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El Tor 型霍乱弧菌分泌性蛋白初步分析

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摘要:【目的】利用高通量蛋白质组分析技术,初步探讨 El Tor 型霍乱弧菌的分泌性蛋白组成,为进一步的功能研究打下基础。【方法】选取 El Tor 型霍乱弧菌流行株 N16961 和非流行株 92-3,用双相电泳和激光辅助解析-飞行时间串联质谱(Matrix Assisted Laser Desorption Ionization-Time of Flight,MALDI-TOF)技术对培养上清的全部蛋白质组份进行鉴定和分析。【结果】从 N16961 株培养上清中可检测到 206 个蛋白点,并鉴定出 49 种蛋白;从 92-3 株培养上清中可检测到 236 个蛋白点,并鉴定出 42 种蛋白,两株菌共鉴定蛋白 68 种,其中经预测含有信号肽的蛋白占总数的 55.88%(38/68)。按功能不同将所鉴定出的蛋白分为 10 类,其中代谢酶、蛋白折叠/伴侣蛋白、蛋白合成以及信号传导相关蛋白占全部培养上清蛋白数的 36.76%,转运蛋白占 14.71%,鞭毛蛋白占 11.76%,降解酶占 10.29%,外膜蛋白占 5.88%,毒素占 1.47%,在所鉴定出的蛋白中有 11 种具有信号肽的假想蛋白和 2 种功能未知的蛋白为首次被实验证实可出现在霍乱弧菌培养上清中,占 19.12%。【结论】初步获得了 El Tor 型霍乱弧菌流行株和非流行株的分泌性蛋白谱及其组成特征,并提示与霍乱弧菌致病关系密切的鞭毛蛋白在胞外释放机制、红血球凝集素/蛋白酶在非流行株中的高表达特征等值得高度关注。

关键词:霍乱弧菌; 蛋白质组; 质谱
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霍乱是一种严重的可以威胁人类生命健康的腹泻性疾病,主要由 O1 群和 O139 群霍乱弧菌引起。O1 群 El Tor 型霍乱弧菌早在 1963 年即引起霍乱的第七次世界大流行,虽然后来又有 O139 菌株的出现,但近几年又重新引起了人们对这类菌株的关注[1-2]。

霍乱弧菌可以在两种完全不同的环境下生存,各种水体环境[3]和人体小肠。细菌为适应不同的生存环境需要表达不同的蛋白,它们可以受渗透压,温度,PH 和某些氨基酸的浓度的调节。分泌性蛋白具有多种功能,如降解底物,细胞壁周转、黏附、防御宿主的免疫系统等。全面了解霍乱弧菌分泌性蛋白可以帮助我们进一步了解其生存、流行特点及致病性,了解细菌与环境的关系。本研究拟采用双相电泳及激光辅助解析-飞行时间串联质谱(Matrix Assisted Laser Desorption Ionization-Time of Flight,MALDI-TOF)技术,对 O1 群 El Tor 型霍乱弧菌流行株 N16961 与非流行株 92-3 培养上清的蛋白进行了鉴定分析,并对相应的分泌蛋白组成及其特征进行分析。

1 材料和方法

1.1 材料

1.1.1 菌株:所用菌株均由中国医学细菌保藏管理中心霍乱弧菌专业实验室(1)保存并提供。O1 群 El Tor 生物型流行株 N16961,为国际测序菌株,1975 年分离自孟加拉;O1 群 El Tor 生物型非流行株 92-3,

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1992 年分离自外环境水体。

1.1.2 主要试剂和仪器：二硫苏糖醇、尿素、十二烷基磺酸钠、甘氨酸、溴酚蓝、CHAPS、干胶条（pH3-10NL）、DALT Gel 12.5（26 × 20 cm）均为 GE Amersham Biosciences 公司产品；四甲基乙二胺、低熔点琼脂糖为普洛麦格（北京）生物技术有限公司产品；胰蛋白酶、TFA、α-氰基-4-羟基肉桂酸，均为 Sigma-Aldrich 公司产品。IPGphor 等电聚焦电泳仪、Ettan DALT Ⅱ垂直电泳仪、扫描仪，切胶仪器为 GE Amersham Biosciences 产品；4700 飞行质谱分析仪为美国应用生物系统公司产品。

1.2 样品制备

将实验用霍乱弧菌单菌落接种于 10 ML 肉汤中，37℃摇床中震荡培养 3h，5000 × g 离心 10 min，用 0.85%生理盐水洗菌，调 OD 值约为 1.0。每 100 mL 山梨醇发酵培养基加入 3.3 mL 上述菌液，37℃摇床中震荡培养至山梨醇明显发酵。将菌液 3000 × g 4℃离心 10min。将上清转入另一离心管中 6000 × g 4℃离心 15min。并用 0.22 μm 孔径的滤器过滤。用三氯醋酸-丙酮法制备蛋白样品，蛋白定量应用 BradFord 法。用蛋白裂解液溶解蛋白，蛋白裂解液成分为 6 mol/L urea，2 mol/L thiourea，4% CHAPS，10mmol/L PMFS，用前加 1% DTT、2% IPG Buffer。N16961 株的样品蛋白浓度为 1.16 g/L，上样量为 430 μL，92-3 株的样品蛋白浓度为 2 g/L，上样量为 250 μL。

1.3 双相电泳

等点聚焦（isoelectric focusing，IEF）使用 24 cm pH3-10 非线性干胶条（GE Healthcare），水化液成分为 6 mol/L urea，2 mol/L thiourea，溴酚蓝少量，用前加 0.5% IPG Buffer、0.28% DTT。等点聚焦程序如下：30V，5 h；60V，5 h；100V，0.5 h；300V，1 h；600V，1 h；1000V，1 h；8000V，10 h（恒定电流，50 μA/胶条，表面温度为 20℃）。总 Vh 达到 80000 左右。等电聚焦结束后在 130 mmol/L DTT 中平衡 15 min，再用 135 mmol/L 碘乙酰胺平衡 15 min。二向电泳采用预制胶，电泳程序为：2.5 W/gel 电泳 30 min，然后 18 W/gel电泳至溴酚蓝移动至胶下缘约 5 mm 处。

1.4 染色、成像及分析及待测点切割

质谱鉴定用胶为考染。染色后凝胶用 Amersham Bioscience 的扫描仪透射扫描，分辨率为 400dpi，图像经 Image Master 2D Platinum 软件自动识别和人工校对后，对全部识别蛋白点用切点仪进行切割。蛋白质斑点的含量被定义为该点的象素占整个胶所有象素强度的百分比，即相对含量。

1.5 胶内酶解及 MALDI-TOF 质谱鉴定

对欲鉴定蛋白进行胰酶消化后[4]，进行 MALDI-TOF 质谱分析，采集质量范围 850-3000 Da。使用 GPS Explorer Workstation 软件，进行数据检索。数据库为 NCBI nr，种属设定为“细菌”，MH + 精确度设定为 0.01%，可能的修饰为半胱氨酸被丙烯酰胺修饰和蛋氨酸被氧化。

