyl-beta-D-thiogalactopyranoside, IPTG), 在16°C条件下诱导表达16 h. 菌体经超高压细胞破碎仪(Constant Systems, 英国)破碎和离心得粗酶液, 随后利用Ni-NTA亲和层析柱分离纯化, 并通过SDS-PAGE检测纯化结果. 重组酶活性采用3,5-二硝基水杨酸(3,5-dinitrosalicylic acid, DNS)比色法进行测定^[12].

(vii) 降解产物薄层色谱(thin layer chromatography, TLC)及电喷雾质谱(electrospray ionization mass spectrometry, ESI-MS)分析. 取降解后的寡糖样品5 μL , 点样于Silica gel 60 F254铝板, 随后置于层析缸中进行展开, 展开剂为正丁醇:甲酸:水(4:6:1), 展开完全后, 取出硅胶板用吹风机吹干, 均匀喷洒二苯胺/苯胺/磷酸混合显色液于硅胶板表面, 并在140°C条件下显色5 min. 采用maXis impact超高分辨飞行时间质谱(Bruker, 德国)在阴离子模式条件下对产物分子量进行分析. 采集参数为: 雾化器压力0.3 bar(1 bar=100 kPa)、毛细管电压3 kV、干燥温度180°C、充电电压2 kV、干气流速4.0 L/min、扫描范围50~1500 m/z .

(viii) AlgSH6、AlgSH7、AlgSH17协同作用分析. 将纯化得到的3种褐藻胶裂解酶AlgSH6、AlgSH7和AlgSH17用Tris-HCl缓冲液(pH 8.0)稀释至相同活性(500 U/mL), 然后将3种酶的稀释液按1:1或1:1:1的方式进行组合, 得到不同组合的酶制剂. 以Tris-HCl缓冲液(pH 8.0)配置的0.5%(w/v)海藻酸钠为底物, 通过DNS法测定不同组合酶的活性. 组合酶降解产物分析: 将不同的酶组合以相同的添加量对1%(w/v)的海藻酸钠底物进行降解, 35°C降解24 h后, 对产物进行TLC及ESI-MS检测.

2 结果与讨论

2.1 SH-1的细胞形态及褐藻降解能力分析

如图1(a), (b)所示, 处于对数生长期(12 h)的SH-1细胞呈长杆状, 细胞长度为1.5~2.5 μm , 宽度为0.4 μm ; 而生长至稳定期的SH-1菌株呈球形, 直径为0.4~0.6 μm (图1(c), (d)). 因此, SH-1菌株形态随生育期变化而呈现“杆-球”态细胞周期. 微泡菌属(*Microbulbifer* sp.)由González等人^[18]在1997年发现并定义, 在系统分类学

上其归属于变形菌门 γ 变形菌纲. 这类菌株具有典型的“杆-球”态细胞周期, 即在生长阶段为长杆状细胞, 在增殖停止后转变为球粒细胞, 如*M. variabilis* sp. nov. Ni-2088T和*M. epialgicus* sp. nov. F-104T^[19]. 已有研究表明, 球粒细胞拥有更强的环境胁迫耐受性, 是生物进化中的一种策略^[19]. 因此, 我们推测SH-1菌株形态的转化可以使其在降解褐藻中拥有更高的菌体密度和应对饥饿的能力. 此外, 有趣的是, SH-1菌株细胞表面并不光滑, 而是存在许多泡状突起和褶皱结构. 这些结构可能有利于其附着在藻体的表面对藻体进行降解.

SH-1对海带、马尾藻、泡叶藻、面条藻、树皮藻、F藻、N藻等褐藻的降解试验表明, SH-1菌株能够在12 h内降解不同褐藻藻体为碎屑状, 并在36 h内几乎将藻体完全液化, 降解率为76.21%~96.31%(图1(e), (f)). 目前, 微泡菌属中已报道的褐藻降解菌株有*Microbulbifer* sp. 6532A、*Microbulbifer* sp. HZ11和*Microbulbifer* sp. ALW1^[20~22]. *Microbulbifer* sp. HZ11和*Microbulbifer* sp. ALW1分别能够在7 d和72 h内将海带片液化^[20,21]; *Microbulbifer* sp. 6532A可在2 d内将多细胞的裙带菜降解为细小的碎屑^[22]. 与之相比, SH-1对褐藻的降解效率更高. 在我们之前的报道中, SH-1拥有比其他微泡属菌株更高的褐藻胶裂解酶活性, 这可能是SH-1菌株实现褐藻高效降解的重要原因^[12].

