

艾丁嗜盐小盒菌 B2 菌株 16S rRNA 核苷酸序列*

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摘 要 艾丁嗜盐小盒菌 B2 菌株 (*Haloarcula aidinensis*, strain B2) 16S rRNA 的核苷酸序列已以双脱氧核苷酸链终止法确定。该菌 16S rRNA 显示出了典型的古生物类 (Archaea) 特性。虽然艾丁嗜盐小盒菌 B2 菌株在序列方面更接近细菌类 (Bacteria) 的 16S rRNA, 但它的序列也显示出与真核生物类 (Eucarya) 的某些特殊的相似性。在序列和结构方面, 该菌与细菌类或真核生物类之间的相似程度要高于细菌类与真核生物类之间的相似程度。另外, 该菌 16S rRNA 的序列与其它嗜盐菌序列相比较支持了以前的结论, 即艾丁嗜盐小盒菌 B2 菌株应属于嗜盐小盒菌属 (*Haloarcula*) 的一新种。

关键词 16S rRNA, 古生物类 (Archaea), 艾丁嗜盐小盒菌 B2 菌株

随着分子生物学的迅速发展和广泛应用, 生物系统分类的基础也发生了重大变化^[1]。经典的以表型标准为主的分类系统已经或正在随着分子标准的不断渗入而完善, 旧的非系统发育体系已开始转向新的以系统发育为主的体系^[2]。早期的系统发育体系主要是建立在分子杂交或寡核苷酸比较的基础上, 但在某些方面如细节和准确度上都是不够的^[2]。现在由于核酸测序方法的改善和其它新技术 (PCR 方法) 的应用, 使得人们用完整的而不是部分的 rRNA 序列来判断生物之间的系统发育关系。

本文报道了艾丁嗜盐小盒菌 B2 菌株 16S rRNA 的核苷酸序列, 比较了它与嗜盐菌各属中其它种的 16S rRNA 序列的相互关系, 并提出了其二级结构的模型, 为其分类地位^[3]进一步提供了有力证据。

1 材料与方法

1.1 菌种

(*Haloarcula aidinensis* strain B2, Ha. B2) (简称 B2) 是从我国新疆盐湖分离的, 引物为:

1. Prime I 包含 EcoR I 切点
5'-GAATTC-3'
3'-CTTAAG-5'
5' GGAATTCATTCCGGTTGATCCTGCCGGA 3'
2. Prime II 包含 Cla I 切点
5'-ATCGAT-3'
3'-TAGCTA-5'

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5' AATCGATAGGAGGTGATCCAGCCGCAG 3'

并经本实验室纯化、鉴定^[3]。

1.2 克隆和亚克隆^[4]产物^[5]

Ha. B2 16S rDNA 的 PCR 先经限制性内切酶 EcoR I 和 Cla I 酶切, 并克隆到经 EcoR I 的 Acc I 酶切过的 pUC19 和 pUC18 载体上, 分别构成 pUC19 和 pUC18 重组体; 然后利用 PCR 产物 (分子量为 1.5kb 左右) 中间位置的 SmaI 酶切位点, 用限制性内切酶 EcoR I 和 SmaI 酶切 pUC19, 用限制性内切酶 SmaI 和 Pst I 酶切 pUC18, 分离出大片段 DNA, 补平后, 经自身酶连构成亚克隆 B-PUC-19-sub 和 B-PUC-18-sub, 最后这四个重组体用双脱氧核苷酸链终止法 (Dideoxynucleotide chain termination method) 进行测序。

1.3 二级结构模型^[6]

使用比较分析法确定二级结构^[7]。首先假设 Ha. B2 的 16S rRNA 的结构类似于 *Haloferax volcanii* 16S rRNA 的结构^[8], 并参照 *Halobacterium cutirubrum*^[9] 和 *Halococcus morhuae* 的 16S rRNA 的二级结构, 然后根据测得的序列数据, 经若干修改后, 再构成该菌的 16S rRNA 二级结构。

