溶藻弧菌全菌可溶性蛋白二维图谱的建立及部分蛋白分子的鉴定

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摘要:本研究采用蛋白质组学技术,建立了溶藻弧菌(*Vibrio alginolyticus*)ZJ03培养至稳定期的蛋白质组双向电泳图谱,并对部分蛋白分子进行了肽质量指纹图谱分析鉴定。首先将溶藻弧菌接种于TSB培养基28 ℃培养24 h,利用裂解液裂解细菌,提取全菌可溶性蛋白,荧光染料标记,24 cm pH4 ~ 7的胶条进行等电聚焦,再用12.5%的胶进行SDS-PAGE。2-D胶经过分析,得到902±8个蛋白斑点,重复胶的匹配点数为866±28,匹配率为96%。从双向电泳图谱中选取68个高丰度蛋白质点进行肽质量指纹图谱鉴定,其中60个在NMPDR数据库中得到鉴定,另外8个在NCBI数据库中得到鉴定。鉴定的蛋白中发现Serine protein kinase、purine nucleoside phosphorylase 和Alkyl hydroperoxide reductase subunit C-like protein存在修饰现象,它们在胶上均有2种表现形式。对68个蛋白进行了细胞功能的分类,发现能量代谢蛋白最多,占43%;其次是转运和结合蛋白,占9%;第三是外膜蛋白,占8%。经CMR操纵子预测软件分析预测到2个操纵子,它们均参与了能量代谢过程。研究结果为溶藻弧菌在不同生长条件下的比较蛋白质组学以及该菌强毒株和无毒株的比较研究提供了基础资料。[中国水产科学,2010,17(3):404-413]

关键词:蛋白质组学;溶藻弧菌;全菌可溶性蛋白;肽质量指纹图谱;双向电泳

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溶藻弧菌(*Vibrio alginolyticus*)是革兰氏阴性菌,它广泛存在于海水中,是海水养殖中重要的致病菌之一;同时它能引起人类食物中毒,是颇受关注的人畜共患菌。许多学者对该病原菌的分离鉴定、快速检测、病理变化、疫苗研制以及毒力因子的寻找等方面进行了较为系统的研究[1-5]。近年来,分子生物学技术的快速发展使得病原菌的研究进入了功能基因组时代,其中具有高通量特性的蛋白质组学起到了十分重要的作用。随着溶藻弧菌全基因组测序的完成,应用蛋白质组学技术对该菌进行研究成为可能。Xu等[6]首先利用双向电泳技术对不同盐度培养下溶藻弧菌的外膜蛋白进行了分析,发现了3个与渗透压相关的蛋白,分别为OMP W、OMPV、OMP TolC。但更多的有关该菌全菌可溶性蛋白的研究还未见报道。本研究建立了溶藻弧菌全菌可溶性蛋白二维图谱,并对高丰度蛋白进行鉴定和功能分析,为进一步研究该菌在不同生长条件下的比较蛋白质组学和该菌强毒株和无毒株的比较研究奠定基础。

1 材料与方法

1.1 菌株

溶藻弧菌ZJ03是本实验室2006年从湛江特呈岛患病红笛鲷(*Lutjanus sanguineus*)上分离,并经Biolog细菌自动鉴定系统鉴定后保藏于本实验室。

1.2 主要试剂

IPG干胶条(pH4 ~ 7)、IPG Buffer(pH4 ~ 7)、蛋

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白酶抑制剂、核酶抑制剂、考马斯亮蓝R350、尿素、硫脲、CHAPS、碘乙酰胺、二硫苏糖醇(DTT)、2-D Clean-up Kit, EttanTM 2-D Quant Kit和Cye 染料均购自GE Healthcare公司；丙烯酰胺、甲叉双丙烯酰胺、TEMED购自AMRESCO公司；蛋白预染Marker购自MBI公司。

1.3　溶藻弧菌的培养和生长曲线的测定

将溶藻弧菌接种于TSB培养基，置于28 ℃恒温振荡生化培养箱中150 r/min分别培养6 h、12 h、18 h、24 h、36 h、48 h、72 h后，检测细菌含量，取1 mL菌液适当稀释后600 nm下测OD值，再对照细菌生长曲线换算成细菌数量。试验重复3次。用软件SPSS 11.0进行单因素方差分析，用Duncan's进行多重比较。

1.4　全菌可溶性蛋白的制备

参照Coelho等[7]的方法，略有改动。将溶藻弧菌接种于TSB培养基，置于28 ℃恒温振荡生化培养箱中150 r/min培养24 h，5 000 r/min离心10 min收集细菌，洗涤缓冲液[10 mmol/L Tris-HCl(pH 8.0)，5 mmol/L醋酸镁]清洗细胞3次。离心，细胞沉淀在细胞裂解缓冲液(7 mol/L尿素，2 mol/L硫脲，4% CHAPS，1%蛋白酶抑制剂，1%核酶抑制剂)中悬浮，使其浓度范围在5～10 mg/mL。置于冰上裂解2 h。13 000 r/min，10 ℃离心1 h取上清，−80 ℃保存。2-D clean-up Kit纯化蛋白，调解样品溶液pH值至8.5，2-D Quant Kit测定样本中蛋白浓度。