1.6 分析用软件及数据库

用 SignalP 3.0 Server 对所有鉴定蛋白进行信号肽预测。用 UniProt 数据库软件进行蛋白功能分析。

2 结果

2.1 2-DE 结果及蛋白质谱鉴定结果

N16961 株样品在胶上共展示了 206 个点，92-3 株样品在胶上展示了 232 个点。大部分蛋白等电点分布在 4 ~ 9 之间，分子量在 20 ~ 120 kDa 之间，见图 1 和 2。对以上蛋白点全部进行质谱鉴定，N16961 株中共鉴定出 95 个蛋白点，对应 49 种蛋白，92-3 共鉴定 144 个蛋白点，对应 42 种蛋白。两株菌共鉴定蛋白 68 种，其中 27 种蛋白为 N16961 株特有蛋白，17 种蛋白为 92-3 株特有蛋白，24 种蛋白为 2 株菌共有蛋白，见表 1 和 2。

2.2 信号肽预测结果

用 SignalP 3.0 Server 对蛋白进行信号肽预测，在 68 种培养上清鉴定的蛋白中预测有信号肽的蛋白 38 种，占全部蛋白的 55.88%；没有信号肽的蛋白 30 种，占全部蛋白的 44.12%。在 38 种具有信号肽的蛋白中，2 株菌共有的蛋白为 18 种，N16961 株特有的蛋白为 11 种，92-3 株特有的蛋白为 9 种，见表 1 和 2。

2.3 蛋白的功能分类

将 2 株菌培养上清鉴定的 68 种蛋白按照功能进行分类，共分为 10 类（图 3）。代谢酶是数量最多的一组蛋白，共包括 18 种蛋白，占全部鉴定蛋白数的 26.47%，这些蛋白主要参与糖酵解、核酸代谢、能量产生等功能。19.12%（13 个）的蛋白为假想蛋白和功能未知的蛋白，其中 11 个假想蛋白均为首次在实验水平能到了验证，并且全部具有信号肽，说明他们可以被细菌分泌到细胞为，并可能具有重要的蛋白功能。10 个预测有信号肽的转运蛋白占全部蛋白的 14.71%，这些蛋白的细胞定位为外周质。8 个鞭毛蛋白成分占全部蛋白的 11.76%，这些蛋白均不具有信号肽。7 个降解酶占全部蛋白的

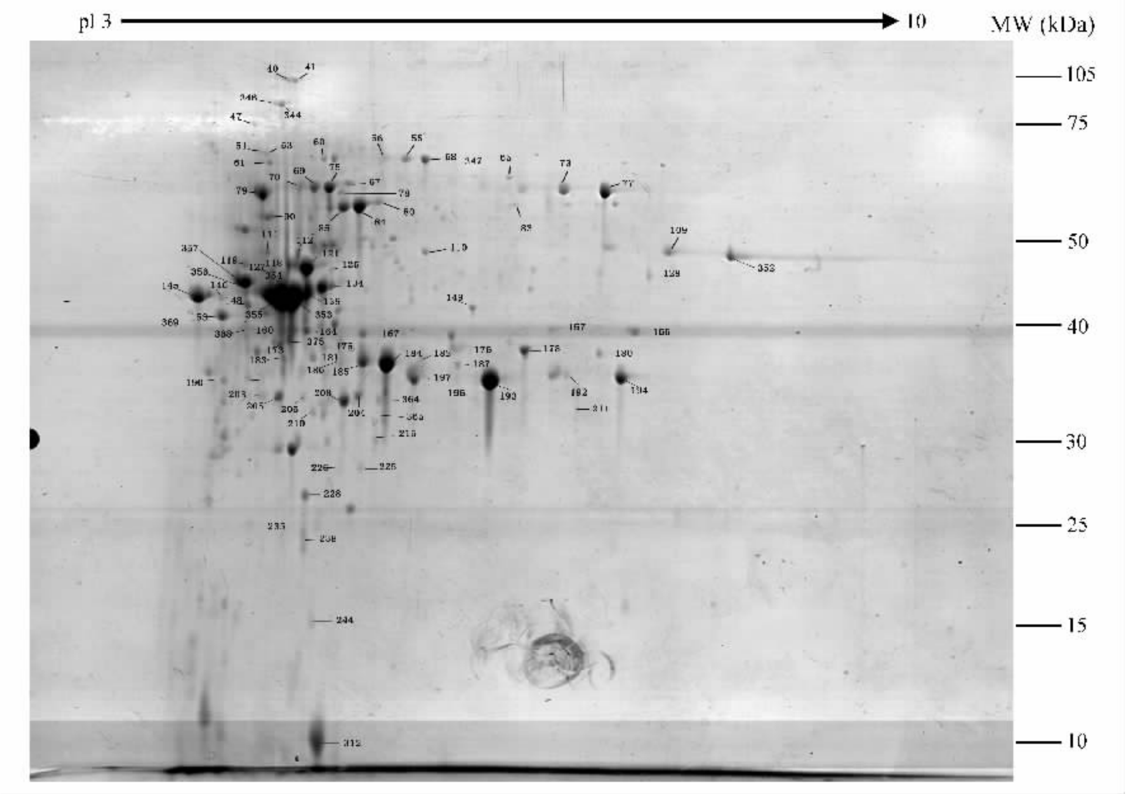

图 1　El Tor 型霍乱弧菌 N16961 培养上清蛋白双相电泳图谱

Fig.1　Overview of 2-DE exoproteins profile of El Tor *V*. *cholerae* strain N16961.