1.4 16S rRNA 序列的分类比较

建立在严格相似性的基础上^[8,11], 序列中核苷酸的位置数目是根据 *E. coli* 16S rRNA 的核苷酸位数确定的。

2 结果和讨论

Ha. B2 的 16S rRNA 的核苷酸序列是从基固序列推导出来的。这一结果分别与同属的其它两株菌 *Haloarcula vallismortis* 和 *Haloarcula marismortui* 16S rRNA 的序列以及真核生物的代表 *Xenopus laevis* 和细菌类的代表 *E. coli* 的 16S rRNA 序列进行了同源性比较, 如图 1, 2 所示。核苷酸序列的排列方式分别参照其他人的报道^[8,9]。比较结果 (表 1, 2, 3) 说明, 该菌的 16S rRNA 初级结构虽总的看来类似于细菌, 但在某些方面又表现出明显的真核生物特性; 另外, 序列分析还表明, Archaea 的 16S rRNA 与 Bacteria 或 18S rRNA 16S rRNA 之间的相似性比 Bacteria 与 Eucarya 之间的相似性更强。Ha. B2 的 16S rRNA 序列由 1473 个核苷酸组成, 比 *E. coli* 的要短; 当两者按最适排列时^[7], 其百分率为 58.7%, 这与其它嗜盐菌和 *E. coli* 16S rRNA 序列之间的 59% 左右的相似率是一致的。而 *E. coli* 和 *Zea mays* 叶绿体 16S rRNA 序列^[12] 之间 75% 的相似率相差甚远 (这两类 rRNA 代表了 Bacteria 类的两个系统发育极端^[13]。这足以说明, Archaea (包括 Ha. B2) 既不同于 Bacteria, 也不同于 Eucarya, 而应归于另一类生物群。从表 3 中还可看出, Ha. B2 与嗜盐菌其它属中不同菌的 16S rRNA 序列之间的相似性以及各属之间的相似性都均为 88% 左右, 这表明了 Ha. B2 与其它菌之间属的差异; 这与极性脂的分类是一致的^[3]。图 1 的比较结果说明了 Ha. B2 与该属内其它两株菌的 16S rRNA 序列比较; 三者之间的差异也是比较明显的, 这表明 Ha. B2 应列为一新种。虽然一级结构表明了这类 Archaea (Ha. B2) 与 Bacteria (*E. coli*) 在很多方面的相似性, 但在序列的某些方面, Archaea 与 Eucarya 表现出了更大的相似性, 比如 (1) 所有 Eucarya 和 Archaea 的 16S rRNA 序列