1.5　蛋白的CyDye染料标记

取2份50 μg的蛋白，避光条件下一份加入1 μL Cy3、另一份加入Cy5荧光染料工作液。标记反应在冰浴、无光的条件下进行30 min，然后加入1 μL 10 mmol/L赖氨酸，继续冰浴10 min，终止标记反应。

1.6　双向电泳

主要是依据GE Healthcare公司双向电泳操作手册进行，试验重复3次。将Cy3标记和Cy5标记的2份蛋白合并后加入水化液(2 mol/L硫脲，7 mol/L尿素，4% CHAPS，0.5% IPG Buffer，1% DTT)至450 μL，上样到1根pH4～7 的24 cm IPG 胶条上，水化12 h后用Ettan IPGphor Ⅲ水平电泳仪进行第一向等电聚焦。等电聚焦后，将IPG 胶条依次用二硫苏糖醇SDS平衡液(6 mmol/L尿素，2% SDS，75 mmol/L Tris-HCl pH 8.8，29.3%甘油，1% DTT，痕量溴酚蓝)、碘乙酰胺SDS平衡液(6 mmol/L尿素，2% SDS，75 mmol/L Tris-HCl pH 8.8，29.3%甘油，2.5%碘乙酰胺)各平衡15 min。将平衡好的IPG胶条置于12.5%的聚丙烯酰胺上端，用EttanTM DALT 6 电泳装置进行第二向电泳，采用恒定功率，首先1 W/胶，电泳1 h，然后17.5 W/胶，至溴酚蓝移出胶外后停止电泳。

1.7　凝胶扫描及图象分析

用TYPHOON TRIO 成像仪对电泳后的胶板图像扫描，Cy3以及Cy5分别进行532 nm、633 nm 波长激光扫描，DeCyder V6.0 软件处理后其图像分别为绿色及红色。

1.8　蛋白选取和肽质量指纹图谱分析

用Ettan自动切点仪随机选取蛋白点，用EttanTM全自动斑点处理工作站进行切点、脱色除盐、干燥、酶解、萃取等步骤处理后，样品放入ABI 4 800 plus TOF-TOF质谱仪进行质谱分析。激光波长为355 nm，射频频率为200 Hz，MS/MS的加速电压为8 000 V。扫描质量范围为700～3 200 D。加入内标对质谱仪进行校正。所有实验样品的肽质量指纹图谱均以用默认模式获得。

1.9　数据库检索

利用软件Mascot distiller过滤基线峰、识别信号峰，再搜索美国国家病原微生物数据库(http://www.nmpdr.org/FIG/wiki/view.cgi)，将未匹配的蛋白再用NCBInr数据库(http://www.matrixscience.com)进行二次搜索。在CVICMR[7]网站(http://www.tigr.org/tigr-scripts/CMR2/gene_table.spl?db=CMR)查询蛋白功能。用CMR操纵子预测软件[8]预测鉴定的蛋白中是否存在操纵子。

2　结果与分析

2.1　溶藻弧菌的生长曲线

从图1可见，随着时间的延长细菌数量呈上升趋势，至24 h达到最大数量，随后缓慢减少。方差分析表明，培养24 h的细菌含量与培养12 h、18 h、48 h、72 h均差异显著($P<0.05$)，确定在24 h时，溶藻弧菌达到稳定生长期。

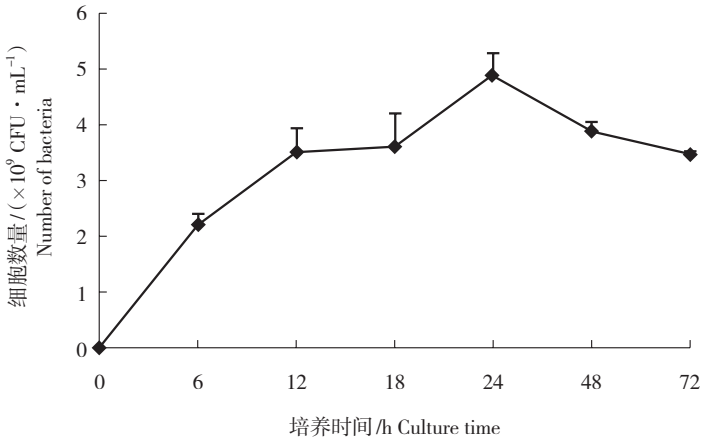

图1 培养时间对溶藻弧菌细菌数量的影响
Fig. 1 Effect of cultural time on the bacterium number of *Vibrio alginolyticus*

2.2 溶藻弧菌蛋白质双向电泳图谱

将2D胶用TYPHOON TRIO扫描后获得绿色及红色斑点图谱(图2)。DeCyder V6.0软件分析得到:图A蛋白质斑点数为894个,图B蛋白质斑点数为909个,白色数字指示质谱鉴定的蛋白,鉴定结果见表1。