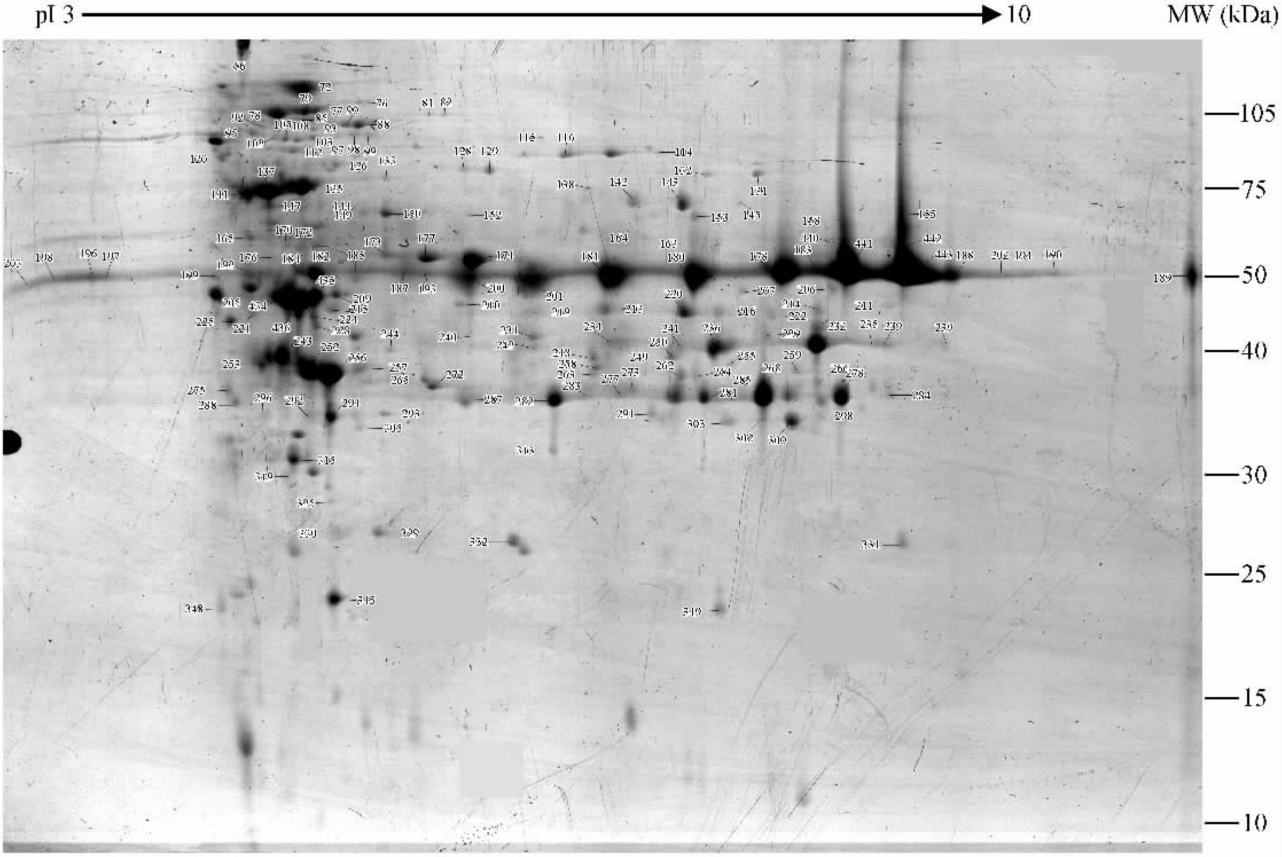

图 2　El Tor 型霍乱弧菌 92-3 培养上清蛋白双相电泳图谱

Fig.2　Overview of 2-DE exoproteins profile of El Tor *V*. *cholerae* strain 92-3.

表 1　**El tor 型霍乱弧菌 N16961 培养上清蛋白鉴定结果**

Table 1　Proteins detected in the supernatant of El tor *vibrio cholerae* N16961

spot NO.	Protein Name	protein function	NCBI Accession No.	PI (cal)	PI (gel)	Mr(cal) Da	Mr(gel) Da	Score MASCOT	Pep. Count	SC%	signal peptide	% vol
60	2′，3′-cyclic-nucleotide 2′-phosphodiesterase	metabolic enzymes	gi|15642557	5.35	5.30	74985	74984	123	17	65%	Y	0.13
225	adenylate kinase	metabolic enzymes	gi|15641001	5.20	5.30	23262	23262	105	11	35%	N	0.16
129	alanine dehydrogenase	metabolic enzymes	gi|15641907	5.88	5.82	39814	46294	84	11	79%	N	0.16
181	amino acid ABC transporter，periplasmic amino acid-binding protein	transporter	gi|15641374	5.19	5.18	36828	37510	95	11	72%	Y	0.23
196	amino acid ABC transporter，periplasmic amino acid-binding protein	transporter	gi|15601790	6.13	5.19	30827	35314	102	12	80%	Y	0.11
208	amino acid ABC transporter，periplasmic amino acid-binding protein	transporter	gi|15641865	5.34	5.20	28694	31755	121	13	35%	Y	0.98
210	amino acid ABC transporter，periplasmic amino acid-binding protein	transporter	gi|15641865	5.34	5.28	28694	31130	87	2	55%	Y	0.27
228	amino acid ABC transporter，periplasmic amino acid-binding protein	transporter	gi|15640042	5.20	5.30	27286	21343	211	18	46%	Y	1.03
238	antioxidant，AhpC/Tsa family	metabolic enzymes	gi|15640750	5.37	5.10	22847	14731	93	10	33%	N	0.35
244	antioxidant，AhpC/Tsa family	metabolic enzymes	gi|15640750	5.37	5.18	22847	9579	51	1	67%	N	0.22
83	ATP synthase F1，alpha subunit	metabolic enzymes	gi|15642759	5.54	5.77	56747	57146	111	15	54%	N	0.15
182	C4-dicarboxylate-binding periplasmic protein	transporter	gi|15641931	5.46	5.30	37072	37510	78	10	35%	Y	0.41
184	C4-dicarboxylate-binding periplasmic protein	transporter	gi|15641931	5.46	5.30	37072	36820	246	22	65%	Y	4.66
185	C4-dicarboxylate-binding periplasmic protein	transporter	gi|15641931	5.46	5.30	37072	36991	217	20	75%	Y	1.31
186	C4-dicarboxylate-binding periplasmic protein	transporter	gi|15641931	5.46	5.22	37072	36906	133	14	50%	Y	0.34
215	C4-dicarboxylate-binding periplasmic protein	transporter	gi|15641931	5.46	5.30	37072	27089	134	14	65%	Y	0.45
364	C4-dicarboxylate-binding periplasmic protein	transporter	gi|15641931	5.46	5.30	37072	33646	156	16	66%	Y	0.06
79	chaperonin，60 Kd subunit	Protein maintenance and folding	gi|15642659	4.78	5.01	57118	58968	72	10	39%	N	1.64
344	chitinase	metabolic enzymes	gi|15600798	4.90	5.30	90257	90257	155	21	70%	Y	0.04
346	chitinase	metabolic enzymes	gi|15600798	4.90	5.21	90257	90921	71	13	35%	Y	0.03
61	chitinase，putative	metabolic enzymes	gi|15640788	4.99	5.13	63122	68010	126	16	60%	Y	0.13
90	chitinase，putative	metabolic enzymes	gi|15601566	4.94	5.10	53594	54948	78	2	30%	Y	0.38
111	chitinase，putative	metabolic enzymes	gi|15601566	4.94	5.09	53594	49104	48	1	65%	Y	0.33
40	chitodextrinase	metabolic enzymes	gi|15601456	5.05	5.30	111645	112247	94	18	75%	Y	0.17
41	chitodextrinase	metabolic enzymes	gi|15601456	5.05	5.30	111645	112247	77	16	44%	Y	0.35
157	cysteine synthase A	metabolic enzymes	gi|15640984	5.93	5.86	34217	40589	194	18	66%	N	0.81
165	cysteine synthase A	metabolic enzymes	gi|15640984	5.93	5.90	34217	40401	207	19	45%	N	0.91
211	DNA-binding response regulator PhoB	Signal transduction mechanisms	gi|15640738	5.75	5.91	26176	29720	81	9	35%	N	0.10
51	dnaK protein	Protein maintenance and folding	gi|15640871	4.80	4.97	68718	76886	115	16	75%	N	0.17
53	dnaK protein	Protein maintenance and folding	gi|15640871	4.80	5.07	68718	76502	159	19	55%	N	0.25