2.2 SH-1的基因组及功能注释

为进一步明确SH-1对褐藻的降解机制, 采用三代PacBio平台对SH-1进行全基因组*de novo*测序. 如图2(a)所示, SH-1基因组大小为4684771 bp(GenBank: CP046948.1), GC含量为57.99%, 共预测得到3961个基因. 图2(a)中不同颜色的基因代表COG(Clusters of Orthologous Groups)数据库注释结果, COG注释编码基因个数为3788个, 占总基因数量的95.63%.

碳水化合物活性酶数据库(carbohydrate-active enzymes database, CAZy)包括能催化碳水化合物降解、修饰、以及生物合成的相关酶系家族. 褐藻胶裂解酶归类于多糖裂解酶家族(polysaccharide lyases, PLs)中的PL5、PL6、PL7、PL14、PL15、PL17、PL18、PL31、PL32、PL34、PL36和PL39家族^[23]. SH-1菌株基因组共鉴定出3个PLs家族酶基因: *Microbulbifer*_2705、*Microbulbifer*_2706和*Microbulbifer*_2712, 其基本性质见表S2. 进化树分析表明, *Microbulbifer*_2705、*Microbulbifer*_2706和*Microbulbifer*_2712分

图 1 SH-1的生长形态及对不同褐藻的降解效果. (a), (c) SH-1菌株在对数期和稳定期的光学显微镜照片, 标尺=5 μ m. (b), (d) SH-1菌株在对数期和稳定期的扫描电子显微镜照片. (e) 不同褐藻降解过程中的藻体形态变化. (f) SH-1菌株对不同褐藻的降解率

Figure 1 Growth morphology of *Microbulbifer* sp. SH-1 and its degradation effect on different brown seaweeds. (a), (c) Optical micrograph of SH-1 strain at exponential and stationary phase. Bar=5 μ m. (b), (d) Scanning electron micrograph of SH-1 strain at exponential and stationary phase. (e) Morphological changes of different brown seaweed during the degradation process. (f) Degradation rate of *Microbulbifer* sp. SH-1 towards different brown seaweeds

别与PL6、PL17和PL7家族的褐藻胶裂解酶OalS6 (AHC69713.1)、OalS17(AHW45238.1)和AlgMsp (BAJ62034.1)共处于同一进化分支(图2(b)). 因此, 这3种酶分别属于PL6、PL17和PL7家族, 将其命名为AlgSH6、AlgSH17和AlgSH7.

在褐藻胶代谢的过程中, 褐藻胶大分子首先在胞外内切酶的作用下, 降解为低聚合度的寡糖. 然后在细菌膜上相关转运蛋白的作用下进入细胞质或细胞内, 再经过外切酶的作用生成不饱和单糖. 不饱和单糖随后非酶促地转化为4-脱氧-L-赤-5-己酮糖醛酸(4-deoxy-L-erythro-5-hexoseulose uronic acid, DEH). DEH在胞内DEH还原酶(DEH reductase, DehR)和2-酮-3-脱氧-葡萄糖酸激酶(2-dehydro-3-deoxygluconokinase, KDGK)的作用下, 转化为2-酮-3-脱氧-6-磷酸葡萄糖酸(2-keto-3-deoxy-6-phosphogluconic acid, KDPG), 进入Entner-Doudoroff途径(ED途径), 然后进入EMP途径(embden-meyerhof-parnas pathway)^[24]. 根据SH-1菌株基因注释结果, 褐藻胶裂解酶基因*algSH6*、*algSH17*和*algSH7*与

其他褐藻胶代谢相关基因构成大小为1.57 kb的褐藻胶利用基因簇(图2(c)). 该基因簇包含3种褐藻胶裂解酶及TonB依赖的转运蛋白(TonB-dependent receptor, TBDR)、内膜上的MFS膜透性酶(MFS permease hexuronate/hexarate, MFS)、短链脱氢/还原酶(short-chain dehydrogenase/reductase, SDR)、2-酮-3-脱氧-葡萄糖酸激酶(2-dehydro-3-deoxygluconokinase, KDGK)等组件. Takase等人^[25]的研究发现, SDR为DEH还原酶DehR的同工酶. Kabisch等人^[26]报道, 在海洋拟杆菌*Gramella forsetii* KT0803中TBDR和MFS分别在寡糖分子转运至细胞质和DEH转运至内膜内具有重要作用. 因此, 该基因簇包含褐藻胶代谢的全部关键组件, 为SH-1高效降解褐藻提供了基因基础.

