文章编号：1004-0374(2012)05-0475-08

海藻酸分解菌研究进展

张 绪[1]，赵 琳[1]，钱 龙[1]，黄庶识[2]，伊日布斯[1]*
(1 昆明理工大学生命科学与技术学院生物转化实验室，昆明 650500；2 广西科学院，南宁 530007)

摘 要：海藻酸分解菌是一类能够自身合成海藻酸裂解酶，能够降解并同化海藻酸的微生物。海藻酸分解菌是海藻酸裂解酶的重要来源，其产生的海藻酸裂解酶具有种类多、反应条件温和、酶活高和易于大规模生产等优点，并且在生物、医疗、化工等领域有重要的应用价值。在过去的几十年里，海藻酸分解菌一直作为海藻酸裂解酶生产者的角色被研究和应用。但随着近年来能源危机的加剧，以海藻酸等海藻生物质为原料转化生物能源成为解决能源危机的潜在途径，因此，海藻酸分解菌又有了崭新的研究领域，即海藻酸分解菌利用海藻酸发酵生产生物能源。本文从海藻酸分解菌及其海藻酸裂解酶的种类和特性、海藻酸分解菌的代谢以及海藻酸分解菌基因工程等方面，介绍海藻酸分解菌的研究现状，并展望未来的发展趋势。

关键词：海藻酸分解菌；海藻酸裂解酶；海藻酸代谢；基因工程
中图分类号：Q557 **文献标志码**：A

Research progress of alginolytic bacteria

ZHANG Xu[1], ZHAO Lin[1], QIAN Long[1], HUANG Shu-Shi[2], Chagan Irbsi[1]*
(1 Laboratory of Bioconvertion, Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, China; 2 Guangxi Academy of Sciences, Nanning 530007, China)

Abstract: Alginolytic bacterium is a kind of microorganism, which can synthesize alginate lyase as well as degrade and assimilate alginate. Alginolytic bacterium is one of the important sources of alginate lyase, the alginate lyases that produced by alginolytic bacteria have many advantages, such as they have many varieties, mild reaction conditions, high activity and are easy for large-scale production. In addition, it has important application value in biological, pharmaceutical, chemical fields and other fields. In the past few decades, alginolytic bacteria, considered as the producer of alginate lyase, have been always studied and applied. However, with the aggravation of energy crisis in recent years, the biofuel converted from seaweed biomass has become a potential way to solve the energy crisis. Therefore, the research on alginolytic bacteria has been further developed. In this review, we introduced the research status of the types and characteristics of alginolytic bacteria and alginate lyase as well as the metabolism and gene engineering of alginolytic bacteria. Furthermore, we also prospected the future development trends of the research on alginolytic bacteria.

Key words: alginolytic bacteria; alginate lyase; alginate metabolism; genetic engineering

海藻酸，又称褐藻胶或褐藻酸，是由 β-D- 甘露糖醛酸 (M) 和它的 C5 位立体异构体 α-L- 古罗糖醛酸 (G) 通过 α-1,4- 糖苷键无规则地连接而成的线性多糖。海藻酸分子中的糖单体 M 和 G 有三种排列方式：M 连续排列形成聚甘露糖醛酸 (polyM)，G 连续排列形成聚古罗糖醛酸 (polyG)，以及 M 和 G 随机交替连接形成聚古罗糖醛酸 - 甘露糖醛酸 (polyMG)[1]。来自不同生物的海藻酸所含 M 和 G 的相对比例也不相同[2-3]。海藻酸是最丰富的海洋生物多糖，也是世界上仅次于纤维素的最丰富的生物高分子聚合物。海藻酸存在于海藻的细胞壁和

收稿日期：2012-03-03； 修回日期：2012-04-08
基金项目：广西自然科学基金重点项目(2010GXNSFD013029)
*通信作者：E-mail: irbisc@gmail.com; Tel：0871-5920211

细胞间质，在褐藻中的含量尤为丰富，泡叶藻(*Ascophyllum*)、公牛藻(*Durvillaea*)、昆布海藻(*Laminaria*)、巨藻(*Macrocystis*)、马尾藻(*Sargassum*)和喇叭藻(*Turbinaria ornata*)是海藻酸主要的商业来源。海藻酸也是一些细菌的胞外多糖，细菌海藻酸主要由在D-甘露糖醛酸的2和(或)3碳上带有O-乙酰基的聚甘露糖醛酸组成[4]。1966年，Linker和Jones[5]首次确认了革兰氏阴性海洋细菌铜绿假单胞菌(*Pseudomonas aeruginosa*)具有合成海藻酸并将其作为胞外多糖分泌到细胞外的现象。具有代表性的合成海藻酸的微生物除铜绿假单胞菌外，还有棕色固氮菌(*Azotobacter vinelandii*)[6]。细菌海藻酸对细菌细胞具有附着、保护等功能，如黏液状铜绿假单胞菌产生的海藻酸能使其附着在气管的上皮细胞和呼吸系统的黏蛋白上，并保护细菌不被吞噬细胞吞噬以及阻止抗生素的伤害[7]。铜绿假单胞菌的海藻酸也是囊性纤维化疾病的主要致病因子[8]。由于海藻酸具有在水介质中形成黏性溶液和凝胶的能力以及对生物无毒性的优点，因此被广泛应用于医药、化妆品、食品和生物技术等行业。根据海藻酸相对分子质量和黏度的不同，其应用范围也不同，低黏度的海藻酸用于造纸和果业，高黏度海藻酸通常用于食品和化妆品[9]。近年来，随着气候变化和化石能源危机的加剧，生物能源越来越受到重视。而且，由于海藻酸储量丰富，生产技术成熟，生产成本低廉等优势，海藻酸很可能作为第三代生物能源的原料加以利用。