续表 1 – 1

spot NO.	Protein Name	protein function	NCBI Accession No.	PI (cal)	PI (gel)	Mr(cal) Da	Mr(gel) Da	Score MASCOT	Pep. Count	SC%	signal peptide	% vol
175	elongation factor Ts	Protein synthesis	gi|15642257	5.16	5.29	29828	38659	89	11	44%	N	0.24
226	elongation factor TU	Protein synthesis	gi|15640389	5.04	5.29	43099	23108	91	12	47%	N	0.17
190	flagellar basal-body rod protein FlgG	Flagellum components	gi|15642194	4.39	4.33	28081	35975	67	1	34%	N	0.56
119	flagellar hook protein FlgE	Flagellum components	gi|15642196	4.71	4.75	46817	47537	50	3	77%	N	0.20
356	flagellar hook protein FlgE	Flagellum components	gi|15642196	4.71	4.79	46817	45564	139	16	29%	N	1.76
357	flagellar hook protein FlgE	Flagellum components	gi|15642196	4.71	4.70	46817	45806	114	14	44%	N	0.75
125	flagellar hook-associated protein FlgL	Flagellum components	gi|15642189	5.06	5.27	44916	46953	74	10	29%	N	0.09
134	flagellar hook-associated protein FlgL	Flagellum components	gi|15642189	5.06	5.26	44916	45323	83	11	35%	N	0.35
368	flagellar hook-associated protein FlgL	Flagellum components	gi|15642189	5.06	4.90	44916	40683	74	10	33%	N	0.01
369	flagellar hook-associated protein FlgL	Flagellum components	gi|15642189	5.06	4.93	44916	42124	72	10	44%	N	0.01
350	flagellar hook-associated protein FlgM	Flagellum components	gi|15642190	5.93	5.73	68646	67222	223	25	55%	N	0.09
127	flagellin core protein A	Flagellum components	gi|15642187	4.85	5.16	40358	46870	64	1	33%	N	0.33
160	flagellin core protein A	Flagellum components	gi|15642187	4.85	5.30	40358	40589	109	13	46%	N	0.76
183	flagellin core protein A	Flagellum components	gi|15642187	4.85	5.30	40358	37510	70	10	29%	N	0.39
354	flagellin core protein A	Flagellum components	gi|15642187	4.85	5.30	40358	45243	129	15	55%	N	4.23
355	flagellin core protein A	Flagellum components	gi|15642187	4.85	5.11	40358	44139	121	14	45%	N	4.63
118	flagellin FlaC	Flagellum components	gi|15642186	4.93	5.30	39881	47537	92	12	70%	N	0.77
121	flagellin FlaC	Flagellum components	gi|15642186	4.93	5.12	39881	47036	109	13	65%	N	3.65
353	flagellin FlaD	Flagellum components	gi|15642142	4.89	5.30	39880	44530	163	17	67%	N	2.91
375	flagellin FlaD	Flagellum components	gi|15642142	4.89	5.30	39880	39474	155	16	43%	N	0.04
164	fructose-bisphosphate aldolase, class II	metabolic enzymes	gi|15640505	4.91	5.11	38895	40495	82	10	56%	N	0.52
180	glyceraldehyde 3-phosphate dehydrogenase	metabolic enzymes	gi|15642002	5.80	5.96	35261	37948	139	14	54%	N	0.16
109	glycerol-3-phosphate ABC transporter, periplasmic glycerol-3-phosphate-binding protein	Transporter	gi|15641557	6.41	5.70	48214	48975	101	13	55%	Y	0.39
352	glycerol-3-phosphate ABC transporter, periplasmic glycerol-3-phosphate-binding protein	Transporter	gi|15641557	6.41	5.30	48214	48213	182	19	65%	Y	0.03
187	haemolysin	toxin	gi|15600988	5.30	5.02	81972	36735	78	1	62%	Y	0.17
203	haemolysin	toxin	gi|15600988	5.30	5.01	81972	33261	95	2	72%	Y	0.37
205	haemolysin	toxin	gi|15600988	5.30	5.26	81972	33041	173	2	54%	Y	0.54
206	haemolysin	toxin	gi|15600988	5.30	5.18	81972	32823	75	1	66%	Y	0.12
312	haemolysin	toxin	gi|15600988	5.30	5.22	81972	1968	159	2	43%	Y	2.79
235	heat shock protein GrpE	Protein maintenance and folding	gi|15640870	4.62	4.70	22645	17498	66	2	44%	N	0.08
204	hypothetical protein VC1101	Hypothetical protein	gi|15641114	5.54	5.30	33262	33261	115	12	45%	Y	0.44
197	hypothetical protein VC1334	Hypothetical protein	gi|15641346	5.77	5.30	34814	35232	97	12	56%	Y	1.18
67	hypothetical protein VC1485	Hypothetical protein	gi|15641494	5.78	5.24	60531	61717	159	18	66%	Y	0.26
69	hypothetical protein VC1485	Hypothetical protein	gi|15641494	5.78	5.19	60531	60372	173	19	60%	Y	0.61
70	hypothetical protein VC1485	Hypothetical protein	gi|15641494	5.78	5.30	60531	60530	90	12	34%	Y	0.52

续表 1 – 2

spot NO.	Protein Name	protein function	NCBI Accession No.	PI （cal）	PI （gel）	Mr（cal） Da	Mr（gel） Da	Score MASCOT	Pep. Count	SC%	signal peptide	% vol
75	hypothetical protein VC1485	Hypothetical protein	gi|15641494	5.78	5.28	60531	60057	214	22	46%	Y	0.95
78	hypothetical protein VC1485	Hypothetical protein	gi|15641494	5.78	5.22	60531	59588	83	12	54%	Y	0.20
365	hypothetical protein VC1523	Hypothetical protein	gi|15641531	5.51	5.30	30242	31755	87	10	43%	Y	0.02
55	hypothetical protein VCA0033	Hypothetical protein	gi|15600804	5.41	5.30	68808	75172	151	19	45%	Y	0.36
56	hypothetical protein VCA0033	Hypothetical protein	gi|15600804	5.41	5.30	68808	75361	107	16	56%	Y	0.46
58	hypothetical protein VCA0033	Hypothetical protein	gi|15600804	5.41	5.30	68808	68807	102	15	55%	Y	0.50
146	hypothetical protein VCA0568	Hypothetical protein	gi|15601327	4.67	4.49	42149	44217	105	2	65%	Y	0.27
192	immunogenic protein	Hypothetical protein	gi|15600914	7.82	5.89	37941	35726	99	12	29%	Y	0.14
194	immunogenic protein	Hypothetical protein	gi|15640457	6.61	6.00	35233	35232	144	15	76%	Y	1.31
155	iron（Ⅲ）ABC transporter，periplasmic iron-compound-binding protein	Transporter	gi|15640628	5.54	5.25	39344	41158	148	2	66%	Y	0.47
47	lipase，putative	Hypothetical protein	gi|15601618	4.88	4.90	83510	83510	42	2	45%	Y	0.11
167	malate dehydrogenase	metabolic enzymes	gi|15640459	6.85	5.30	36935	40121	101	12	56%	N	0.44
148	maltose ABC transporter，periplasmic maltose-binding protein	Transporter	gi|15601698	4.89	4.80	43779	43519	78	1	45%	Y	0.70
73	oligopeptide ABC transporter，periplasmic oligopeptide-binding protein	Transporter	gi|15641104	6.02	5.88	61102	59744	109	15	56%	Y	0.64
77	oligopeptide ABC transporter，periplasmic oligopeptide-binding protein	Transporter	gi|15641104	6.02	5.97	61102	59122	109	15	34%	Y	1.53
173	outer membrane protein OmpA	Outer membrane proteins	gi|15642211	5.07	5.29	34265	39110	80	10	56%	Y	0.34
145	outer membrane protein OmpU	Outer membrane proteins	gi|15640653	4.51	4.20	37636	44139	130	14	39%	Y	2.32
112	outer membrane protein TolC	Outer membrane proteins	gi|15642433	5.10	5.30	47722	48848	84	12	43%	Y	0.54
153	outer membrane protein，putative	Outer membrane proteins	gi|15601761	4.68	4.49	35892	42222	69	10	29%	Y	0.78
80	peptide ABC transporter，periplasmic peptide-binding protein	Transporter	gi|15640201	5.76	5.30	60576	57596	83	12	70%	Y	0.40
84	peptide ABC transporter，periplasmic peptide-binding protein	Transporter	gi|15640201	5.76	5.30	60576	56551	94	13	86%	Y	2.32
85	peptide ABC transporter，periplasmic peptide-binding protein	Transporter	gi|15640201	5.76	5.20	60576	56551	94	13	45%	Y	0.96
193	phosphate ABC transporter，periplasmic phosphate-binding protein	Transporter	gi|15600841	8.76	5.70	31652	34929	105	12	43%	Y	5.54
65	phosphoenolpyruvate carboxykinase	metabolic enzymes	gi|15642731	5.60	5.75	59806	63418	159	19	56%	N	0.08
347	phosphoenolpyruvate carboxykinase	metabolic enzymes	gi|15642731	5.60	5.03	59806	63912	114	15	55%	N	0.02
110	protease DO	degradative enzymes	gi|15640588	5.74	5.29	48337	49104	120	14	34%	Y	0.15
139	spindolin-related protein	metabolic enzymes	gi|15600910	5.39	5.13	44356	44609	59	1	67%	Y	3.09
176	thiosulfate ABC transporter，periplasmic thiosulfate-binding protein	Transporter	gi|15640560	6.14	5.06	36898	38569	190	19	34%	Y	0.42
178	thiosulfate ABC transporter，periplasmic thiosulfate-binding protein	Transporter	gi|15640560	6.14	5.80	36898	38213	244	22	32%	Y	0.72
149	trypsin，putative	degradative enzymes	gi|15641213	6.30	4.90	43214	43213	125	14	34%	Y	0.18