2.3 褐藻胶裂解酶三维结构及降解模式预测

在PDB数据库中筛选与AlgSH6、AlgSH7和AlgSH17相似性最高的5GKQ(Identity: 58.6%)、5ZU5 (48.25%)和4OJZ(49.7%)为模板进行同源建模, 结果如

图2 SH-1基因组分析。(a) 菌株SH-1的基因组圈图；(b) 菌株SH-1中褐藻胶裂解酶的系统进化树；(c) 菌株SH-1中褐藻胶利用基因簇
Figure 2 Genomic analysis of *Microbulifer* sp. SH-1. (a) Circular representation of the genome map of SH-1 strain; (b) phylogenetic tree of alginate lyases in SH-1 strain; (c) gene cluster of alginate utilization in SH-1 strain

图3所示。AlgSH6由 β -螺旋折叠结构构成的N端结构域和C端结构域通过Loop连接而成。催化位点Lys248和Arg269位于C端催化腔的两侧，与 Ca^{2+} 共同构成活性中心，表明AlgSH6活性可能依赖于 Ca^{2+} (图3(a))。分子对接结果表明，AlgSH6与5GKQ催化口袋并无显著差异，但Ser151、Ala156和Val175发生了改变。催化口袋中催化残基Lys248和Arg269可识别底物(甘露糖醛酸四糖)非还原性末端第一个糖苷键，表明AlgSH6可能具有外切酶活性(图3(a))。类似地，PL6家族中来源于*Shewanella* sp. Kz7的OalS6^[27]和来源于*Cellulophaga* sp. SY116的OalC6^[28]同样被报道具有外切酶活性。

如图3(b)所示，AlgSH17的C端结构域由 $(\alpha/\alpha)_n$ 桶状结构组成。催化位点Tyr270与Tyr461位于 $(\alpha/\alpha)_n$ 桶状结构形成的催化腔两侧， Zn^{2+} 离子结合位点位于活性中心附近。AlgSH17的N端由 β 片层结构组成。目前已报道的PL17家族褐藻胶裂解酶均为外切型褐藻胶裂解酶，能够断裂褐藻胶糖链非还原性的末端的糖苷键，逐个释放单糖^[29]。分子对接显示，底物进入催化口袋，Tyr270与Tyr461完成对底物-1和+1位糖苷键的识别和断裂。此外，甘露糖醛酸三糖(MMM)作为底物时，AlgSH17可以降解MMM产生单糖，表明AlgSH17具有

外切酶活性(图3(b))。

AlgSH7的C端由多个 β 片层结构弯曲形成催化口袋，而N端折叠为碳水化合物结合结构域(carbohydrate binding module, CBM)，为PL7家族中CBM_PL7类型酶。在5ZU5中CBM和PL7家族结构域之间通过 α 螺旋组成的Linker连接，而AlgSH7中的Linker是由丝氨酸(Ser)组成的Loop构成(图3(c))。AlgSH7与甘露糖醛酸十糖分子对接结果表明，C端催化残基Tyr575能够剪切底物内部的糖苷键，CBM结构域中Asp177和Trp282对底物结合和锚定起关键作用，表明AlgSH17为内切型褐藻胶裂解酶(图3(c))。CBM_PL7类型褐藻胶裂解酶AlyB和Alg7A通过CBM结构域及 α 螺旋组成的Linker完成对降解产物的精准调控，使得三糖为主要降解产物^[15,30]。对AlgSH7而言，其Linker由丝氨酸组成的Loop构成，与 α 螺旋相比，柔韧性更强，因此降解产物可能更为复杂。

2.4 SH-1中褐藻胶裂解酶基因的异源表达及活性分析

将重组质粒pET28a(+)-*algsh6*、pET28a(+)-*algsh7*和pET28a(+)-*algsh17*转入表达宿主*E. coli* BL21(DE3)，

图3 褐藻胶裂解酶AlgSH6(a)、AlgSH7(b)和AlgSH17(c)的三维结构预测及与底物的分子对接分析

Figure 3 Three-dimensional structure prediction and molecular docking analysis of alginate lyase AlgSH6 (a), AlgSH7 (b) and AlgSH17 (c)

重组菌株在16℃条件下, 经0.1 mmol/L的IPTG诱导培养16 h成功表达目的基因. 如图4(a)所示, 3种酶通过Ni-NTA柱亲和层析纯化可得到较高纯度的重组蛋白. SDS-PAGE分析表明, AlgSH6和AlgSH17的分子量约为80.0 kDa, AlgSH7分子量约为60.0 kDa, 与其理论分子量一致(表S2).