1987年，Gacesa[10]提出了海藻酸的酶解机理。海藻酸裂解酶通过β消去反应裂解海藻酸的4-O-糖基键，同时在C-4和C-5之间形成双键，在产生的寡糖的非还原端产生4-deoxy-L-erythro-hex-4-ene pyranosyluronate，其在230~240 nm有强吸收峰。按底物的特异性，海藻酸裂解酶分为三种：第一种是聚甘露糖醛酸裂解酶poly(β-D-mannuronate) lyase [EC 4.2.2.3]，对polyM有特异性；第二种是聚古罗糖醛酸裂解酶poly(α-L-guluronate) lyase [EC 4.2.2.11]，对polyG有特异性；第三种是对polyM和polyG两种底物均有活性的聚古罗糖醛酸和聚甘露糖醛酸裂解酶[11]。按作用方式又可分为内切海藻酸裂解酶和外切海藻酸裂解酶。根据氨基酸序列的相似性，海藻酸裂解酶分属于七个多糖裂解酶(PL)家族，即家族5、6、7、14、15、17和18[12]。海藻酸裂解酶的来源广泛，主要有三大类：第一类是微生物，如海洋细菌、土壤细菌、真菌等[13-15]；第二类是海洋软体动物和棘皮动物，如海螺、海参、鲍鱼等[16-17]；第三类是植物，如巨藻、泡叶藻、海带等[18]。

海藻酸裂解酶可用来制备海藻的原生质体[19]，被广泛运用于藻类的细胞工程、基因工程等领域。另外，由于海藻酸裂解酶能够降解铜绿假单胞菌的生物膜中的海藻酸成分，因此，它协助抗生素抑制铜绿假单胞菌的生长[20]。此外，海藻酸裂解酶还能够将海藻酸裂解为具有生物活性的低聚海藻酸和海藻寡糖，有关不同相对分子质量海藻酸的生理活性的研究已有较多报道，尤其在免疫赋活作用[21-22]和对癌细胞形态变化的影响[23]等方面引人注目。如平均相对分子质量2 000的低聚海藻酸能促进人的内皮细胞增长[24]，特定相对分子质量的海藻酸寡糖能刺激人类巨噬细胞分泌毒性细胞因子[25]；海藻酸的降解产物还能够提高植物萌芽效率和幼芽的伸长[26]。

海藻酸分解菌是一类能够自身合成海藻酸裂解酶，能够降解并同化海藻酸的微生物。海藻酸分解菌作为海藻酸裂解酶的重要来源之一，其产生的海藻酸裂解酶具有种类多、反应条件温和、酶活高和易于大规模生产等优点。因此，对于海藻酸分解菌的研究有着深远的理论意义和广泛的应用前景。

2 海藻酸分解菌的研究现状

2.1 海藻酸分解菌及其海藻酸裂解酶的种类和特性

目前，国内外所发现的海藻酸分解菌的种类众多(表1)，主要分布于海洋细菌、土壤细菌和真菌等，还有一些属于病原微生物，如大肠杆菌、肺炎克里伯氏菌、铜绿假单胞菌。其中鞘氨醇单胞菌属中的*Sphingomonas* sp. A1、铜绿假单胞菌*P. aeruginosa*和克里伯氏菌*Klebsiella* sp.是研究较多，备受关注的明星菌株。而曲霉(*Aspergillus oryzae*)和苍白杆菌(*Ochrobactrum* sp.)[49]研究很少。随着新的海藻酸分解菌的发现，其种类还将不断增加。

由于海藻酸分解菌的种类众多，因此其海藻酸裂解酶的类型多样，如棕色固氮菌产生的胞内海藻酸裂解酶可断裂M-M和M-G间的1, 4-糖苷键，能够降解alginate、polyM、polyMG和乙酰化的polyM[31]。克里伯氏菌*K. pneumoniae*能够产生胞外聚古罗糖醛酸海藻酸裂解酶[37]。铜绿假单胞菌*P. aeruginosa*产生的是胞内聚甘露糖醛酸裂解酶[39]。鞘氨酸单胞菌*Sphingomonas* sp. A1能产生一种胞内外切寡聚海藻酸裂解酶[42]。解海藻酸弧菌*Vibrio alginolyticus* AL-9来源的海藻酸裂解酶既具有甘露