<i>Haloarcula</i> B2:	ATUCCGGTUG	AUCCUGCCGG	AGGCCAATGC	UAUCGGAGUC	CGAATTAGCC	AUGCUAGTUG	CACGAGTULA	GACUCGUAGC	AUAUAGCUC	GUAAACAGGUG
<i>Ha. mari.</i> :	ATUCCGGTUG	AUCCUGCCGG	AGGCCAATGC	UAUCGGAGUC	CGAATTAGCC	AUMCTAGTUG	CACGNCY TUA	GAATUCGUNG	AUAUAGCUC	GUAAACAGGUG
<i>Ha. ralis.</i> :	ATUCCGGTUG	AUCCUGCCGG	AGGCCAATGC	UAUCGGAGUC	CGAATTAGCC	ATNYCAGTUG	CACGNGY TUA	GACUHGUNG	ATUAUAGCUC	GUAAACAGGUG
<i>Haloarcula</i> B2:	GCCAAACUAC	CCUACAGACC	GCGAUAACCU	CGGGAACACUG	AGGCCAAUAG	CGGAUFAUAC	UUCUAUGCUG	GAGUGCCAGAG	AGUAGAAGAC	GUUCCGGCCGC
<i>Ha. mari.</i> :	GCCAAACUAC	CCUACAGACC	GCGAUAACCU	CGGGAACACUG	AGGCCAAUAG	CGGAUFAUAC	UUCUAKGYUG	GAGUGCCAGAG	AGUKAGAAAC	GUUCCGGCCGC
<i>Ha. ralis.</i> :	GCCAAACUAC	CCUACAGACC	GCGAUAACCU	CGGGAACACUG	AGGCCAAUAG	CGGAUFAUAC	UUCUACGGCUG	GAGUGCCAGAG	AGUGAGAAAC	GUUCCGGCCGC
<i>Haloarcula</i> B2:	UGUAGGAUGU	GGCUCCGGCC	GAUAGGUAAG	AUGGUGGGGU	AACGGCCCCAC	CAUCCCGGUA	AUCCGATACAG	GUUGUGAGAG	CAAGAGCCUG	GAGACGGUAU
<i>Ha. mari.</i> :	UGUAGGAUGU	GGCUCCGGCC	GAUAGGUAAG	AUGGUGGGGU	AACGGCCCCAC	CAUCCCGGUA	AUCCGATACAG	GUUGUGAGAG	CAAGAGCCUG	GAGACGGUAU
<i>Ha. ralis.</i> :	UGUAGGAUGU	GGCUCCGGCC	GAUAGGUAAG	AUGGUGGGGU	AACGGCCCCAC	CAUCCCGGUA	AUCCGATACAG	GUUGUGAGAG	CAAGAGCCUG	GAGACGGUAU
<i>Haloarcula</i> B2:	CUGAGACAAG	AUACCGGGGCC	CUACGGGGGGC	CAGCAGGGGC	GAAACCUUUA	CACUCCACGA	CAGUCCGUAU	CGGGGACUCC	GAGUGUGAGG	GCATUAUAGCC
<i>Ha. mari.</i> :	CUGAGGNNNN	nnnnnnnnnn	nnnnnnnnnn	CAGCAGGGGC	GAAACCUUUA	CACUCCACGA	CAGUCCGUAU	CGGGGACUCC	GAGUGUGAGG	GCATUAUAGCC
<i>Ha. ralis.</i> :	CUGAGACAAG	AUACCGGGGCC	CUACGGGGGGC	CAGCAGGGGC	GAAACCUUUA	CACUCCACGA	CAGUCCGUAU	CGGGGACUCC	GAGUGUGAGG	GCATUAUAGCC