如图4(b)所示, 重组酶AlgSH6、AlgSH7和AlgSH17均具有明显的褐藻胶裂解酶活性. AlgSH6的

粗酶液活性为311.75 U/mg, 纯化后酶活性为1577.29 U/mg, 酶活性提高了5.06倍. AlgSH17的酶活性略高于AlgSH6, 粗酶液活性为380.82 U/mg, 纯化后酶活性提高了4.49倍, 为1709.18 U/mg. AlgSH7酶活性最高, 其粗酶液活性为1964.24 U/mg, 经纯化后酶活性为15608.53 U/mg, 酶活性提高了7.94倍. 内切型褐藻胶裂解酶从糖链内部断裂糖苷键, 而外切型褐藻胶裂解酶只能以“一个接一个”的方式从糖链非还原性末端逐个释放褐藻

图 4 重组酶AlgSH6、AlgSH17和AlgSH7的SDS-PAGE(a)及酶活性(b)分析

Figure 4 SDS-PAGE (a) and enzymatic activity analysis (b) of recombinant enzyme AlgSH6, AlgSH17 and AlgSH7

胶单糖, 因此, 内切型褐藻胶裂解酶活性通常高于外切型褐藻胶裂解酶^[29,31]. AlgSH7的酶活性分别为AlgSH6和AlgSH17纯酶液活性的9.89和9.13倍, 这为AlgSH7可能为内切酶提供了新的证据.

2.5 AlgSH6、AlgSH7和AlgSH17对褐藻胶的协同降解

通常条件下, 外切型褐藻胶裂解酶降解底物产生的单糖会非酶促地转化为DEH^[31]. 如图5(a)所示, AlgSH6和AlgSH17降解海藻酸钠的产物为褐藻胶单体(单糖或DEH)和DP 2~5的寡糖, 经ESI-MS进一步分析后, 确定AlgSH6和AlgSH17产物中包含不饱和单糖或DEH($[\Delta DP-H]^- = 175 \text{ m/z}$)、不饱和二糖($[\Delta DP2-H]^- = 351 \text{ m/z}$ 、 $[\Delta DP2+Na-2H]^- = 373 \text{ m/z}$)、不饱和三糖($[\Delta DP3-H]^- = 527 \text{ m/z}$ 或 $[\Delta DP3+Na-2H]^- = 549 \text{ m/z}$)、不饱和四糖($[\Delta DP4-H]^- = 703 \text{ m/z}$ 、 $[\Delta DP4+2Na-3H]^- = 747 \text{ m/z}$)和不饱和五糖($[\Delta DP5-H]^- = 879 \text{ m/z}$ 、 $[\Delta DP5+2Na-3H]^- = 923 \text{ m/z}$)(图5(b)). 两者降解的产物中存在不饱和单糖及DEH表明, AlgSH6和AlgSH17具有外切酶活性, 支撑了分子对接预测结果(图3). 在以往报道中, 外切型褐藻胶裂解酶在降解褐藻胶的过程中不会产生寡糖中间体, 而是直接攻击褐藻胶糖链的非还原性末端释放单糖^[32]. 然而AlgSH6和AlgSH17的降解产物中除了单糖外, 还包含一定量的寡糖. 这表明AlgSH6和AlgSH17还具有内切活性. 因此, AlgSH6和AlgSH17具

有独特的内切和外切两种作用模式, 目前具有两种酶切方式的酶鲜有报道. 虽然两者降解模式具有一定相似性, 但AlgSH6与AlgSH17的降解产物存在明显差异. AlgSH6降解产物中单糖组分含量较少, 寡糖组分占比较多, 且奇数聚合度糖组分(三糖)含量较多(图5(a), (b)). 因此, 我们推测, AlgSH6优先以内切的方式降解底物, 且偏好断裂于褐藻胶糖链奇数位的糖苷键, 从而释放奇数聚合度寡糖. AlgSH17降解产物中单糖为主要组分, 寡糖含量较少, 因此AlgSH17优先以内切的方式降解底物. 由图5(a), (b)可知, AlgSH7的降解产物主要为不饱和二糖($[\Delta DP2-H]^- = 351 \text{ m/z}$)、不饱和三糖($[\Delta DP3-H]^- = 527 \text{ m/z}$)、不饱和四糖($[\Delta DP4-H]^- = 703 \text{ m/z}$)和不饱和五糖($[\Delta DP5+2Na-3H]^- = 923 \text{ m/z}$), 表明AlgSH7为内切型褐藻胶裂解酶, 与分子对接预测结果一致(图3).