2.3 图谱的重复性

在相同实验条件及参数设置情况下,3次双向电泳图谱非常相近。经过背景消减处理后,将其中任一块胶作为参考进行匹配,匹配点数为866±28,匹配率为96%。

2.4 质谱分析和数据库检索

对图2B标注的68个蛋白点进行质谱分析,数据搜索结果列为表1。检索结果发现,其中60个蛋白点在NMPDR数据库中得到鉴定,其余8个蛋白点在NCBInr数据库中得到鉴定。在JCVICMR网站查询这68个蛋白的功能,结果发现,能量代谢和运输蛋白最多,占43%,其中5个蛋白参与TCA循环,7个蛋白参与糖酵解信号通路;转运和结合蛋白占9%;外膜蛋白占8%(图3)。再经过CMR数据库检索,发现了4个由邻近基因编码的蛋白(表2),这4个蛋白均参与了能量代谢过程。

3 讨论

本研究首次应用双向电泳和质谱技术建立了重复性好、分辨率高的溶藻弧菌全菌可溶性蛋白的2-DE图谱,并选取了68个高丰度的蛋白点进行质谱鉴定。实验结果将为该菌的研究提供蛋白质组学方面的信息。

3.1 双向电泳

样品制备是双向电泳中最为关键的一步,这一步处理结果的理想程度将直接影响2-DE 结果。样品缓冲液中组分含量的变化会显著影响样品制备结果,因此针对不同生物材料,必须首先探索出能尽可能多地溶解细胞总蛋白的溶解缓冲液。鉴于霍乱弧菌(*Vibrio cholerae*)能分泌很多黏液,会影响蛋白的溶解度,陈守义等[9]把裂解缓冲液中的CHAPS 质量分数降至3%,并且增大溶解缓冲液和随后水化缓冲液中还原剂的浓度,检测到1 081个蛋白点,但小分子量蛋白较少,而且一些蛋白有拖尾或污点现象。本研究吸取上述研究人员的经验,用洗涤缓冲液代替生理盐水,避免了盐离子的残留所造成的拖带现象。并且在破碎细胞及以后的操作中始终在尽可能低的温度(冰浴)条件下处理样品,减少蛋白质的降解,获得清晰的图谱及 902±8个蛋白点,略少于陈守义等人所做的图谱,原因可能是陈守义等人未进行洗涤细菌这一操作步骤,而是用液氮直接研磨,所获蛋白是菌体蛋白和胞外蛋白的总和。