<i>Haloarcula</i> B2:	CUCGCTUUTUC	UGUACCGUAA	GGUGUACAG	GAACAAGGAC	UGGGCAAGAC	CGGUGCCAGC	CGCCGCCGUA	AUACCGGGCAG	UCCAAGUGAU	GCCTCGAUATU
<i>Ha. mari.</i> :	CUCGCTUUTUC	UGUACCGUAA	GGUGUACAG	GAACAAGGAC	UGGGCAAGAC	CGG				
<i>Ha. ralis.</i> :	CUCGCTUUTUC	UGUACCGUAA	GGUGUACAG	GAACAAGGAC	UGGGCAAGAC	CGG				
<i>Haloarcula</i> B2:	AUUUGGGCCUA	AAGCGUCCGU	AGCUUGCUGU	GUAGUCCAU	UGGGAUAUCC	ACCAGCUCAA	CUGGUGCCGC	UCCGGUGGAA	ACUACACAGC	UUGGGGCCGA
<i>Ha. mari.</i> :				AGUACA	UGGGAUAUCC	AYCAGCUCAA	CUGGUGCCGC	UCCGGUGGAA	ACUACAMINN	UUGGGGCCGC
<i>Ha. ralis.</i> :				AAGUACA	UGGGAUAUCC	NYAAGCUCAA	CUGGUGCCGC	UCCGGUGGAA	ACCAMNUNNC	UUGGGGCCGC
<i>Haloarcula</i> B2:	GAGGCUCAAC	GGGUACGUCC	GGGUAGGAG	UGAAAUCCUG	UAAUCCUGGA	CGGACCACCA	AUGGGGAUAC	CACGUGACA	GACCGGACCC	GACAGUGACG
<i>Ha. mari.</i> :	GAGACUUUAG	GGGUACGUCC	GGGUAGGAG	UGAAAUCCUG	UAAUCCUGGA	CGGACCACCA	AUGGGGAUAC	CACGUTAGCN	NGANGGACCC	GACNUGACG
<i>Ha. ralis.</i> :	NACACUYNAS	GGGUACGUCC	GGGUAGGAG	UGAAAUCCUG	UAAUCCUGGA	CGGACCACCA	AUGGGGAUAC	CACGUTAGCA	GACCGGACCC	GACXGUGACG
<i>Haloarcula</i> B2:	GACGAAGACC	AGGGUCUCGA	ACCGGAUUAG	AUACCCGGGU	AGUCCUUGGU	GUAAACAUAUG	CUCGCUAGGU	AUGUCACGGC	CCAU GAGCA	CGUUGUGUGC
<i>Ha. mari.</i> :	GACGAAGACU	AGGGUCUCGA	ACCGGAUUAG	AUACCCGGGU	AGUCCUAGGU	GUAAACAUAUG	CUCGCUAGGU	GUNNNNNN G	CUAUCGAGCC	CGUUCUNYGC
<i>Ha. ralis.</i> :	GACGAAGACU	AGGGUCUCGA	ACCGGAUUAG	AUACCCGGGU	AGUCCUAGGU	GUAAACAUAUG	CUCGCUAGGU	GUNNNNNN G	CUAUCGAGCC	CGUUCUNYGC
<i>Haloarcula</i> B2:	CGUAGUGAAG	ACGAUAAGCG	AGCCGCCUUG	GAAGUACGUC	CGCAAGGAUG	AAACUUAAG	GAUUGGGCG	GGGAGCACCA	CAACCGGAGG	AGCCUGCGGU
<i>Ha. mari.</i> :	CCUAGKGAAG	CCGAKAAGCG	AGCCGCCUUG	GAAGUACGUC	UGCAAGGA					
<i>Ha. ralis.</i> :	CCUAGCGAAG	CCGAGAAGCG	AGCCGCCUUG	GAAGUACGUC	U				ACCGGAGG	AGNCUGCGGU