如图5(a), (b)所示, 与单一AlgSH6或AlgSH17的降解产物相比, AlgSH7的加入使AlgSH6+AlgSH7和AlgSH7+AlgSH17酶组合产物中寡糖的含量及单糖的含量均明显提高, 且几乎不存在大分子寡糖(DP>4), 表明内切酶AlgSH7的加入提高了褐藻胶的降解效率. 与单一AlgSH7降解产物相比, AlgSH6+AlgSH7及AlgSH7+AlgSH17组合的降解产物中单糖及DEH组分增加, 寡糖组分有所减少, 但仍残留一定数量的寡糖. 这表明AlgSH6或AlgSH17能够依赖外切酶活性进一步将内切酶AlgSH7产生的寡糖降解为单糖, 但不能将寡

图 5 褐藻胶裂解酶AlgSH6、AlgSH7和AlgSH17协同作用分析. (a) 不同酶组合降解产物的TLC分析; (b) 不同酶组合降解产物的ESI-MS分析; (c) 不同酶组合的活性分析

Figure 5 Synergistic analysis of alginate lyase AlgSH6, AlgSH7 and AlgSH17. (a) TLC analysis of degradation products with different enzyme combinations; (b) ESI-MS analysis of degradation products with different enzyme combinations; (c) enzyme activity analysis of different enzyme combinations

糖完全转化为单糖. 令人惊奇的是, 当AlgSH6与AlgSH17进行组合时, 降解产物中只有DEH, 与AlgSH6+AlgSH7+AlgSH17的降解产物相同, 表明AlgSH6和AlgSH17在降解底物生成单糖的过程中具有良好的协同作用(图5(a)). 上文提到, AlgSH6与AlgSH17同时兼具内切和外切两种作用方式, 且AlgSH6优先以内切方式降解底物, AlgSH17优先以外切方式降解底物, 两者作用方式既有相似性又有互补性. 当两者进行组合时, AlgSH6可以内切的方式产生小分子寡糖为AlgSH17供给底物, 同时其外切活性也可作为AlgSH17的补充. Li等人^[28]报道来源于*Cellulophaga* sp. SY116的寡糖降解酶OalC6和OalC17在降解褐藻胶及褐藻寡糖的过程中具有协同作用, 该结果与本研究相近. 然而, 与AlgSH6+AlgSH17组合相比, OalC6+OalC17组合降解产物中仍含有未降解的低聚物, 这可能与“OalC6和OalC17只具有外切活性, 而AlgSH6和AlgSH7同时具有内切和外切两种活性”有关.

将3种酶调至相同活性(500 U/mL), 在相同加酶量的条件下研究不同酶组合的活性变化. 如图5(b)所示, 3种酶组合(AlgSH6+AlgSH7+AlgSH17)的酶活性最高, 比单

一酶的活性提高了136.41%. 而且, AlgSH6+AlgSH7和AlgSH17+AlgSH7酶组合的活性比单一酶的活性分别增加了64.18%和61.55%. 令人意外的是, 虽然AlgSH6+AlgSH17可完全降解褐藻胶为单糖, 与AlgSH6+AlgSH7+AlgSH17降解产物相同, 但该组合酶活性仅比单一酶活性增加了20.51%. 因此, AlgSH6、AlgSH7、AlgSH17在降解褐藻胶中的协同作用, 一方面表现为对降解产物的调控, 另一方面表现为对酶活性的提升.

3 结论

SH-1含有3个褐藻胶裂解酶基因*algsh6*、*algsh7*、*algsh17*, 分别编码不同剪切方式的褐藻胶裂解酶AlgSH6、AlgSH7、AlgSH17. AlgSH7为内切型褐藻胶裂解酶, 其酶活性高, 能够高效降解褐藻胶为寡糖; 褐藻胶裂解酶AlgSH6和AlgSH17兼具内切活性和外切活性, 可协同高效降解褐藻胶寡糖为单糖. 因此, 3种酶对褐藻胶的协同降解是SH-1实现海带、马尾藻、泡叶藻等褐藻高效降解的重要作用机制. 本文研究结果为褐藻的高效降解、褐藻寡糖的制备及褐藻胶的糖化提供了理论依据.