理论上蛋白质组应该包含一种组织或细胞所表达的全部蛋白质,但是双向电泳有其固有局限性,如低丰度蛋白以及极端酸性、碱性和难溶性蛋白质

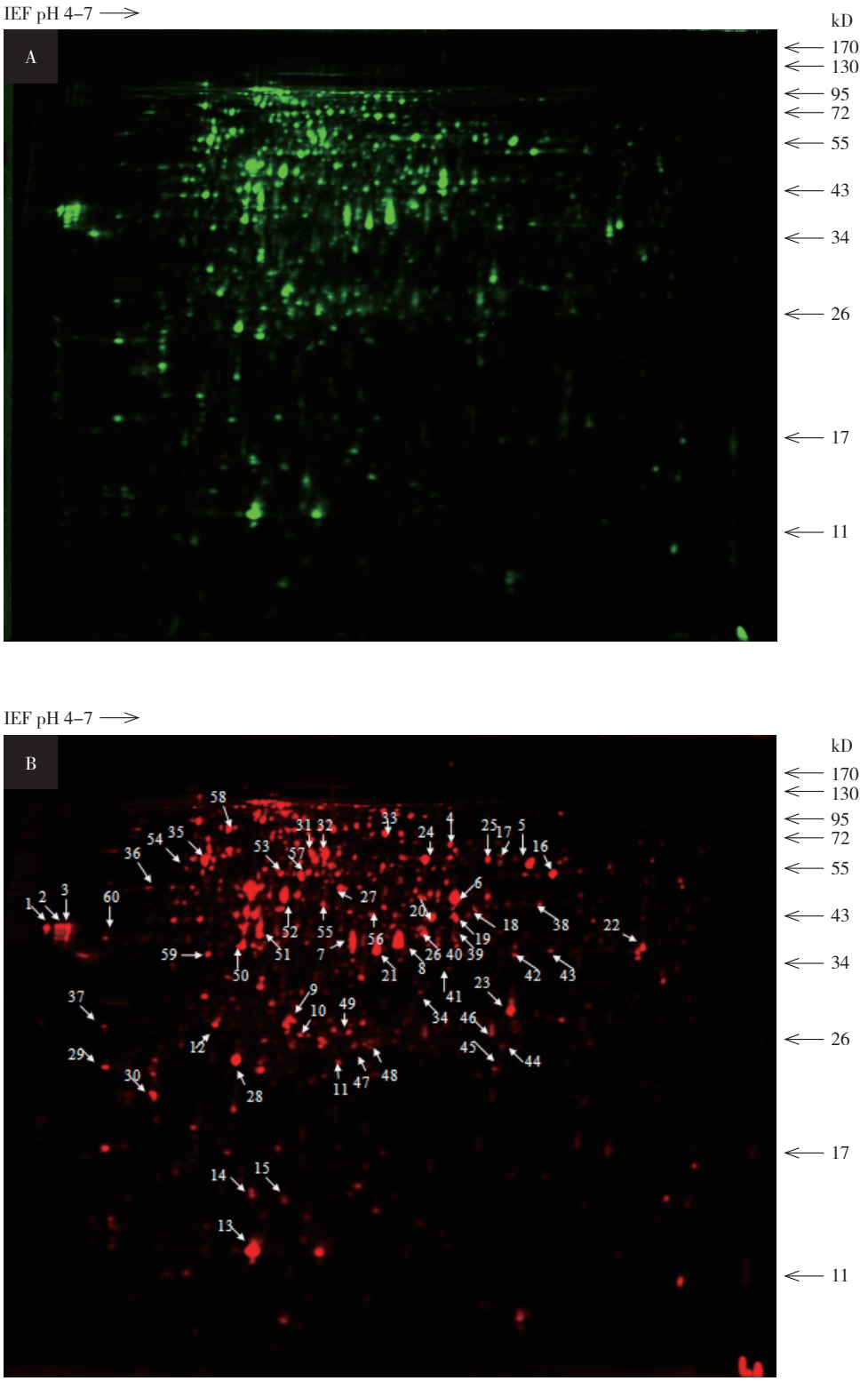

图2　溶藻弧菌全菌可溶性蛋白双向电泳图（2个重复）

A：Cy3标记的2-D图谱，蛋白质斑点数为894个；B：Cy5标记的2-D图谱，蛋白质斑点数为909个；白色数字标记了68个高丰度的蛋白点.

Fig. 2　2-D map for total soluble proteins of *Vibrio alginolyticus* (repeat)

A: 2-D map of proteins labeled with Cy3. Total of 894 spots were found. B: 2-D map of proteins labeled with Cy5. Total of 909 spots were found. 68 high-abundant protein spots targeted with mass fingerprint analysis are labeled with white figures.

表1 溶藻弧菌高丰度蛋白质肽质量指纹图谱鉴定结果

Tab. 1 Identification of high-abundant *Vibrio alginolyticus* ZJ03 proteins by mass fingerprint analysis

编号 No.	蛋白名称 Protein name	基因名称 Gene symbol	登录号 Accession No.	分数 Protein score	分子量/D Protein MW	等电点 Protein PI	功能 Function
1	Outer membrane protein OmpA[Vibrio harveyi HY01]	*ompA*	gi|153833224	235	35843.1	4.23	Cell envelop
2	Outer membrane protein[Vibrio harveyi HY01]	*omp*	gi|153835145	115	34930.8	4.31	Cell envelop
3	Outer membrane protein N, non-specific porin [Vibrio harveyi HY01]	*ompN*	gi|153835150	236	37501.8	4.51	Cell envelop
4	Succinate dehydrogenase flavoprotein subunit	*sdhA*	fig|314288.3.peg.3947	557	64184.4	5.63	Energy metabolism: TCA cycle
5	Peptide ABC transporter, periplasmic peptide-binding		fig|314288.3.peg.183	73	57422.8	5.71	Transport and binding proteins
6	Alanine dehydrogenase	*ald*	fig|314288.3.peg.483	503	39899.7	5.41	Energy metabolism: amino acids and amines
7	Elongation factor Ts	*tsf*	fig|314288.3.peg.504	245	29832.4	5.12	Protein synthesis; translation factors
8	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase		fig|314288.3.peg.688	308	35274.9	5.17	Energy metabolism: glycolysis/gluconeogenesis
9	Purine nucleoside phosphorylase	*deoD*	fig|314288.3.peg.3826	590	25921.8	4.86	Purines, pyrimidines, nucleosides, and nucleotides: nucleotide and nucleoside interconversions
10	Lysine-arginine-ornithine-binding periplasmic protein		fig|314288.3.peg.2094	332	28481.5	5.05	Transport and binding proteins: Amino acids, peptides and amines
11	Acetoacetyl-CoA reductase	*phbB*	fig|314288.3.peg.939	149	26173.2	5.56	Fatty acid and phospholipid metabolism: Biosynthesis
12	Triosephosphate isomerase	*tpiA*	fig|314288.3.peg.2969	160	26957.7	4.84	Energy metabolism: Glycolysis/ gluconeogenesis
13	unknown		fig|314288.3.peg.919	56	13143.7	5.18	unknown
14	unknown function		fig|314288.3.rna.106	271	19989.6	4.79	unknown
15	LSU ribosomal protein L9p		fig|314288.3.peg.3319	245	15699.3	5.03	Ribosomal proteins: synthesis and modification
16	Dihydrolipoamide dehydrogenase of pyruvate	*lpdA*	fig|314288.3.peg.4128	348	50964.6	5.65	Energy metabolism: Pyruvate dehydrogenase
17	Alkaline phosphatase	*phoA*	fig|314288.3.peg.681	126	57673.8	5.72	Energy metabolism: dephosphorylation.
18	Glucose-1-phosphate adenylyltransferase	*glgC*	fig|314288.3.peg.3792	209	45443.9	5.41	Energy metabolism: TCA cycle
19	Acetylornithine aminotransferase		fig|314288.3.peg.3411	284	43354.9	5.32	Amino acid biosynthesis: Glutamate family
20	3-ketoacyl-CoA thiolase		fig|314288.3.peg.9	229	41627.4	5.28	Energy metabolism: Amino acids and amines
21	Cysteine synthase		fig|314288.3.peg.2510	374	34191	5.1	Energy metabolism: O3-acetyl-L-serine
22	unknown function		fig|314288.3.peg.1220	511	36716.4	6.97	unknown
23	Phosphate ABC transporter, periplasmic		fig|314288.3.peg.324	224	28660.9	8.84	Transport and binding proteins