Numbers correspond to the protein spot numbers in Fig. 2. The figures corresponding to Mr（cal）and pI（cal）were calculated by the MASCOT software. The Mr（gel）and pI（gel）values were estimated from the spot position in the 2-DE gel. SC% means sequence coverage（percentage）. It includes the results of the secretion signal search using the Signal P software. Y: Yes; N: No.

表 2　**El tor** 型霍乱弧菌 **92-3** 培养上清蛋白鉴定结果

Table 2　Proteins detected in the supernatant of El tor *vibrio cholerae* 92-3

spot NO.	Protein Name	protein function	NCBI Accession No.	PI（cal）	PI（gel）	Mr（cal）Da	Mr（gel）Da	Score MASCOT	Pep. Count	SC%	signal peptide	%vol
126	2′,3′-cyclic-nucleotide 2′-phosphodiesterase	metabolic enzymes	gi|15642557	5.16	8.80	77275	74984	142	17	29%	Y	0.11
207	acetyl-CoA acetyltransferase	metabolic enzymes	gi|15601447	5.94	6.09	41374	48590	76	10	33%	N	0.06
283	amino acid ABC transporter, periplasmic amino acid-binding protein,	transporter	gi|153801946	6.45	6.73	30786	35474	90	10	30%	Y	0.35
292	amino acid ABC transporter, periplasmic amino acid-binding protein	transporter	gi|15641865	5.34	6.58	28694	33155	130	14	45%	Y	0.17
294	amino acid ABC transporter, periplasmic amino acid-binding protein	transporter	gi|15641865	5.34	7.71	28694	32945	109	12	50%	Y	0.57
315	amino acid ABC transporter, periplasmic amino acid-binding protein	transporter	gi|15601731	5.05	7.20	26729	29004	89	13	44%	Y	0.34
152	aminopeptidase	degradative enzymes	gi|15601568	5.24	6.51	54510	59134	47	18	24%	Y	0.07
173	aminopeptidase	degradative enzymes	gi|15601568	5.24	8.80	54510	53312	74	11	45%	Y	0.16
174	aminopeptidase	degradative enzymes	gi|15601568	5.24	5.60	54510	52590	89	13	60%	Y	1.67
177	aminopeptidase	degradative enzymes	gi|15601568	5.24	5.40	54510	52877	137	17	30%	Y	0.48
215	aminopeptidase	degradative enzymes	gi|15601568	5.24	8.15	54510	46389	110	15	45%	Y	0.16
252	aminopeptidase	degradative enzymes	gi|15601568	5.24	7.32	54510	38373	99	14	44%	Y	2.30
256	aminopeptidase	degradative enzymes	gi|15601568	5.24	7.93	54510	37789	75	12	30%	Y	2.75
257	aminopeptidase	degradative enzymes	gi|15601568	5.24	6.40	54510	38520	75	12	45%	Y	0.33
265	C4-dicarboxylate-binding periplasmic protein	transporter	gi|15641931	5.46	8.80	37072	37072	143	15	50%	Y	0.08
78	chitinase	metabolic enzymes	gi|15600798	4.9	4.72	90257	97772	203	25	33%	Y	0.72
92	chitinase	metabolic enzymes	gi|15600798	4.9	4.49	90257	91496	132	19	29%	Y	0.06
112	chitinase	metabolic enzymes	gi|15600798	4.9	6.67	90257	81018	169	22	60%	Y	0.10
135	chitinase	metabolic enzymes	gi|15600798	4.9	8.80	90257	64468	156	21	65%	Y	1.78
141	chitinase	metabolic enzymes	gi|15641954	4.9	4.22	90257	62762	176	8	6%	Y	0.75
147	chitinase	metabolic enzymes	gi|15600798	4.9	4.59	90257	60438	126	19	39%	Y	0.20
330	chitinase	metabolic enzymes	gi|15600798	4.9	8.58	90257	23282	53	1	30%	Y	0.07
72	chitodextrinase	metabolic enzymes	gi|15601456	5.05	8.80	111645	111645	141	22	45%	Y	1.55
76	chitodextrinase	metabolic enzymes	gi|15601456	5.05	6.52	111645	103903	101	19	55%	Y	0.06
77	chitodextrinase	metabolic enzymes	gi|15601456	5.05	5.32	111645	99407	213	28	33%	Y	0.22
79	chitodextrinase	metabolic enzymes	gi|15601456	5.05	8.06	111645	98859	193	27	56%	Y	0.32
85	chitodextrinase	metabolic enzymes	gi|15601456	5.05	7.32	111645	94582	205	28	70%	Y	0.26
88	chitodextrinase	metabolic enzymes	gi|15601456	5.05	6.40	111645	92004	209	28	65%	Y	0.21
90	chitodextrinase	metabolic enzymes	gi|15601456	5.05	5.20	111645	92514	236	30	55%	Y	0.11
98	chitodextrinase	metabolic enzymes	gi|15601456	5.05	6.04	111645	87538	199	27	39%	Y	0.07
99	chitodextrinase	metabolic enzymes	gi|15601456	5.05	7.48	111645	87055	73	16	44%	Y	0.07
54	flagellar hook protein FlgE	flagellum components	gi|15642196	4.71	4.20	46817	142391	90	12	45%	N	1.07