表1 海藻酸分解菌的海藻酸裂解酶的酶学性质

代表菌株	酶名称	相对分子质量(kDa)	最适温度(℃)	最适pH	粗酶相对活性(U/mg)	纯化酶相对活性(U/mg)	酶的类型	酶的底物特异性	参考文献
Agarivorans sp. JAM-A1	A1m	31	30	10.0	0.142	108.5	extracellular	alginate, polyMG polyG, polyM	[27]
Agrobacterium tumefaciens C58	Atu3025	88	30	7.3	0.065	13.1	extracellular endotype	polyM, polyG, polyMG	[28]
Alginovibrio aquatilis	alginate lyase	110	25	8.0	0.93	57.0	extracellular exotype	alginate	[29]
Alteromonas sp. 272	alginate lyase	33.9	30	7.5	8.3	1122.8	extracellular endotype	polyM, polyG	[30]
Aspergillus oryzae	alginate lyase	95	35	6.5	0.48	67.24	extracellular	alginate	[12]
Azotobacte chroococcum 4A1M	alginate lyase	24	60	6.0	1.93	32.4	extracelluar	polyM	[31]
Azotobacter vinelandii NCIB8789	alginases	39	30	6.8	0.53	——	intracellular	polyM, polyMG, alginate, O-acetyl-polyM	[32]
Bacillus sp. ATB-1015	alginate lyase	41	37	7.5	2.0	51.3	extracelluar endotype	polyG, polyM	[33]
Corynebacterium sp. Strain ALY-1	AlgPG	27	55	7.0	3.8	57	extracelluar	polyG	[34]
Enterobacter cloacae M-1	alginate lyase	38	30	7.8	0.0017	35.0	extracelluar endotype	polyG	[35]
Flavobacterrum multwolum K-11	alginate lyase	33	40	7.5	1.07	32.3	extracelluar	polyM, polyG	[36]
Klebsiella pneumonia	AlyA	31.4	35	7.6	0.078	——	extracelluar	polyG	[37]
Pseudoalteromonas sp. SM0524	Aly-SJ02	32	50	8.5	65.4	4802.7	extracelluar	polyM, polyG	[38]
Pseudomonas aeruginosa WcM#2	ALG	43	37	9.0	56.0	1386.4	intracellular	polyM	[39]
Sphingomonas sp. A1	A1-I	63	45	8.0	6.55	73.1	intracellular endotype	polyG, polyM	[40]
	A1-II	25	70	8.0	4.27	109	intracellular endotype	polyG	[40]
	A1-III	40	30	8.0	9.20	45.0	intracellular endotype	polyM, polyMG	[41]
Sphingomonas sp. A1	oligoalginate lyase	85	37	8.0	0.071	2.89	intracellular exolytic	oligoalginate	[42]
Streptomyces sp. A5	alginate lyase	32	37	7.5	7180	101600	extracellular	polyG, polyM, alginate	[43]
Vibrio alginolyticus AL-9	alginate lyase alginate lyase	25 30	37 37	9.0 8.0	0.014	2.83	extracellular	polyG polyM	[44]
Vibrio harveyi AL-128	alginate lyase	57	37	7.8	0.026	1.89	extracelluar	polyG	[45-46]
Vibrio sp. YKW-34	alginate lyase	60	40	7.0	2.22	55.93	extracellular	polyG, polyM, polyMG	[47]
Vibrio sp. QY102	alginate lyase	28.5	40	7.1	16	254	extracellular	polyM	[48]

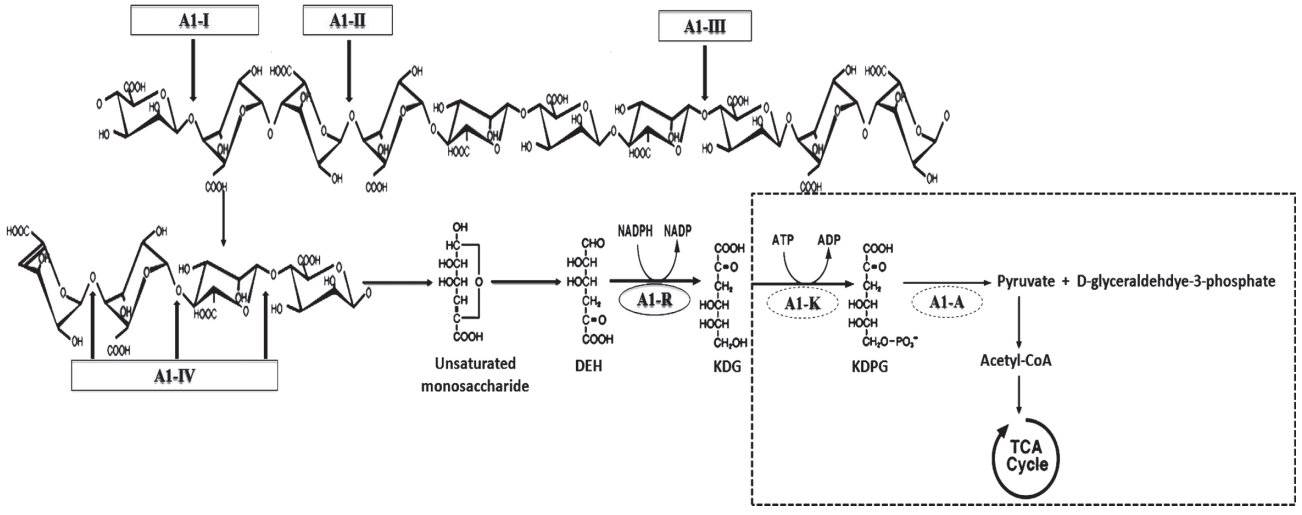
糖醛酸裂解酶活性，又具有古罗糖醛酸裂解酶活性[44]。海藻酸分解菌的海藻酸裂解酶的反应条件比较温和，pH 值范围主要为 6~12，温度在 20~70 ℃之间。1976 年，Stevens 和 Levin[29] 发现的 *Alginovibrio aquatilis* 的胞外海藻酸裂解酶的最适温度为 25 ℃。而 2000 年，Yoon 等 [40] 表达并纯化了 *Sphingomonas* sp. A1 的海藻酸裂解酶 A1-II，其最适温度为 70 ℃。1996 年，Haraguchi 和 Kodama[32] 发现的 *Azotobacte chroococcum* 4A1M 的海藻酸裂解酶的最适 pH 为 6.0；而 2009 年，Kobayashi 等 [27] 发现的 *Agarivorans* sp. JAM-A1 的海藻酸裂解酶最适 pH 为 10.0。另外，海藻酸分解菌产生的海藻酸裂解酶还具有酶活高的特点，2011 年，Li 等 [38] 纯化并研究了 *Pseudoalteromonas* sp. SM0524 的海藻酸裂解酶 Aly-SJ02，其酶活高达 65.4 U/mg。