续表

24	Serine protein kinase		fig|314288.3.peg.2103	451	74034.2	5.3	Amino acid biosynthesis：Serine family
25	Arylsulfatase A	*atsA*	fig|314288.3.peg.3649	87	58296	6.01	Central intermediary metabolism：Sulfur metabolism
26	2-amino-3-ketobutyrate coenzyme A ligase	*kbl*	fig|314288.3.peg.276	78	43177.8	5.43	Energy metabolism：Amino acids and amines
27	Tryptophanase	*tnaA*	fig|314288.3.peg.3988	338	52458.1	5.25	Energy metabolism：Amino acids and amines
28	Alkyl hydroperoxide reductase subunit C-like protein	*ahpC*	fig|314288.3.peg.2066	492	22223.1	5.03	Cellular processes：Detoxification
29	Outer membrane protein W precursor	*ompW*	fig|314288.3.peg.302	96	23284.6	4.62	Cell envelop
30	PTS system，glucose-specific IIA component		fig|314288.3.peg.2507	293	17943.4	4.47	Transport and binding proteins：Carbohydrates，organic alcohols，and acids
31	Pyruvate kinase	*pyk*	fig|314288.3.peg.3232	599	49926.9	5.12	Energy metabolism：Glycolysis/gluconeogenesis
32	Phosphoenolpyruvate carboxykinase		fig|314288.3.peg.1912	494	60090	5.04	Energy metabolism：Glycolysis/gluconeogenesis
33	Serine protein kinase		fig|314288.3.peg.2103	508	74034.2	5.3	Amino acid biosynthesis：Serine family
34	esterase，putative		fig|314288.3.peg.3031	148	30977.1	5.22	Central intermediary metabolism：Other
35	ATP synthase beta chain	*atpD*	fig|314288.3.peg.2722	460	50667.9	4.72	Energy metabolism：ATP-proton motive force interconversion
36	Type I secretion outer membrane protein，TolC	*tolC*	fig|314288.3.peg.3897	129	47846.3	4.7	Cell envelop
37	peptidyl-prolyl cis-trans isomerase，FKBP-type [Vibrio parahaemolyticus RIMD 2210633]		gi|28897059	504	21826.1	4.43	Protein fate：Protein folding and stabilization
38	Citrate synthase		fig|314288.3.peg.3944	349	48188.8	5.51	Energy metabolism：TCA cycle
39	Phenylalanyl-tRNA synthetase alpha chain	*pheS*	fig|314288.3.peg.966	535	36919.7	5.32	Protein synthesis：tRNA aminoacylation
40	Universal stress protein E		fig|314288.3.peg.728	356	35272.2	5.66	Cellular processes：Adaptations to atypical conditions
41	Oligopeptide transport ATP-binding protein oppD	*oppD*	fig|314288.3.peg.3365	219	36085.3	5.55	Transport and binding proteins
42	6-phosphofructokinase	*pfkA*	fig|314288.3.peg.3936	294	34560.9	6.02	Energy metabolism：Glycolysis/gluconeogenesis
43	Transcriptional regulator，LysR family		fig|314288.3.peg.3226	312	36137.3	6.2	Regulatory functions：DNA interactions
44	3-oxoacyl-[acyl-carrier-protein] reductase [Vibrio harveyi]	*fabG*	gi|1706756	807	25503.1	5.59	Fatty acid and phospholipid metabolism：Biosynthesis
45	RNA polymerase sigma factor RpoE	*rpoE*	fig|314288.3.peg.3640	90	21754.2	5.4	Transcription：DNA-dependent RNA polymerase
46	pyruvate formate lyase-activating enzyme 1 [Vibrio fischeri ES114]	*pflA*	gi|59712198	131	27984.7	5.6	Energy metabolism：Fermentation
47	Stringent starvation protein A	*sspA*	fig|314288.3.peg.4011	407	24317.5	5.18	Cellular processes：Adaptations to atypical conditions