(图 1 Fig. 1)

<i>Haloarecula</i> B2:	UUAUUUGGAC	UCAACGCCCG	ACAUCUCACC	GGUCCCGACA	GUAGUAUGA	CGUCAGGUU	GACGACUUUA	CCCGACGGUA	CUGAGGGGAG	GUGCAUGGCC
<i>Ha. mari.</i> :		AACGCCCG	ACAUCUCACC	GGUCCCGACC	GUAGUAUGA	CAGUCAGGUU	GACGACUUUA	CUCGACGGUA	CUGAGAGGAG	GUGCAUGGCC
<i>Ha. valis.</i> :	UUAUUUGnnn	nnAACGCCCG	ACAUCUCACC	GGUCCCGACC	GUAGUAUGA	CNGUCAGGUU	GACGACUUUA	CUCGACGGUA	CYGAGAGGAG	GUGCAUGGCC
<i>Haloarecula</i> B2:	GCCGUCAGCU	CGUACCGUGA	GGCGUCCUGU	UAAGUCAGGC	AACGAGCGAG	ACCCGCACUU	CUAGUUGCCA	GCAAUACCCC	UUGA...GGU	AGUUGGGUAC
<i>Ha. mari.</i> :	GCCGUCAGCU	CGUACCGUGA	GGCGUCCUGn	nnnnnnnnnn	nnnngAGCGAG	ACCCGCACUU	CUAGUUGCCA	GCAAACACCCC	U...GAn...GGUn	GGUUGGGUAC
<i>Ha. valis.</i> :	GCCGUCAGCU	CGUACCGUGA	GGCGUCCUGU	UAAGUCAGGC	AACGAGCGAG	ACCCGCACUU	CUAGUUGCCA	GCAAACACCCC	UUGAnGGGU	XGUUGGGUAC
<i>Haloarecula</i> B2:	ACUAGGAGGA	CUGCCCGUGC	UAAAGCGGAG	GAAGGAACGG	GCAAACGGUAG	GUCAGUAUCC	CCCGAAUGGA	CCGGGCAACA	CCGGGGCUAC	AAUGGCUAUG
<i>Ha. mari.</i> :	ACUAGGAGGA	CUGCnnnnUGC	UAAAUUGGAG	GAAGGAUUG	GCAAACGGUAG	GUCAGUAUCC	CCCGAAUGGA	CCGGGCAACA	CCGGGGCUAC	AAUGGCUAUG
<i>Ha. valis.</i> :	ACUAGGAGGA	CUGCCAUUGC	UAAAUUGGAG	GAAGGAUUG	GCAAACGGUAG	GUCAGUAUCC	CCCGAAUGGA	CCGGGCAACA	CCGGGGCUAC	AAUGGCUAUG
<i>Haloarecula</i> B2:	ACAGUGGGAU	GCAACGCCGA	GAGCGCGAGC	CUAAUCUCCA	AACGUAGUCC	UAGUCCGGAU	UCCGGGCUGA	AACCCGCCCG	CAUGAAGCUU	GAUUCGGUAG
<i>Ha. mari.</i> :	ACAGUGCGAU	GCAAACGCCGA	NAGGCGGA...G	CUAAUCUCCA	AACGUAGUCC	UAGUCCGGAU	UCCGGGCUGA	AACCCGCCCG	CAUGAAGCUU	GAUUCGGUAG
<i>Ha. valis.</i> :	ACAGUGGGAU	GCAACGCCGA	GAGGCGGA...G	CUAAUCUCCA	AACGUAGUCC	UAGUCCGGAU	UCCGGGCUGA	AACCCGCCCG	CAUGAAGCUU	GAUUCGGUAG
<i>Haloarecula</i> B2:	UAAUCGCGUG	UCAGAAGCGC	CGGUGAUUA	CGUCCCGUCU	CCUUGCACAC	ACCGCCCGUC	AAAGCACCCG	AGUGGGGUCC	GGAGAGGCC	GUGAUGCGAC
<i>Ha. mari.</i> :	UAAUCGCGUG	UCAGAAGCGC	CGGUGAUUA	CGUCCCGUCU	CCUUGCACAC	ACCGCCCGUC	AAAGCACCCG	AGUGGGGUCC	GGAGAGGCC	GUGAUGCGAC
<i>Ha. valis.</i> :	UAAUCGCGUG	UCAGAAGCGC	CGGUGAUUA	CGUCCCGUCU	CCUUGCACAC	ACCGCCCGUC	AAAGCACCCG	AGUGGGGUCC	GGAGAGGCC	GUGAUGCGAC
<i>Haloarecula</i> B2:	GGUCCGAUUCU	GGUCCCGCAA	GGGGGCUUAA	GUGGUAACAA	GGUAGCCGUA	GAGGAUUCUG	CGGCUUGAUC	ACCUUCU		
<i>Ha. mari.</i> :	GGUCCGAUUCU	GGCnCGCnn	GGGGGCUUnn	nnnnnnnnnn	nnnnnnnnnn					
<i>Ha. valis.</i> :	GGUCCGAUUCU	ngCUCCGCAA	GGGGGCUnnn	nnnnnnnnnn	nnnnnnnnnn					