续表 2－1

spot NO.	Protein Name	protein function	NCBI Accession No.	PI (cal)	PI (gel)	Mr(cal) Da	Mr(gel) Da	Score MASCOT	Pep. Count	SC%	signal peptide	%vol
165	flagellar hook protein FlgE	flagellum components	gi|15642196	4.71	4.28	46817	55842	130	3	30%	N	0.23
205	flagellar hook protein FlgE	flagellum components	gi|15642196	4.71	4.30	46817	48989	136	16	55%	N	0.37
131	flagellar hook-associated protein FlgM	flagellum components	gi|15642190	5.93	6.11	68646	67760	238	26	65%	N	0.07
132	flagellar hook-associated protein FlgM	flagellum components	gi|15642190	5.93	6.04	68646	67501	177	22	33%	N	0.04
105	flagellin A	flagellum components	gi|15642187	4.85	5.33	40358	85623	121	14	44%	N	0.06
170	flagellin A	flagellum components	gi|15642187	4.85	5.87	40358	54637	91	12	55%	N	0.06
434	flagellin A	flagellum components	gi|15642187	4.85	4.80	40358	48326	95	12	50%	N	1.41
93	flagellin C	flagellum components	gi|15642186	4.93	5.47	39881	90490	145	16	40%	N	0.04
182	flagellin C	flagellum components	gi|15642186	4.93	5.10	39881	50896	105	13	29%	N	0.94
190	flagellin C	flagellum components	gi|15642186	4.93	7.66	39881	50896	82	11	30%	N	0.17
108	flagellin D	flagellum components	gi|15642142	4.89	7.73	39880	85151	105	12	33%	N	0.06
97	flagellin FlaB	flagellum components	gi|15642141	5.05	8.80	39493	87055	78	10	45%	N	0.05
209	flagellin FlaB	flagellum components	gi|15642141	5.05	8.29	39493	48194	135	15	65%	N	0.15
262	glyceraldehyde 3-phosphate dehydrogenase	metabolic enzymes	gi|15642002	5.8	6.07	35261	37645	179	17	60%	N	0.16
263	glyceraldehyde 3-phosphate dehydrogenase	metabolic enzymes	gi|15642002	5.8	6.54	35261	37789	86	10	55%	N	0.07
259	haemolysin	toxin	gi|32698438	5.52	6.17	54749	38226	124	16	45%	Y	0.06
325	haemolysin	toxin	gi|122893689	4.8	7.93	34506	25535	124	10	29%	Y	0.05
332	haemolysin	toxin	gi|150420310	5.31	7.78	82309	22696	114	5	9%	Y	0.20
345	haemolysin	toxin	gi|23321281	4.97	8.36	36436	19049	112	13	30%	Y	0.43
28	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.65	65850	165314	118	16	25%	Y	2.93
32	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.80	65850	166230	106	15	30%	Y	0.53
44	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.17	65850	164402	130	17	45%	Y	0.55
47	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.21	65850	163496	153	19	55%	Y	0.42
155	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.75	65850	57543	130	17	65%	Y	1.54
158	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.50	65850	57543	95	14	45%	Y	0.47
163	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.02	65850	55690	129	17	33%	Y	0.20
164	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.14	65850	55842	96	14	56%	Y	0.05
176	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	4.61	65850	53022	86	13	25%	Y	0.07
178	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.15	65850	51314	167	20	35%	Y	4.13
180	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.02	65850	50619	142	18	65%	Y	3.40
181	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	5.80	65850	50481	126	17	45%	Y	3.90
183	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.20	65850	51455	133	17	60%	Y	0.73
184	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.00	65850	51314	85	13	25%	Y	0.35
185	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.16	65850	51035	115	16	35%	Y	0.21
187	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.80	65850	51035	91	14	45%	Y	0.72
188	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.80	65850	50619	118	16	40%	Y	1.05
189	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.80	65850	50619	130	17	35%	Y	1.02
192	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	4.28	65850	50758	93	14	45%	Y	0.63
193	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	7.10	65850	50896	141	18	30%	Y	0.36
194	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	7.42	65850	50758	114	16	25%	Y	0.21

续表 2－2

spot NO.	Protein Name	protein function	NCBI Accession No.	PI (cal)	PI (gel)	Mr(cal) Da	Mr(gel) Da	Score MASCOT	Pep. Count	SC%	signal peptide	%vol
196	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	3.76	65850	50344	141	18	35%	Y	0.31
197	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	3.80	65850	50481	116	16	45%	Y	0.24
198	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	3.67	65850	50207	117	16	55%	Y	0.34
199	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	4.12	65850	50481	83	13	56%	Y	0.30
200	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.51	65850	50344	125	17	66%	Y	1.77
201	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.73	65850	50070	81	13	35%	Y	3.16
202	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	7.24	65850	50619	117	16	56%	Y	0.13
203	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	3.60	65850	49527	128	17	35%	Y	0.23
206	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.33	65850	48989	82	13	45%	Y	0.10
210	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.45	65850	47026	124	17	30%	Y	0.07
211	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.25	65850	46770	104	15	39%	Y	0.04
213	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.00	65850	46389	83	13	45%	Y	0.37
214	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.20	65850	46262	143	18	35%	Y	0.16
216	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.05	65850	46011	86	13	45%	Y	0.11
222	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.13	65850	45885	94	14	55%	Y	0.06
228	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	5.32	65850	44408	131	14	20%	Y	0.19
229	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.13	65850	43331	152	17	30%	Y	0.23
232	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.20	65850	42069	97	14	35%	Y	1.52
234	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.15	65850	42393	92	14	45%	Y	0.70
235	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	7.80	65850	42231	106	15	55%	Y	0.10
236	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.05	65850	41429	65	11	36%	Y	0.91
238	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.15	65850	42069	87	13	29%	Y	0.17
239	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	7.11	65850	41748	82	13	30%	Y	0.12
241	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.07	65850	41748	105	15	44%	Y	0.18
242	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.75	65850	41748	85	13	28%	Y	0.13
244	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.80	65850	41271	79	12	50%	Y	0.06
264	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.02	65850	37501	97	14	68%	Y	0.04
285	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.08	65850	34996	54	11	26%	Y	0.14
438	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.60	65850	162595	143	18	69%	Y	1.37
439	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	7.88	65850	165314	94	14	45%	Y	1.07
441	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	6.95	65850	50481	139	18	80%	Y	2.53
442	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.55	65850	53750	141	18	78%	Y	1.70
443	hemagglutinin/protease	degradative enzymes	gi|15601620	5.27	8.59	65850	50481	142	18	90%	Y	4.66
293	hypothetical protein VC1101	hypothetical protein	gi|15641114	5.54	8.80	33262	33261	141	14	75%	Y	0.05
137	hypothetical protein VC1485	hypothetical protein	gi|15641494	5.78	4.55	60531	63488	53	9	60%	Y	1.89
319	hypothetical protein VC1603	hypothetical protein	gi|15641611	5.23	5.60	29169	27475	86	10	67%	Y	0.08
128	hypothetical protein VCA0033	hypothetical protein	gi|15600804	5.41	8.80	68808	68807	161	20	56%	Y	0.06
129	hypothetical protein VCA0033	hypothetical protein	gi|15600804	5.41	6.49	68808	68544	161	20	66%	Y	0.11
278	hypothetical protein VCA0096	hypothetical protein	gi|15600867	5.7	6.40	44711	35115	171	21	26%	Y	1.01
349	hypothetical protein VCA0539	hypothetical protein	gi|15601298	6.29	6.06	20409	18511	114	11	78%	Y	0.13