续表

48	Purine nucleoside phosphorylase	*deoD*	fig|314288.3.peg.311	147	25639.9	5.17	Purines, pyrimidines, nucleosides, and nucleotides: nucleotide and nucleoside interconversions
49	Succinate dehydrogenase iron-sulfur protein	*sdhB*	fig|314288.3.peg.3948	559	26293.9	5.1	Energy metabolism: TCA cycle
50	Malate dehydrogenase	*mdh*	fig|314288.3.peg.4316	542	32172.9	4.9	Energy metabolism: TCA cycle
51	Fructose-bisphosphate aldolase class II	*fba*	fig|314288.3.peg.4433	409	38723.3	4.75	Energy metabolism: Glycolysis/gluconeogenesis
52	Enolase	*eno*	fig|314288.3.peg.3624	1250	45590.3	4.88	Energy metabolism: Glycolysis/gluconeogenesis
53	Glycerol kinase	*glpK*	fig|314288.3.peg.430	620	55599.7	5	Energy metabolism: Other
54	cell division protein FtsZ [Vibrio parahaemolyticus RIMD 2210633]	*ftsZ*	gi|28897238	169	42546.8	4.7	Cellular processes: Cell division
55	Alcohol dehydrogenase class III	*adhI*	fig|314288.3.peg.3030	198	40694.4	5.11	Energy metabolism: Fermentation
56	Aspartate aminotransferase	*aspC*	fig|314288.3.peg.3300	322	43467.2	5.22	Amino acid biosynthesis: Aspartate family
57	ATP synthase alpha chain	*atpA*	fig|314288.3.peg.2720	376	55543	5.05	Energy metabolism: ATP−proton motive force interconversion
58	Phosphoenolpyruvate-protein phosphotransferase of PTS system		fig|314288.3.peg.2508	298	63361.4	4.69	Energy metabolism:
59	Transaldolase	*tal*	fig|314288.3.peg.1820	425	34824.8	4.86	Energy metabolism: Pentose phosphate pathway
60	Zinc finger protein 275		gi|18203321	55	51991.5	9.13	unknown
61	Maltose/maltodextrin ABC transporter, substrate binding		fig|314288.3.peg.385	444	42132.2	4.84	Transport and binding proteins
62	4-hydroxyphenylpyruvate dioxygenase	*hppD*	fig|314288.3.peg.916	619	40283.1	4.86	Energy metabolism: Amino acids and amines
63	Succinyl-CoA ligase [ADP-forming] beta chain		fig|314288.3.peg.3951	745	41502.3	4.8	Energy metabolism: TCA cycle
64	Translation elongation factor Tu	*tuf*	fig|314288.3.peg.3352	386	43081.7	4.8	Protein synthesis: Translation factors
65	3,4-dihydroxy-2-butanone 4-phosphate synthase/GTP cyclohydrolase II		fig|314288.3.peg.1974	112	40291.4	5.04	Biosynthesis of cofactors, prosthetic groups, and carriers: Riboflavin, FMN, and FAD
66	Alcohol dehydrogenase		fig|314288.3.peg.3187	578	40359.4	4.8	Energy metabolism: Fermentation
67	unknown function		fig|314288.3.peg.919	425	13143.7	5.18	unknown
68	Alkyl hydroperoxide reductase subunit C-like protein	*ahpC*	fig|314288.3.peg.2066	833	22223.1	5.03	Cellular processes: Detoxification

表2 鉴定出的由邻近基因编码的高丰度蛋白

Tab. 2 High-abundant identified proteins coded by adjacent genes

蛋白编号 Protein No.	蛋白名称 Protein name	基因名 Gene symbol	功能 Function
4	succinate dehydrogenase flavoprotein subunit	*sdhA*	Energy metabolism: TCA cycle
49	Succinate dehydrogenase iron-sulfur protein	*sdhB*	Energy metabolism: TCA cycle
57	ATP synthase alpha chain	*atpA*	Energy metabolism: ATP-proton motive force interconversion
35	ATP synthase beta chain	*atpD*	Energy metabolism: ATP-proton motive force interconversion

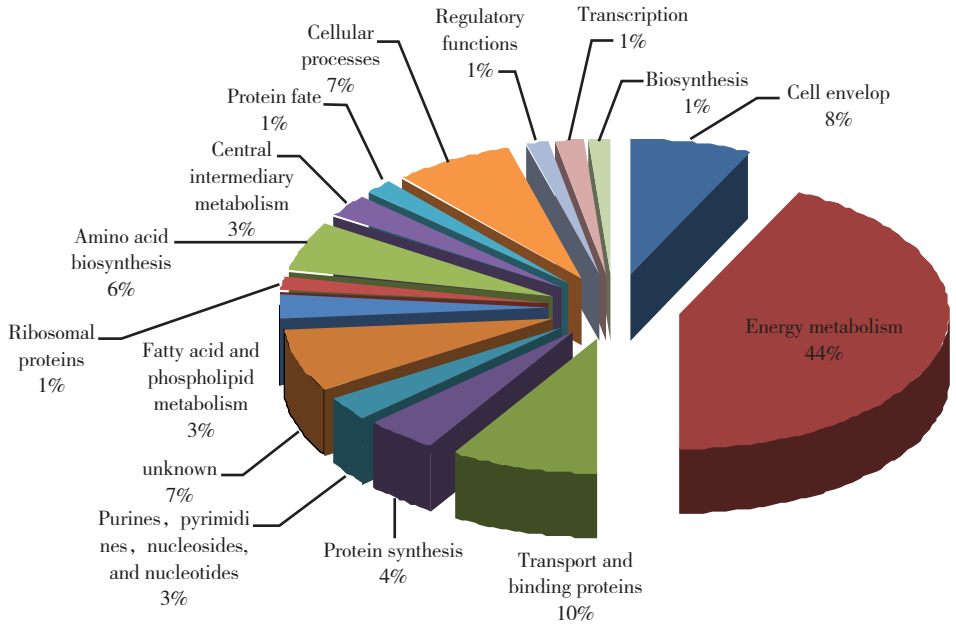

图3　溶藻弧菌68种高丰度可溶性蛋白的功能分类

Fig. 3　Cellular role categories of 68 high-abundant soluble proteins of *Vibrio alginolyticus*