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补充材料

表S1 用于扩增目的基因的引物

表S2 *Microbulbifer* sp. SH-1菌株中3个褐藻胶裂解酶的基本特性

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Summary for “*Microbulbifer* sp. SH-1 依赖褐藻胶裂解酶 AlgSH6、AlgSH7 和 AlgSH17 协同增效降解褐藻”

Microbulbifer sp. SH-1 degrades brown seaweed via the synergistic effect of alginate lyase AlgSH6, AlgSH7 and AlgSH17

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Brown seaweed contains a range of natural bioactive compounds that are of commercial interest, including polysaccharides, polyphenols, terpenoids and unique pigments. Due to its great prospects of application and development in food, pharmaceutical, cosmetics and biostimulant industries, brown seaweed has attracted great interest among researchers in recent decades. However, due to the lack of efficient processing technologies, brown seaweed is still considered as an underused resource. Recently, the biodegradation of brown seaweed, such as microbial enzymolysis and microbial fermentation, has been perceived as a promising and cost-effective technique to alleviate the bottleneck of brown seaweed processing. Here, we report an efficient brown seaweed-degrading bacterium *Microbulbifer* sp. SH-1 isolated from coastal soil. *Microbulbifer* sp. SH-1 has a typical “rod-coccus” cell cycle and a rough cell surface, which contributes to adhering to the interface of the seaweed. The strain SH-1 could degrade brown seaweed, such as *Laminaria japonica*, *Sargassum fusiforme*, *Ascophyllum nodosum* and *Durvillaea Antarctica*, at degradation rates of 76.21% to 96.31% within 36 h. The results from whole genome sequencing and gene annotation demonstrated that the genome size of the strain was 4.68 Mbp with a 57.99% GC content, and encoding 3788 genes. Three putative alginate lyase genes, *algsh6*, *algsh7* and *algsh17*, which played crucial roles in the enzymatic degradation of brown seaweed, were identified by analyzing the carbohydrate active enzyme (CAZy) database. Moreover, the recombinant proteins AlgSH6, AlgSH7 and AlgSH17 were successfully expressed using the *E. coli* heterologous expression system, and the recombinant enzymes AlgSH6, AlgSH7 and AlgSH17 exhibited obvious alginate degradation activities of 1577.29, 15608.53 and 1709.18 U/mg, respectively. The results from molecular docking indicated that AlgSH7 was an endolytic alginate lyase, and that AlgSH6 and AlgSH17 might have exolytic activity. Thin layer chromatography (TLC) and electrospray ionization mass spectrometry (ESI-MS) analysis showed that the degradation products of AlgSH7 were unsaturated oligosaccharides with degree of polymerization (DP) of 2 to 5, implying it was an endolytic alginate lyase, which was consistent with the molecular docking results. The degradation products of AlgSH6 and AlgSH17 were similar, both containing monosaccharides and oligosaccharides (DP 2–5). This indicates that AlgSH6 and AlgSH17 may possess both endolytic and exolytic activity. It is worth noting that monosaccharides were predominant in the AlgSH17 products, while the AlgSH6 products contained more oligosaccharides (DP 2–5), indicating that AlgSH6 and AlgSH17 have different emphases on endolytic and exolytic cleavage activity. Although AlgSH6 and AlgSH17 could not entirely digest alginate into monosaccharides on their own, the combination of AlgSH6 and AlgSH17 as well as the combination of AlgSH6, AlgSH7 and AlgSH17 could. Additionally, compared with the activity of a single enzyme, the activities of the pair-to-pair enzyme combinations increased by 20.5%–61.2%, and that of the three-enzyme combination increased by 136.41%. The above results indicate that AlgSH6, AlgSH7 and AlgSH17 have different digestion patterns and exhibit a good synergistic effect, which helps *Microbulbifer* sp. SH-1 to achieve high efficiency degradation of brown seaweed. The results of this study provide an important theoretical basis for the efficient preparation of bioactive compounds from brown seaweed.

brown seaweed-degrading bacterium, *Microbulbifer* sp. SH-1, alginate lyase, endolytic and exolytic activity, synergistic effect

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