续表 2 – 3

spot NO.	Protein Name	protein function	NCBI Accession No.	PI (cal)	PI (gel)	Mr(cal) Da	Mr(gel) Da	Score MASCOT	Pep. Count	SC%	signal peptide	% vol
273	immunogenic protein	hypothetical protein	gi|15640457	6.61	8.80	35233	35958	75	9	25%	Y	0.21
277	immunogenic protein	hypothetical protein	gi|15640457	6.61	6.86	35233	35354	103	12	54%	Y	0.31
281	immunogenic protein	hypothetical protein	gi|15640457	6.61	6.03	35233	34996	141	15	45%	Y	0.48
300	lactonizing lipase	degradative enzymes	gi|15600990	6.04	6.16	35984	32528	277	12	42%	N	0.38
303	lactonizing lipase	degradative enzymes	gi|15600990	6.04	6.07	35984	32424	92	11	70%	N	0.15
220	leucine aminopeptidase-related protein	degradative enzymes	gi|15601567	5.29	6.00	51972	46011	143	9	60%	Y	0.43
268	leucine aminopeptidase-related protein	degradative enzymes	gi|15601567	5.29	6.12	51972	35354	81	11	65%	Y	1.98
302	leucine aminopeptidase-related protein	degradative enzymes	gi|15601567	5.29	6.12	51972	32631	73	10	70%	Y	0.13
221	maltose ABC transporter, periplasmic maltose-binding protein	transporter	gi|15601698	4.89	4.32	43779	45885	96	13	35%	Y	0.14
142	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein	transporter	gi|15641104	6.02	8.80	61102	61101	120	16	65%	Y	0.21
143	oligopeptide ABC transporter, periplasmic oligopeptide-binding protein	transporter	gi|15641104	6.02	6.00	61102	60603	77	12	55%	Y	0.33
225	outer membrane protein, putative	outer membrane proteins	gi|15601761	4.68	4.16	35892	45018	121	16	43%	Y	0.18
133	peptide ABC transporter, periplasmic peptide-binding protein	transporter	gi|15640201	5.76	8.80	60576	66475	83	12	45%	Y	0.05
149	peptide ABC transporter, periplasmic peptide-binding protein	transporter	gi|15640201	5.76	6.88	60576	59457	94	13	65%	Y	0.13
150	peptide ABC transporter, periplasmic peptide-binding protein	transporter	gi|15640201	5.76	8.80	60576	59295	82	12	54%	Y	0.21
282	phosphate ABC transporter, periplasmic phosphate-binding protein	transporter	gi|15600841	8.76	7.96	31652	34760	132	14	65%	Y	0.86
287	phosphate ABC transporter, periplasmic phosphate-binding protein	transporter	gi|15600841	8.76	7.89	31652	34760	155	14	49%	Y	0.50
224	protease	degradative enzymes	gi|15600992	5.06	5.47	101819	45511	79	15	65%	Y	0.19
313	purine nucleoside phosphorylase	metabolic enzymes	gi|15600824	5.97	8.01	26723	29752	90	10	44%	N	0.07
253	spermidine/putrescine ABC transporter, periplasmic spermidine/putrescine-binding protein	transporter	gi|15641436	4.95	4.47	40756	39115	157	17	70%	Y	0.30
243	spindolin-related protein	metabolic enzymes	gi|15600910	5.39	4.80	44356	40333	118	16	29%	Y	1.46
435	spindolin-related protein	metabolic enzymes	gi|15600910	5.39	5.54	44356	48194	120	1	45%	Y	0.68
329	superoxide dismutase, Fe	metabolic enzymes	gi|15642047	5.24	8.20	21470	23282	80	8	29%	N	0.13
82	TagA-related protein	unknown	gi|15600918	5.4	7.04	149428	97233	83	17	45%	N	0.03
331	thiol: disulfide interchange protein	protein maintenance and folding	gi|2624890	6.1	8.80	20487	22552	104	10	65%	Y	0.13
258	thiosulfate ABC transporter, periplasmic thiosulfate-binding protein	protein maintenance and folding	gi|15640560	6.14	6.34	36898	38668	168	17	50%	Y	0.19
231	trypsin, putative	degradative enzymes	gi|15641213	6.3	8.80	43214	43213	77	10	45%	Y	0.19
291	uridine phosphorylase	metabolic enzymes	gi|15641047	5.71	7.77	27645	33261	87	9	44%	N	0.08
248	vgrG protein	unknown	gi|15600789	5.62	6.49	75657	40179	334	18	29%	N	0.29
249	vgrG protein	unknown	gi|153829283	5.78	5.95	65466	40333	94	11	25%	N	0.06
250	vgrG protein	unknown	gi|153829283	5.78	6.59	65466	40333	205	17	36%	N	0.22
255	vgrG protein	unknown	gi|153829283	5.78	6.07	65466	39115	96	14	30%	N	0.03

Numbers correspond to the protein spot numbers in Fig. 2. The figures corresponding to Mr(cal) and pI(cal) were calculated by the MASCOT software. The Mr(gel) and pI(gel) values were estimated from the spot position in the 2-DE gel. SC% means sequence coverage (percentage). It includes the results of the secretion signal search using the Signal P software. Y: Yes; N: No.

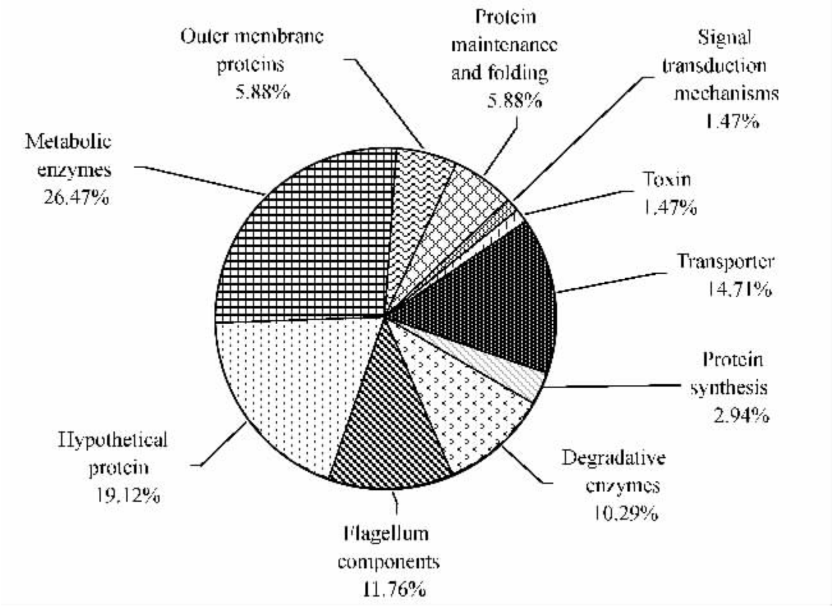

图 3 El tor 型霍乱弧菌培养上清蛋白功能分类

Fig.3 Proteins in the supernatant of El Tor *Vibrio cholerae* were divided into ten different subgroups according to their function.