的分离和鉴定仍很困难。本研究在采用pH 4～7的IPGF胶条之前，曾用pH 3～10的线性胶条对溶藻弧菌全菌可溶性蛋白进行分离（数据未附），发现蛋白点密集在pH 4～7之间，后改用pH 4～7的胶条，蛋白的分离度明显优于pH 3～10的胶条。根据NCBI上提供的数据，溶藻弧菌12G01株全菌约有4 732个可溶性蛋白（ORFs），本研究检测到902个，占理论全菌蛋白的19%，比Coelho等[7]检测到的14.1%略高。若想得到更高的蛋白检出率，则需要将现有的技术手段取长补短综合运用。据报道，应用一系列窄pH范围的胶条可使大肠杆菌双向电泳图谱的蛋白检出率提高到理论蛋白数的70%[10]。因此，为了得到更完整的溶藻弧菌分子解剖图谱，需采用一系列窄pH范围的胶条。

3.2　质谱鉴定和数据分析

本实验挑选了68个在所有图谱中都存在的高丰度蛋白进行质谱鉴定。在68个蛋白中，绝大部分蛋白的等电点均落在4～7之间，只有2个蛋白（编号为23和60）的等电点位于4～7范围外，这可能是由于蛋白质翻译后获得不同的修饰导致蛋白等电点发生较大的变化。另外有3对蛋白——purine nucleoside phosphorylase（编号为9和48）、serine protein kinase（编号为24和33）和alkyl hydroperoxide reductase subunit C-like protein（编号为28和68），它们两两在胶上的位置不同，但鉴定为同一种蛋白，推测这些蛋白中存在修饰现象[11]。

在鉴定的高丰度蛋白当中，能量代谢和运输蛋白最多，所占比重超过1/3；其次是转运和结合蛋白，约占1/10。这两项结果均与霍乱弧菌的蛋白质组学研究结果相似[7]。

通过CMR软件发现，*sdhA*和*sdhB*、*atpA*和*atpD*分别位于同一个操纵子上。对照霍乱弧菌的蛋白质组学研究报道，在*sdhA*和*sdhB*所属的操纵子上还有*sucB*、*sucC*和*sucD*，它们均参与TCA循环[7]，但本研究并没有鉴定出后三者。在*atpA*和*atpD*的操纵子上，Coelho等[7]还鉴定得到1个邻近的基因*atpH*，它们均参与了同一代谢过程。

经过分析还发现了10个致病性相关蛋白，其中OMP TolC和OMP W是已知的参与溶藻弧菌渗透调节的蛋白[6]。有报道指出OMP TolC是部分溶

血素和毒素的重要分泌通道,可以将外源蛋白呈递到细胞表面[12]。OMP W是溶藻弧菌的主要外膜蛋白,具有免疫反应性[13],但其是否具有免疫原性还未见报道。另外有8个蛋白是已知的其他细菌的抗原。OMP A 和 OMP N曾多次被证明具有免疫反应性[14-16]。Peptide ABC transporter 是包柔氏螺旋体菌(*Borrelia garinii*)的抗原[17]。Elongation factor Ts (EF-Ts), GAPDH, alanine dehydrogenase, dihydrolipoamide dehydrogenase, alkaline phosphatase 均可被葡萄状球菌(*Staphylococcus epidermidis*)的抗血清识别[18]。下一步可通过免疫印迹在可溶性蛋白中进一步筛选出具有免疫反应的蛋白质,再进行质谱测序,鉴定出与致病性相关的蛋白,为开发新的抗溶藻弧菌的疫苗提供帮助。

参考文献:

[1] 李永芹,简纪常,吴灶和. 红鳍笛鲷弧菌病病原的研究[J]. 湛江海洋大学学报,2004,24(6): 1-5.

[2] 王军,鄢庆枇,苏永全,等.溶藻弧菌的间接荧光抗体快速检测[J].海洋科学,2002,26(7): 1-4.

[3] 左凤琴,简纪常,吴灶和. 溶藻弧菌胞外产物注射赤点石斑鱼后的组织病理学观察[J]. 湛江海洋大学学报,2006,26(3): 13-16.

[4] 曹剑香,简纪常,吴灶和. 溶藻弧菌疫苗对凡纳滨对虾免疫功能的影响[J]. 湛江海洋大学学报,2004,24(6): 11-17.

[5] 叶剑敏,简纪常,吴后波,等. 溶藻弧菌脂多糖的化学成分分析及其对石斑鱼的毒性[J]. 水生生物学报,2004,28(5): 574-576.