2.3 海藻酸分解菌的代谢途径研究

虽然对海藻酸分解菌的研究已经有几十年的历史，但是海藻酸分解菌的海藻酸代谢研究进展缓慢。1962 年，Preiss 和 Ashwell[50] 研究了假单胞菌的海藻酸代谢，发现了海藻酸裂解酶降解海藻酸的机制，海藻酸裂解酶将海藻酸裂解为寡糖，寡糖的非还原端带有不饱和糖醛酸糖残基，然后进一步将不饱和糖醛酸裂解为 4-deoxy-n-erythro-hexoseulose uranic acid。但是直到 2010 年，Takase 等 [51] 首次在 *Sphingomonas* A1 菌株细胞中发现了不饱和糖醛酸还原酶 A1-R，从而提高了对 *Sphingomonas* A1 的海藻酸代谢途径的认识。*Sphingomonas* A1 在细胞表面形成具有“superchannel”的海藻素摄入系统，能够直接将高分子海藻酸摄入细胞质，相对分子质量较大的海藻酸在细胞质中被进一步代谢。如图 1 所示，首先相对分子质量较大的的海藻素被内切海藻酸裂解酶 A1-I、A1-II 和 A1-III 解聚为二糖、三糖和四糖，这些寡糖再被细胞质中的内切海藻酸裂解酶 A1-IV 进一步降解为单糖，经非酶促反应转变为 4-deoxy-L-erythro-5-hexoseulose uronic acid (DEH)，DEH 被 DEH 还原酶 A1-R 还原为 2- 酮 -3- 脱氧 -D- 葡糖酸 (2-keto-3-deoxy-D-gluconic acid, KDG)。另外，Takase 等 [51] 推测可能存在 2- 酮 -3- 脱氧葡糖酸激酶 A1-K 将 KDG 磷酸化，生成 2- 酮 -3- 脱氧 -6- 磷酸 - 葡糖酸 (2-keto-3-deoxy-6-phosphogluconic acid, KDPG)，然后 KDPG 被醛缩酶 A1-A 催化分解为 D- 甘油醛 -3- 磷酸 (D- glyceraldehdye-3-phosphate) 和丙酮酸 (pyruvate)。

2.4 海藻酸分解菌基因工程研究

海藻酸分解菌的基因工程研究主要集中于海藻酸裂解酶基因的克隆和表达。目前，已有二十多种海藻酸分解菌的海藻酸裂解酶基因被克隆，而且其中大部分基因已成功地进行了异源表达 (表 2)。重组海藻酸裂解酶的表达量均高于野生菌株。1993 年，Maki 等 [54] 在大肠杆菌中表达了 *Pseudomonas* sp. OS-ALG-9 的海藻酸裂解酶，其表达量约是野生型菌株的 53 倍。此外，岳明等 [55] 以铜绿假单胞菌 (*P. aeruginosa*) 基因组 DNA 为模板，克隆出约 1.0 kb 的海藻酸裂解酶基因 *algL*，并将其插入巴斯德毕赤酵母表达载体 pPIC9K 中，获得重组质粒 pPIC9K-*algL*。重组质粒线性化后用聚乙二醇 (PEG) 法导入



图中粗箭头表示海藻酸裂解酶的裂解位点，虚线框部分为假想反应。

图1 *Sphingomonas* A1胞内海藻酸代谢途径[51]