10.29%,其中 6 个蛋白具有信号肽,主要参与蛋白水解。4 个具有信号肽的外膜蛋白占全部蛋白的 5.88%,这些外膜蛋白主要参与蛋白和其它大分子的转运。另四类蛋白包括分子伴侣蛋白,蛋白合成、信号传导蛋白和毒素分别占全部蛋白的 5.88%、2.94%、1.47%和 1.47%。

3 讨论

致病性细菌分泌性蛋白的研究工作无论在细菌致病性方面,还是对于将来药物和疫苗的研制都具有非常重要的意义。目前已经进行了幽门螺杆菌[5]、李斯特菌[6]和金黄色葡萄球菌[7]等细菌的分泌性蛋白质组研究,研究表明分泌性蛋白的研究对于鉴定未知毒力因子或寻找疫苗侯选抗原是非常有效的方法。目前尚未见有关于霍乱弧菌的分泌性蛋白组的研究报道。

分泌性蛋白的研究最大的困难是无法避免由于菌体自身裂解产生的菌体蛋白在其中的干扰。本研究在蛋白制备的过程中采用梯度低速离心,并将培养上清用滤器过滤(0.22 μm)。本研究在排除由于软件识别过程中将一些较大的蛋白点识别为多个点和由于上样量较大造成的脱尾现象等因素后,发现 2DE 胶上存在同一个蛋白的多点现象,表明存在蛋白转录后的修饰,蛋白的部分降解,电泳前蛋白的不完全变性等因素。这些都是以往研究中对蛋白数目、种类和功能分析的难点,由于本研究对所涉及的全部蛋白点进行了 MALDI-TOF 质谱分析,从根本上解决了对上清中各种蛋白的判断客观依据问题,保证了结果的客观可信性。

本研究共鉴定出 239 个蛋白点,对应 68 种蛋白,这些蛋白按功能归为 10 类,具有信号肽的蛋白占全部蛋白的 55.88%。代谢酶,蛋白折叠/伴侣,蛋白合成,信号传导蛋白占据了全部培养上清蛋白的 36.76%,除个别蛋白(如几丁质酶等)外,这些蛋白通常在细胞内执行各自的功能,如糖代谢,氧化还原反应,能量合成,并且也不具有信号肽,似乎这些蛋白不应该在培养上清中检出。它们在各自胶上的相对含量非常低,可能是少量菌体裂解产生,通过 MALDI-TOF 质谱分析获得的准确蛋白判断信息有助于将分泌性蛋白和非分泌性蛋白进行初步区分。

目前革兰氏阴性菌已经发现了至少有六种不同的分泌系统,霍乱弧菌可以通过 VI 型分泌系统分泌不具有信号肽的 vgrG 蛋白[8],该蛋白在本研究的非流行株 92-3 中也被检出。同样提示我们这些细胞内蛋白也存在被分泌到细胞外的可能。本研究在流行株与非流行株均鉴定出几丁质酶和几丁质解聚酶,它可以帮助霍乱弧菌离开水中的壳质颗粒[9],正是这种酶类的存在得以使霍乱在水中长期生存。

虽然软件预测外膜蛋白和外周质结合蛋白均具有信号肽,但它们通常定位在外膜上和与外膜结合的外周质间隙,而不是细胞外。在对其他细菌分泌蛋白的研究中也发现了这些蛋白,认为可能是蛋白脱离了外膜,没有嵌合到外膜或从外周质渗漏[10]。这类蛋白是否在释放后发挥重要的功能尚需探讨。

除了霍乱毒素，霍乱弧菌的运动能力已经被认为是一种重要的毒力特性。许多研究表明不具有运动能力、但具有肠毒性的霍乱突变体毒力被减弱了[11－12]。在沙门氏菌已经证明这些鞭毛蛋白在进行组装鞭毛之前，首先通过Ⅲ型分泌系统分泌到菌体外[13]。Nidia[14]等人研究发现，在霍乱弧菌中，FlgM 蛋白的功能与沙门氏菌相似，而且带有鞘的鞭毛也可以分泌组装鞭毛的非结构蛋白。鞭毛蛋白 FlaC，FlaD 及鞘蛋白 OmpU 均可在霍乱弧菌的培养上清中检出，这些鞭毛蛋白可以诱导肠上皮细胞 IL-8 的分泌，与霍乱疫苗的反应原性有关[15]。本研究在 2 株菌共发现 8 种鞭毛蛋白，其中 5 种在两类菌株均表达，包括鞭毛丝状体的 4 种蛋白 FlaA，FlaB，FlaC，FlaD，钩状体蛋白 FlgE 及相关蛋白 FlgM，FlgL，和基础小体杆蛋白 FlgG。证明这些蛋白虽然不具有信号肽，但却可以分泌到菌体外。

本研究发现的降解酶有 6 个具有信号肽，这些蛋白基本已经明确可以分泌到细胞外。其中红血球凝集素/蛋白酶（hemagglutinin/protease，HAP）仅在非流行株中大量表达，其在胶上含量占所有蛋白含量的 46.49%。红血球凝集素/蛋白酶（HAP）可以帮助霍乱弧菌离开宿主的上皮细胞[16]。在霍乱弧菌的生长过程中，完整的 CTXϕ 溶原性细菌可以产生感染颗粒，然而到了稳定生长期后期，CTXφ 溶原性细菌可以被霍乱弧菌分泌的大量 HAP 抑制，表明 HAP 可以阻止 CTXφ 对环境和宿主的再感染[17]。本研究在非流行株中发现大量表达的 HAP，而流行株中却未发现，这可能是一个很有价值的现象，这些 HAP 蛋白对于非流行株的意义是什么，在两类菌株间有无鉴别诊断价值，值得关注。

溶血素是本研究发现的唯一一个毒素，并且流行株的表达量远远高于非流行株。溶血素是一种孔形成毒素，可以引起细胞离子的渗漏，最终导致细胞裂解。在兔肠结扎试验中，溶血素可以导致液体的堆积，提示我们这种蛋白有助于霍乱的肠毒性作用[18]。这种蛋白在流行株的高表达分泌是否与两类菌株的致病特点有关，有待深入研究。

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Analysis of exoproteins of El Tor *Vibrio cholerae* by 2DE and MALDI-TOF-MS/MS

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Abstract: [**Objective**] Our aim is to analyze the exoproteins of the epidemic strain and nonepidemic strain of El Tor *Vibrio cholerae*. [**Methods**] We separated the exoproteins of two El Tor strains, one epidemic strain N16961 and one nonepidemic strain 92-3, by two dimension electrophoresis. All protein spots were identified by MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) mass spectrometry and database referencing. [**Results**] We identified 49 exoproteins from 206 protein spots in epidemic strain and 42 exoproteins from 236 protein spots in nonepidemic strain. Sixty-eight exoproteins were totally identified in two strains, and the proteins with signal peptides were about 55.88% in total. We classified the exoproteins into 10 subgroups according to their functions. Among them were metabolic enzymes, protein maintenance and folding, protein synthesis and signal transduction proteins, which accounted for 36.76% of all the proteins identified. We identified 11 hypothetical proteins and 2 unknown function proteins with signal peptides at the first time, accounting for 19.12%. Transporter, flagellum components, degradation enzymes, outer membrane proteins and toxin accounted for 14.71%, 11.76%, 10.29%, 5.88%, 1.47% separately. [**Conclusion**] We obtained the exoprotein profiles of epidemic strain and nonepidemic strain of El Tor *Vibrio cholerae*. The reasons of why the flagellum components without signal peptides that are associated closely with virulence could be released out of the bacteria, and why with high hemagglutinin/protease secretion in nonepidemic strains should be analyzed in the future.

Keywords: *Vibrio cholerae*; proteomics; Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF)

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