(续图 1 Continued Fig. 1)

图 1 嗜盐小合菌属三个成员: *Ha. B2*, *Ha. marismortui* 和 *Ha. vallismortis* 16S rRNAs 的比较

Fig. 1 Comparison of 16S rRNA sequences from three members of Haloarcula: *Ha. aidinensis* strain B2, *Ha. marismortui*, and *Ha. vallismortis* n, unknown base; y, purimi-dine; x, purine.

<i>E. coli</i>	AAUUGAAGA	GUUGAUAU	GGCUCAGAU	GAACGCUGCC	GGCA-GG-CCUAACACAUCCAA	GUC-GAACGGU	AACAGGAAGA	AGCUTUGCTUC	UUUGCUGACG	100
<i>Ha. B2</i>	-----AUUCC	GGUGAUCCU	GCCGGAGCC	--AUUGCUAUC	GGAGUCCGATUAGCCAUCCUA	GUU-GCACGAG	UUUA-----	-----GACU	-----GACU	
<i>X. laevis</i>	-----UACCU	GGUGAUCCU	GCCAGUAGCA	--UAUGCTUGU	CUCA--AAGAUUAAGCCAUCCAC	GUG*CCACGGC	CGU-----	-----ACAGUGA	-----ACAGUGA	
<i>E. coli</i>	AGUCGGGAC	GGUGAGUAA	U-GUCUGGAA	--ACUGCCUGAU	GGAGGGGGAU	AACUACUGGA	AUACCGCAU	AACGUCGCA	GACCAAGAG	200
<i>Ha. B2</i>	CGUAGCAUAU	AGCUCAGUAA	CACGUGGCAA	--ACUACCCUAC	AGACCGCGAU	AACUCGGGA	AUACCGGAU	AUAACTUCUA	UGCUGGAGUG	
<i>X. laevis</i>	AACUGCGAAU	GGCUCAUUAA	AUCAGUUAUGG	UUCCUUGAUC	GC*CUUGAU	AACUGUGUA	AUUCUAGAGCU	GACGAGCGCU	GACCCCCAGG	
<i>E. coli</i>	G-----GGGACCUUGCGGCUUGCCAUUGGGAUG			--UGCCCAGAU-G	GGAUUAGCUA	-----GUAGGUGGGUAACGGCUCA	CCUAGCGGAC	GAUCCCUA-GC	UGGUCUGAGA	300
<i>Ha. B2</i>	CAGAGAUUAGAAACGUCGGCGCUGUAGGAUG			--UGG-CUGCGG	CGAUUAGGUA	---GAUGGUGGGUAACGGCCCA	CCAUCCGGAU	AAUCCGUA-CA	GGUUGUGAGA	
<i>X. laevis</i>	GAUGCGUCCA*	GACGGC	ACGAUACAUI	CGGAUGUUG-C	CCUAUCAU	UUCGAUGGUACUUCUUGCGCCUA	CCAUUGGAG	CACGGGUAAACG	GGGAUUCAGG	
<i>E. coli</i>	GGAUGACCAG	CCACACUUGA	ACUGAGACAC	GGUCCAGACU	CCUACGGGAG	GCACGAGUGG	ACAALUGGCC	CAAGCCUGAU	GCAGCCAUCC	400
<i>Ha. B2</i>	GCAAGAGCCU	GGAGACGGUA	UCUGAGACAA	GAUACCGGC	CCUACGGGGC	GCACGAGGGC	ACAACCCUUI	ACAGUCGGAU	AGGGGACUC	
<i>X. laevis</i>	GUUCGAUUC	GGAGAGGGAG	CCUGAGAAAC	GGCUACCA	UCCAAGGAAG	GCACGAGGGC	CGCAAAUUAAC	C-CGCGGGAG	GUAGUGACGA	
<i>E. coli</i>	CGCGUGUAUG	AAGAAGGCCU	UCGGGUUGUA	AAGUACUUC	AGCGGGGAGG	AAGGAGUAA	AGUUAUUAAC	UUGUCUCUAU	GCAGAAAGAG	500
<i>Ha. B2</i>	CGAGUGUGAG	GGCAUAUA----	-----GC	CUUCGCGUUI	CUUAACCGU--	AAGUG-----	-----GUA	-----AAC	CAGGAACAAG	
<i>X. laevis</i>	*								GAGGAUCUAU	
<i>E. coli</i>	CACC-GGCCUA	CUCGUGCCA	GCAGCCCGG	UAUAACGGAG	GGUGCAAAGC	UUUAUCCGAA	UUACUGGGG	GCAGCGGUTU	UGUUAAGUCA	600
<i>Ha. B2</i>	GACUGGGCAAG	ACCGUGCCA	GCCGCGCGG	UAUAACCGG	AGUCCAAGUG	AUGGCCGUA	UUUAUUGGGC	GUAGCUTUGCU	GUGUAAGUCC	
<i>X. laevis</i>	UGGAGGGCAAG	UCUGGUGCCA	GCAGCCCGC	UAUUCGAGC	UCCAUAAGCG	UAUAUUAAG	UUGCUGCAGU	UAAAAAGCUC	GUAGUUGGAU*	
<i>E. coli</i>	GAUGUGAAU	CCCGGGGCU	AACUUGGAA	C-UGCAUUGA	UACUUGCAAG	CUUGAGUUC	GUA-GAGGGG	AGGUUAGCG	GUGAAAUCCG	700
<i>Ha. B2</i>	AUUGGGAUU	CGACCAGCUC	AACUGGUCG	CGUCCGGUGGA	AACUACACAC	CUUGGGGCG	AGA-GACUCA	CGGUUACGUC	GUGAAAUCCU	
<i>X. laevis</i>					GAU	UAAAGAGGAC	GGCGGGGGCA	UUCGUUUGU	GUGAAAUUCU	
<i>E. coli</i>	UAGAGAUUCG	GAGCAUUAAC	GGUGGCGAAG	GCGGCCCCU	GGACGAAGAC	UGACGCUACAG	GUGCGAAAGC	AACAGAUUA	GAUACCCUGC	800
<i>Ha. B2</i>	GUAAUCCUG	ACGACACACC	AAUGGGA	CCACGUUGAC	AGACCGGACC	CGACAGUGAG	GGACGAAAGC	AACCGAUUA	GAUACCCGGG	
<i>X. laevis</i>	UGACCCGGC	CAAGACGAAC	CAAAAGGAA	GCAUUGCCA	AGAAUGUUU	CAUUAUUA	GAACGAAAGU	AAGACGAUCA	GAUACCCUGC	
<i>E. coli</i>	UAGUCCACGC	CGUAAACGAU	GUCGACUUG	AGGUUGGCC	CUUGAGGGGU	GG-----CUUCCCGAGCUAACCGGUUAAGUCGACC		GCUCGGGGAG	UACGGGCCGA	900
<i>Ha. B2</i>	UAGUCCUGGC	UGUAAACAUA	GCUCGUAAG	UAUGUCACGC	GCCAUUGAGCA	CGUUGUGUCCGUAAGUAGAGCAAGCAUAGCGAGCC		GCUCGGGAAC	UACUCCGCA	
<i>X. laevis</i>	UAGUCCGAC	CAUAAACGAU	GCCGACUAGC	*				CCGGGGGAC	UAUGGUGCA	

(图 2 Fig. 2)