表2 海藻酸分解菌基因工程研究

海藻酸裂解酶基因	原始菌株	表达载体	表达菌株	重组酶活性	参考文献
aly	*Klebsiella pneumoniae*	pHG327	*E. coli* JM107	3.82 U/mg	[52]
algL	*Pseudomonas aeruginosa* FRD1	pKK223-3	*E. coli* JM109	145.83 U/mg	[53]
aly	*Pseudomonas* sp. OS-ALG-9	pUC18	*E. coli* JM109	117 U/ml	[54]
alyA	*Klebsiella aerogenes*	pBluescript SK$^-$	*E. coli*	——	[56]
algL	*Azotobacter vinelandii*	pTrc99A	*E. coli* JM109	1.25 U/mg	[14]
algL	*Azotobacter chroococcum*	pET3a	*E. coli* BL-21(DE3)	9.25 U/mg	[57]
alyPG	*Corynebacterium* sp. ALY- 1	pBluescript II SK$^-$	*E. coli XL-l blue MRF'*	——	[58]
alyVG1	*Vibrio halioticoli* IAM 14596^T	pUC18	*E. coli* DH5α	——	[59]
alyVG2	*Vibrio halioticoli* IAM 14596^T	pUC18	*E. coli* DH5α	——	[59]
alyVG3	*Vibrio halioticoli* IAM 14596^T	pUC18	*E. coli* DH5α	——	[59]
A1-I	*Sphingomonas* sp. A1	pET3a	*E. coli* BL21(DE3)pLysE	6.55 U/mg	[40]
A1-II	*Sphingomonas* sp. A1	pET17b	*E. coli* BL21(DE3)pLysE	4.27 U/mg	[40]
A1-III	*Sphingomonas* sp. A1	pET3a	*E. coli* HMS174(DE3)	9.20 U/mg	[40]
alyPEEC	*Pseudoalteromonas elyakoii* IAM14594(*Alteromonas* sp. H4)	pUC18	*E. coli* JM109	7.37×10^{-6} U/OD280	[60]
AALyase	*Pseudoalteromonas* sp. 272 (*Alteromonas* sp. 272)	pCR™ 2.1	*E. coli* INVαF9'	——	[61]
A1-IV	*Sphingomonas* sp. A1	pET3a	*E. coli* HMS174(DE3)	8.6 U/L	[62]
A1-IV'	*Sphingomonas* sp. A1	pET21b	*E. coli BL21(DE3)pLysE*	89.0 U/L	[63]
Atu3025	*Agrobacterium tumefaciens* C58	pET21b	*E. coli* HMS174(DE3)	5.9 U/mg	[28]
alyVOA	*Vibrio* sp. O2	pT7Blue	*E. coli* DH5α	——	[64]
alyVOB	*Vibrio* sp. O2	pBluescript II SK+	*E. coli* DH5α	——	[64]
Alg-5	*Streptomyces sp. ALG-5*	pColdI	*E. coli* BL21(DE3)	——	[65]
a lyA	*Pseudoalteromonas atlantica AR06*	pNIT6012	*E. coli* DH5α	——	[66]

毕赤酵母菌株 GS115 中表达。用甲醇诱导培养基进行摇瓶发酵，表达得到了相对分子质量为 40 000 的重组海藻酸裂解酶，酶活力可达 540 U/mL。

Sphingomonas A1 的代谢工程研究也有新的突破。2011 年，Takeda 等 [67] 利用新发现的强启动子 *sph2987* 在 *Sphingomonas* A1 中过表达运动发酵单胞菌 *Zymomonas mobilis* 的丙酮酸脱羧酶基因 *pdc* 和乙醇脱氢酶基因 *adhB*，使重组后的 A1 能够生产乙醇，同时还破坏了乳酸脱氢酶基因 *ldh*，阻断乳酸的代谢途径，提高了重组菌株 A1 的乙醇产量。经过代谢工程改造，重组菌株 A1 以海藻酸作为唯一碳源三天累计生产乙醇达 13.0 g/L。此研究开创了海藻酸分解菌利用海藻酸生产生物乙醇的先河。

3　展望

目前，海藻酸分解菌的研究主要集中于海藻酸分解菌的筛选、鉴定和海藻酸裂解酶的克隆与表达。对于海藻酸分解菌的代谢工程方面的研究还相对薄弱，不能发挥海藻酸分解菌的应用潜力。因此，未来海藻酸分解菌的研究将在分离、鉴定新菌种的同时，优选海藻酸裂解酶产量高、分解海藻酸能力强的菌株，并对其进行深入的研究，阐明海藻酸及糖醛酸在细胞内的代谢途径，发现并研究有应用价值的代谢产物。

另外，海藻酸分解菌的基因工程必将成为今后的研究热点。首先是海藻酸裂解酶基因的异源表达，筛选并克隆海藻酸裂解酶的基因，将其导入酵母、大肠杆菌等工程菌，高效表达海藻酸裂解酶，这方面的研究如上所述已经取得了一些成果。但是，以海藻酸为底物进行发酵的研究极为少见，原因是酵母、大肠杆菌等常用的发酵菌株不能直接利用海藻酸酶解产物糖醛酸。因此，阐明糖醛酸在海藻酸分解菌细胞内的代谢途径，并将糖醛酸代谢途径的关键酶基因一同导入工程菌，才能使其利用海藻酸生产有价值的生物制品。其次是海藻酸分解菌代谢的改造，将外源基因导入海藻酸分解菌，如丙酮酸脱羧酶 *pdc* 和乙醇脱氢酶 *adhB* 基因；或者使用分子生物学手段对其代谢途径进行改造，使其能够利用海藻生物质生产生物能源等具有应用价值的产品。目前，这方面的研究还仅有 Takeda 等对 *Sphingo-*

monas A1 的改造 [67]。

至今报道的海藻酸分解菌仅局限在好氧菌，未见有关厌氧海藻酸分解菌的报道。厌氧海藻酸分解菌进行厌氧呼吸，与好氧菌相比，无需将底物彻底氧化即可获得自身生命活动所需的能量，而且通过发酵代谢产生乙醇等有价值的代谢产物并再生细胞内的还原力，厌氧海藻酸分解菌更适合用于利用海藻生物质生产生物能源。本研究室已筛选到两株厌氧海藻酸分解菌，并正在对其海藻酸裂解酶、代谢产物和代谢途径等方面进行研究，从而为利用厌氧海藻酸分解菌进行海藻生物质转化提供技术支持和理论依据。