[6] Xu C X, Wang S Y, Ren H X, et al. Proteomic analysis on the expression of outer membrane proteins of *Vibrio alginolyticus* at different sodium concentrations [J]. Proteomics,2005,5: 3142-3152.

[7] Coelho A, de Oliveira S E, Faria M L, et al. A proteome reference map for *Vibrio cholerae* El Tor [J]. Proteomics ,2004,4: 1491-1504.

[8] Ermolaeva M D, White O , Salzberg S L. Prediction of operons in microbial genomes [J]. Nucl Acids Res,2001,29: 1216-1221.

[9] 陈守义,张欣强,刘俊华,等. 霍乱弧菌菌体蛋白双向电泳技术的建立[J]. 热带医学杂志,2008,8(4): 335-337.

[10] Tonella L, Hoogland C, Binz P A, et al. New perspectives in the *Escherichia coli* proteome investigation [J]. Proteomics,2001,1: 409-423.

[11] 王三英,吴谋胜,陈晋安,等.嗜水气单胞菌蛋白质组分子解剖图谱的初步建立[J].厦门大学学报,2003,42(2): 139-143.

[12] Spreng S, Dietrich G, Goebel W, et al. Protection against murine listeriosis by oral vaccination with recombinant Salmonella expressing protective listerial epitopes within a surface-exposed loop of the TolC-protein [J]. Vaccine,2003,21(7-8): 746-752.

[13] 钱荣华.溶藻弧菌主要毒力相关基因的克隆、表达及其免疫原性研究[D].杭州: 浙江大学,2007: 1-143.

[14] Jennison A V, Raqib R, Verma N K. Immunoproteome analysis of soluble and membrane proteins of *Shigella flexneri* 2457T [J]. World J Gastroenterol,2006,12(41): 6683-6688.

[15] Dabo S M, Confer A, Montelongo M, et al. Vaccination with *Pasteurella multocida* recombinant OmpA induces strong but non-protective and deleterious Th2-type immune response in mice [J]. Vaccine,2008,26(34): 4345-4351.

[16] Prilipov A, Phale P S, Koebnik R, et al. Identification and characterization of two quiescent porin genes, *nmpC* and *ompN*, in *Escherichia coli* [J].1998,180(13): 3388-3392.

[17] Biemans-Oldehinkel E, Doeven M K, Poolman B. ABC transporter architecture and regulatory roles of accessory domains [J]. FEBS Lett,2006,580(4): 1023-1035.

[18] Sellman B R, Howell A P, Kelly-Boyd C, et al. Identification of immunogenic and serum binding proteins of *Staphylococcus epidermidi* [J]. Infect Immun,2005,73(10): 6591-6600.

Proteome reference map for total soluble proteins of *Vibrio alginolyticus*

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Abstract: *Vibrio alginolyticus* is the main *Vibrio* species isolated from diseased mariculture animals with clinical symptoms of bacterial septicemia and skin ulcer in the South China Sea. This bacterium belongs to the family of Vibrionaceae and is normal inhabitant in estuarine and marine environments. Proteome analysis by two dimensional electrophoresis (2-DE) together with mass finger print is a powerful approach for protein resolution and identification of complex biological samples. In this study, a proteome reference map has been constructed for *V. alginolyticus* in the pI range from 4.0 to 7.0. The techniques for proteome analysis were used to establish reference map of *V. alginolyticus* in a stationary phase. *V. alginolyticus* strain ZJ03 was cultured in TSB medium for 24 h at 28 ℃. Total soluble proteins were extracted with lysis buffer and purified with a 2-D clean-up kit. Then the purified proteins were labeled with Cy3 or Cy5 and separated by 2-DE. 2-DE gels were scanned with Typhoon 9410 and analyzed by DeCyder 6.0. 2-DE image analysis revealed 902±8 protein spots. The matching spots were 866±28 in two repeat electrophoregrams, with matching rate of 96%. The results were obtained from three gels run with 24 cm immobilized pH gradient strips and 12.5% SDS-PAGE gels. Sixty-eight high-abundant spots were chosen for mass spectrometry identification. Among the 68 spots from the 2-DE map, 60 spots could match with the proteins in NMPDR Database, and the other 8 spots matched with the proteins in NCBInr Database. Among the 68 proteins, energy metabolism components are the most, accounting for 43%; The second is the transport and binding proteins, accounting for 9%; The third is the outer membrane proteins, accounting for 8%. Two isoforms of serine protein kinase, purine nucleoside phosphorylase and alkyl hydroperoxide reductase subunit C-like protein were proposed. Two operons are proposed which involved in energy metabolism. Ten pathogenicity associated proteins were found. They are OMP TolC, OMP W, OMP A, OMP N, peptide ABC transporter, elongation factor Ts (EF-Ts), GAPDH, alanine dehydrogenase, dihydrolipoamide dehydrogenase and alkaline phosphatase. The primary proteome reference map established in this study is helpful in future comparative proteomic investigations on bacterium growth under various experimental conditions or on different bacterial strains. [Journal of Fishery Sciences of China, 2010, 17(3): 404–413]

Key words: proteome; *Vibrio alginolyticus*; total soluble proteins; peptide mass fingerprint; two dimensional electrophoresis

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