<i>E. coli</i>	AGGUUAAAAC	UCAAUUGAAU	UGACGGGGG	CCGC-ACAAGC	GGUGGAGCAU	GUGGUUUAU	UCGAGUCAC	GCGAAGAACC	UUACCUUGUC	UUAGACAUCA	1000
<i>Ha. B2</i>	AGGAUGAAAC	UUAAAGGAU	UGCGGGGGA	GCACCACAACC	GGAGGAGCU	GCGGUUUAU	UGGACUUAAC	GCGGACAUUC	UCACCGUCC	-CGA---CAGU	
<i>X. laevis</i>	AACGUGAAAC	UUAAAGGAU	UGACGGAGG	GCACCACAGG	AGUGCAGCU	GCGGCUUAU	ULGACUUAAC	ACGGAAACC	UCACCGGCC	CGGA-----CAC	
<i>E. coli</i>	CGGAAGUUUU	CAGAGUAG	AUUGUGCUU	CGGAACCGU	GAGACAGGUG	CUGCAGGCU	GUCLUCAGCU	CGUUGUGUA	AUUGUUGGU	UAAUCCCGC	1100
<i>Ha. B2</i>	AGUUAUGAG	GUCAGGUGA	CGACUUA-C	CGACGGUAC	-UGAGGGAG	GUGCAGGCG	GCGUACAGCU	CGUACCGUGA	GGGUGCCUGU	UAAGUCAGGC	
<i>X. laevis</i>	GGAAAGGAU	GACAGAUUA	UAGCUCUUC	UCGALUCU	-GGGUGUG	GUGCAGGCG	GUUCUUAU	GGUGGAGCGA	UUUGUCUGGU	UAAUCCCGAU	
<i>E. coli</i>	AAAGAGCGCA	ACCUUAUCC	UUUGUUGCA	GGGUCC---GGC	-----	CGGAACUCAAAGG	AGACUGCCAGUG	AUAAUCUGGAGG	AAAGUGGGGUAU	ACGUCAAGUC	1200
<i>Ha. B2</i>	AACGAGCGAG	ACCGGACU	-CUAGUUGCA	GCAUAACCCU	GAGGUAGUUGG	UACACUAG	AGGAGACUCCG	CUUAAAGCGGAGG	AAACCGGCA	ACGGUAGGUC	
<i>X. laevis</i>	AACGAAACGAG	ACUCCUCCAU	GCUAAACUAGU	ACCGGAC---CCC	CGG+GGUCCAAU	CUUUAAGAGG	ACAGUGGCUU	UACGCCACACG	AGAU---CGAGCA	AUAACAGGUC	
<i>E. coli</i>	-AUCAUGGCC	-UUACGACCAG	GGCUACACAC	GUGCGACAU	GGCGCAUACA	AAGAGAAGCG	ACCUCCGAG	A-GCAAGCGGA	-CCUACAUAAG	UGCGUCCUAG	1300
<i>Ha. B2</i>	-AGUAUGCCCC	-GAUUGACCG	GGCAACACGC	GGCUACAAU	GGCUGAGACA	GUGGGAUGCA	ACGCCGAGAG	GCGGACGCUAA	-UCUCC--AAAC	GUAGUGGUAU	
<i>X. laevis</i>	UGUGAUGCCU	UAGAUUGCCG	GGCUGACGC	GGCUACACU	GAACGGAUCA	CGGUGUGUCU	ACCCUGCGCC	U-GCGGG-UAA	CCCGCUGAAC	CCGUUGGUA	
<i>E. coli</i>	UCCGGAUUGG	AGUCUGCAAC	UCGACUCCAU	GAAGUCGGAA	UCGGUAGUA	UCGUGGAUCA	GAUUGCCACG	GUAAUACGU	UCCCGGGGCCU	UGUACACACC	1400
<i>Ha. B2</i>	UUCGGAUUUC	GGCGUGAAAC	CCGCCGCCAU	GAAGCUGGAU	UCGGUAGUA	UCGCGUGUCA	GAAGCGCGCG	GUGAAUACGU	CCCUGCUCCU	UGCACACACC	
<i>X. laevis</i>	UAGGGAUCGG	GGAUUGCAAU	UAUUCUCCAU	GAAGCUGGAU	UCGGUAGUA	GUGCGGGUCA	UAAGCUCGCG	UUGAUUAAGU	CCCGGCCCUU	UGUACACACC	
<i>E. coli</i>	GCCCGUCACA	CCAUGGGAGU	-GGGUUGCAA	AGAAGUAGGU	AGCUUAACCU	UCGGGAACCU	GUUUAACCAU	UUGUGAUUA	UGACUGGGGU	GAAGUGCUAA	1500
<i>Ha. B2</i>	GCCCGUCAAA	GCACCCGAGU	-GGGUUCCGGA	UGA-GGCCGU	C-----	AUGC	GACGGUCGAA	UCUGGCUCCG	CAAGGGGCU	UAAGUGCUAA	
<i>X. laevis</i>	GCCCGUCGU	ACUACCGAU	GGAUGGUUAG	*	-----	-----	ACGAUCAAAC	UUGACUAUCU	AGAGGAAGUA	AAAGUGCUAA	
<i>E. coli</i>	CAAGGUAACC	GUAGGGCAAC	CUGCGGUUGG	AUCACCUCCU	UA						1542
<i>Ha. B2</i>	CAAGGUAAGCC	GUAGGGAU	CUGCGGCUUG	AUCACCUCCU	---						
<i>X. laevis</i>	CAAGGUAUCC	GUAGGUAAC	CUGCGGAAGG	AUCA-----U	UA						

(续图 2 Continued Fig. 2)

图 2 三个主要类群的每一代表之间的 16S-like rRNA 的比较

(此排列是建立在初级和二级结构同源性“最佳”基础上)

Fig. 2 Comparison of 16S-like rRNA sequences from one representative of each of the three primary kingdoms (1) The alignment is based on the 'best fit' with regard to both primary and secondary structural homologies (7).