随着海藻酸分解菌研究不断深入，海藻酸分解菌自身所具有的巨大应用潜力将不断显现，从而对能源、医药、化工等领域产生重要影响。

[参 考 文 献]

[1] Gacesa P. Alginates. Carbohydrate Polymers, 1988, 8:161-82

[2] Tako M, Yoza E, Tohma S. Chemical characterization of acetyl fucoidan and alginate from commercially cultured *Cladosiphon okamuranus*. Bot Mar, 2000, 43: 393-8

[3] Tako M, Kiyuna S, Uechi S, et al. Isolation and characterization of alginic acid from commercially cultured *Nemacystus decipiens* (Itomozuku). Biosci Biotechnol Biochem, 2001, 65: 654-7

[4] Skjak-Bræk G, Grasdalen H, Larsen B. Monomer sequence and acetylation pattern in some bacterial alginates. Carbohydrate Res, 1986, 154: 239-50

[5] Linker A, Jones RS. A new polysaccharide resembling alginic acid isolated from *Pseudomonads*. J Biol Chem, 1966, 241: 3845-51

[6] Sabra W, Zeng AP, Deckwer WD. Bacterial alginate: physiology, product quality and process aspects. Appl Microbiol Biotechnol, 2001, 56: 315-25

[7] Nichols WW, Evans MJ, Slack MPE, et al. The penetration of antibiotics into aggregates of mucoid and non-mucoid *Pseudomonas aeruginosa*. J Gen Microbiol, 1989, 135: 1291-303

[8] Boyd A, Chakrabarty AM. Role of alginate lyase in cell detachment of *Pseudomonas aeruginosa*. Appl Environ Microbiol, 1994, 60: 2355-9

[9] Hernandez-Carmona G, McHugh DJ, Lopez-Gutierrez F. Pilot plant scale extraction of alginates from *Macrocystis pyrifera*.2. Studies on extraction conditions and methods of separating the alkaline-insoluble residues. J Appl Phycol, 2000, 11: 493-502

[10] Gacesa P. Alginate-modifying enzymes: a proposed unified mechanism of action for the lyases and epimerases. FEBS Lett, 1987, 212: 199-202

[11] Haugen F, Kortner F, Larsen B. Kinetics and specificity of alginate lyases: Part I, a case study. Carbohydrate Res, 1990, 198: 101-9

[12] Singh RP, Gupta V, Kumari P, et al. Purification and partial characterization of an extracellular alginate lyase from *Aspergillus oryzae* isolated from brown seaweed. J Appl Phycol, 23(4): 755-62

[13] Sawabe T, Sugimura I, Ohtsuka M, et al. *Vibrio halioticoli* sp. nov., a non-motile alginolytic marine bacterium isolated from the gut of the abalone *Haliotis discus hannai*. Int J Syst Bacteriol, 1998, 48: 573-80

[14] Ertesvåg H, Erlien F, Skjåk-Bræk G, et al. Biochemical properties and substrate specificities of a recombinantly produced *Azotobacter vinelandii* alginate lyase. J Bacterol, 1998, 180(15): 3779-84

[15] Schaumann K, Weide G. Efficiency of uronic acid uptake in marine alginate-degrading fungi. Helgoländer meeresunters, 1995, 49: 159-67

[16] 朱仁华. 三种海螺解壁酶组分的比较研究. 中国水产科学, 1983, 5: 513-8

[17] Boyen C, Kloareg B, Polne-Fuller M, et al. Preparation of alginate lyases from marine molluscs for protoplast isolation in brown algae. Phycologia, 1990, 29(2): 173-81

[18] Lin TY, Hass WZ. Pathway of alginic acid synthesis in the marine brown alga, *Fucus gardneri* Silva. J Biol Chem, 1966, 241: 5284-97

[19] Boyen C, Bertheau Y, Barbeyron T, et al. Preparation of guluronate lyase from *Pseudomonas alginovora* for protoplast isolation in Laminaria. Enzyme Microb Technol, 1990, 12: 885-90

[20] Alkawash MA, Soothill JS, Schiller NL. Alginate lyase enhances antibiotic killing of mucoid *Pseudomonas aeruginosa* in biofilms. APMIS, 2006, 114: 131-8

[21] Otterlei M, Sandan A, Skjåk-Braek G, et al. Induction of cytokine production from human monocytes stimulated with alginate. J Immunother, 1991, 10: 286-91

[22] Otterlei M, Sundan A, Skjåk-Braek G, et al. Similar mechanisms of action of defined polysaccharides and lipo polysaccharides:characterization of binding and tumor necrosis factor α induction. Infect Immun, 1993, 61: 1917-25

[23] Stevan FR, Oliveira MBM, Noseda MD, et al. Cytotoxic effects against HeLa cells of polysaccharides from seaweeds. J Submicrosc Cytol Pathol, 2001, 33: 477-84

[24] Kawada A, Hiura N, Tajima S, et al. Alginate oligosaccharides stimulate VEGF-mediated growth and migration of human endothelial cells. Arch Dermatol Res, 1999, 291: 542-7

[25] Iwamoto M, Kurachi M, Nakashima T, et al. Structure–activity relationship of alginate oligosaccharides in the induction of cytokine production from RAW264.7 cells. FEBS Lett, 2005, 579: 4423-9

[26] Yonemoto Y, Tanaka H, Yamashita T, et al. Promotion of germination and shoot elongation of some plants by alginate oligomers prepared with bacterial alginate lyase. J Ferment Bioeng, 1993, 75: 68-70

[27] Kobayashi T, Uchimura K, Miyazaki M, et al. A new high-alkaline alginate lyase from a deep-sea bacterium *Agarivorans* sp. Extremophiles, 2009, 13: 121-9

[28] Ochiai A, Hashimoto W, Murata K. A biosystem for alginate metabolism in *Agrobacterium tumefaciens* strain C58: Molecular identification of Atu3025 as an exotype family PL-15 alginate lyase. Res Microb, 2006, 157: 642-9

[29] Stevens RA, Levin RE. Purification and characteristics of an alginase from *Alginovibrio aquatilis*. Appl Environ Microbiol, 1977, 3: 1156-61

[30] Iwamoto Y, Araki R, Iriyama K, et al. Purification and characterization of bifunction alginate lyase from *Alteromonas* sp. strain No. 272 and its action on saturated oligomeric substrates. Biosci Biotechnol Biochem, 2001, 65(1): 133-42

[31] Kennedy L, Mcdowell K, Sutherland IW. Alginases from Azotobacter species. J Gen Microb, 1992, 138: 2465-71

[32] Haraguchi K, Kodama T. Purification and properties of poly (β-D-mannuronate) lyase from *Azotobacter chroococcum*. Appl Microbiol Biotechnol, 1996, 44: 576-81

[33] Nakagawa A, Ozaki T, Chubachi K, et al. An effective method for isolating alginate lyase-producing *Bacillus* sp. ATB-1015 strain and purification and characterization of the lyase. J Appl Microbiol, 1998, 84: 328-35

[34] Matsubara Y, Kawada R, Iwasaki KI, et al. Extracellular poly(α-L-guluronate)lyase from *Corynebacterium* sp.: purification, characteristics, and conformational properties. Protein Chem, 1998, 17(1): 31-6

[35] Nibu Y, Satoh T, Nishi Y, et al. Purification and characterization of extracellular alginate lyase from *Enterobacter cloacae* M-1. Biosci Biotechnol Biochem, 1995, 59(4): 632-7

[36] Takeuchi T, Nibu Y, Murata K, et al. Characterization of a novel alginate lyase from *Flavobacterium multivolum* K-11. Food Sci Technol, 1997, 3(4): 388-92

[37] Dyrset N, Lystad KQ, Levine DW. Development of a fermentation process for production of an alginate G-lyase from *Klebsiella pneumonia*. Appl Microbiol Biotechnol, 1994, 41: 523-30

[38] Li JW, Dong S, Song J, et al. Purification and characterization of a bifunctional alginate lyase from *Pseudoalteromonas* sp. SM0524. Mar Drugs, 2011, 9(1): 109-23

[39] Eftekhar F, Schille NL. Partial purification and characterization of a mannuronan-specific alginate lyase from *Pseudomonas aeruginosa*. Curr Microbiol, 1994, (29): 37-42

[40] Yoon HJ, Hashimoto W, Miyake O, et al. Overexpression in *Escherichia coli*, purification, and characterization of *Sphingomonas* sp. A1 alginate lyases. Protein Expr Purif, 2000, 19: 84-90

[41] Hashimoto W, Okamoto M, Hisano T, et al. *Sphingomonas* sp. A1 lyase active on both poly-β-d-mannuronate and heteropolymeric regions in alginate. Ferment Bioengin, 1998, 86(2): 236-8

[42] Hashimoto W, Miyake O, Momma K, et al. Molecular identification of oligoalginate lyase of *Sphingomonas* sp. strain A1 as one of the enzymes required for complete depolymerization of alginate. J Bacteriol, 2000, 182(16): 4572-7

[43] Cao LX, Xie LJ, Xue XL, et al. Purification and characterization of alginate lyase from *Streptomyces* species strain A5 isolated from banana rhizosphere. J Agri Food Chem, 2007, 55: 5113-7

[44] Tseng CH, Kuniko Y, Kitamikado M. Two types of alginate lyase from a marine bacterium *Vibrio* sp. AL-9. Nippon Suisan Gakkaishi, 1992, 58(4): 743-9

[45] Tseng CH, Kuniko Y, Kitamikado M. Isolation and some properties of alginate lyase from a marine bacterium *Vibrio* sp. AL-128. Nippon Suisan Gakkaishi, 1992, 58(3): 533-8

[46] Kitamikado M, Tseng CH, Kuniko Y, et al. Two types of bacterial alginate lyases. Appl Environ Microbiol,1992(8): 2474-8

[47] Fu XT, Lin H, Kim SM. Purification and characterization of a Na^+/K^+ dependent alginate lyase from turban shell gut *Vibrio* sp. YKW-34. Enzyme Microb Technol, 2007, 41: 828-34

[48] 李京宝, 于文功, 韩峰, 等. 从海洋中分离的弧菌YQ102褐藻胶裂解酶的纯化和性质研究. 微生物学报, 2003, 43(6): 753-7

[49] Zhou MH, Han FF, Li J, et al. Isolation and identification of a novel alginate-degrading bacterium, *Ochrobactrum* sp. Songklanakarin J Sci Technol, 2008, 30 (2): 135-40

[50] Preiss J, Ashwell G. Alginic acid metabolism in bacteria: I. enzymatic formation of unsaturated oligosaccharides and 4-deoxy-L-erythro-5-hexoseulose uronic acid. J Biol Chem, 1962, 237: 309-16

[51] Takase R, Ochiai A, Mikami B, et al. Molecular identification of unsaturated uronate reductase prerequisite for alginate metabolism in *Sphingomonas* sp. A1. Biochim Biophys Acta, 2010, 1804: 1925-36

[52] Gacesa P, Caswell RC. Control and heterologous expression in *Escherichia coli* of the *Klebsiella pneumoniae* gene encoding alginate lyase. Hydrobiologia, 1990, 204/205(1): 661-5

[53] Schiller N, Monday S, Boyd C, et al. Characterization of the *Pseudomonas aeruginosa* alginate lyase gene (*algL*): cloning, sequencing, and expression in *Escherichia coli*. J Bacteriol, 1993, 175(15): 4780-9

[54] Maki H, Mori A, Fujiyama K, et al. Cloning, sequence analysis and expression in *Escherichia coli* of a gene encoding an alginate lyase from *Pseudomonas* sp. OS-ALG-9. Microbiology, 1993, 139: 987-93

[55] 岳明, 丁宏标, 乔宇. 铜绿假单胞菌*algL*基因在毕赤酵母中的表达及其性质研究. 中国农业科学, 2008, 41(4): 1192-8

[56] Baron AJ, Wong TY, Hicks SJ, et al. Alginate lyase from *Klebsiella pneumonia* subsp. *aerogenes*: gene cloning, sequence analysis and high-level production in *Escherichia coli*. Gene, 1994, 143: 61-6

[57] Peciña A, Pascual A, Paneque A. Cloning and expression of the *algL* gene, encoding the *Azotobacter chroococcum* alginate lyase: purification and characterization of the enzyme. J Bacteriol, 1999, 181(5): 1409-14

[58] Matsubara Y, Kawada R, Iwasaki K, et al. Cloning and

sequence analysis of a gene(*alyPG*)encoding poly(a-L-guluronate) lyase from *Corynebacterium* sp. strain ALY-1. J Biosci Bioeng, 2000, 89(2): 199-202

[59] Sugimura I, Sawabe T, Ezura Y. Cloning and sequence analysis of *Vibrio halioticoli* genes encoding three types of polyguluronate lyase. Mar Biotechnol, 2000, 2: 65-73

[60] Sawabe T, Takahashi H, Ezura Y, et al. Cloning, sequence analysis and expression of *Pseudoalteromonas elyakoii* IAM 14594 gene (*alyPEEC*) encoding the extracellular alginate lyase. Carbohydrate Res, 2001, 335: 11-21

[61] Iwamoto Y, Iriyama K, Osatomi K, et al. Primary structure and chemical modification of some amino acid residues of bifunctional alginate lyase from a marine bacterium *Pseudoalteromonas* Sp. strain No. 272. Protein Chem, 2002, 21(7): 455-63

[62] Miyake O, Hashimoto W, Murata K. An exotype alginate lyase in *Sphingomonas* sp. A1: overexpression in *Escherichia coli*, purification, and characterization of alginate lyase IV (A1-IV). Protein Expr Purif, 2003, 29: 33-41

[63] Hashimoto W, Miyake O, Ochiai A, et al. Molecular identification of *Sphingomonas* sp. A1 alginate lyase (A1-IV') as a member of novel polysaccharide lyase family 15 and implications in alginate lyase evolution. Biosci Bioeng, 2005, 99(1): 48-54

[64] Kawamoto H, Horibe A, Miki Y, et al. Cloning and sequencing analysis of alginate lyase genes from the marine bacterium *Vibrio* sp. O2. Mar Biotechnol, 2006, 8(5): 481-90

[65] Kim DE, Lee EY, Kim HS. Cloning and characterization of alginate lyase from a marine bacterium *Streptomyces* sp. ALG-5. Mar Biotechnol, 2009, 11: 10-6

[66] Matsushima R, Danno H, Uchida M, et al. Analysis of extracellular alginate lyase and its gene from a marine bacterial strain, *Pseudoalteromonas atlantica* AR06. Appl Microb Biotechnol, 2010, 86: 567-76

[67] Takeda H, Yoneyama F, Kawai S, et al. Bioethanol production from marine biomass alginate by metabolically engineered bacteria. Energy Environ Sci, 2011, 4: 2575-81