An asterisk denotes the start of a deleted portion that is nonhomologous. The numbering in the figure is according to that of the *E. coli* sequence.

表 1 区分嗜盐菌不同属的三个成员菌的 16S rRNA 序列特性
Table 1 16S rRNA sequence signatures distinguishing the three members
from various halobacterial genera respectively

碱基位点* Position in sequence	碱基组成 Composition		
	<i>Halobacterium cutirubrum</i>	<i>Haloferax volcanii</i>	<i>Haloarcula aidinensis</i> strain B2
108	G	A	U
145	G	A	C
177	C	U	G
182	A	C	U
187	U	G	C
206	G	C	A
443	A	C	G
483	G	C	A
592	A	C	U
594	C	A	U
628	A	G	C
835	G	A	U
1260	C	U	A
1263	U	C	G
1362	C	U	A
1440	G	C	U
1462	C	G	A

* 碱基的位数以 *E. coli* 为准。

Numbering is according to the *E. coli* sequence.

表 2 古生物类和真核生物类 16S rRNA 之间的序列相似性
Table 2 Sequence similarities between the archaeal and eucaryal 16S rRNA

碱基位点* Position	生物类 Archaea	细菌类 Bacteria	真核生物类 Eucarya	碱基位点 Position	古生物类 Archaea	细菌类 Bacteria	真核生物类 Eucarya
9	C	G	C	931	G	C	G
25	G	C	G	952	C	U	C
47	A	C	A	966	U	G	U
53	C	A (R)	C	975	G	A	G
113	C	G	C	1060	C	U	C
314	G	C	G	1098	G	C	G
358	G	U	G	1109	A	C	A
361	C	G (R)	C	1110	G	A	G
377	C	G	C	1197	G	A	G
386	G	C	G	1212	A	U	A
514	G	C (Y)	G	1229	G	A	G
537	C	G (R)	C	1381	C	U	C
540	C	G	C	1384	U	C	U
675	G	A	G	1386	C	G	C
684	G	U	G	1415	C	G	C
716	C	A	C	1485	G	U	G
912	U	C	U				

* Numbering is according to the *E. coli* sequence; Y: pyrimidine (嘧啶) R: purine (嘌呤).

第 6 位开始^[14], (2) 如表 2 所示, 表明了 Archaea 与 Eucarya 在某些位置上的相似性, 以及两者与细菌类的差别; (3) Bacteria 中不变的序列片段 GCACA936 在其它两类生物中都含有吡啶核苷酸 C 的插入等等。

表 3 不同生物 16S rRNA 之间的百分相似性

Table 3 Homologies among 16S rRNA sequences from various organisms

菌 种 Species	百分相似性 Percent homology			
	1	2	3	<i>E. coli</i>
<i>Hb. cutirubrum</i>	—			59.6
<i>Hf. volcanii</i>	88	—		59.3
<i>Ha. aidinensis strain B2</i>	88.4	87.8	—	58.7
<i>Hc. morrhuae</i>	88	89	86.6	60

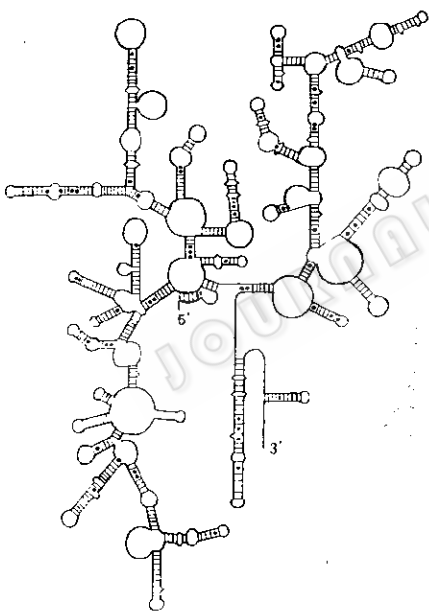


图 3 艾丁嗜盐小盒菌属 B2 菌株
16S rRNA 的二级结构模型

Fig. 3 Secondary structure model of 16S rRNA
from *Haloarcula aidinensis* strain B2

在三级结构 (图 3) 的模型方面, 虽然 Archaea 比较类似于 Bacteria^[15], 但序列方面, Archaea 更接近 Eucarya, 特别是在二级结构的 双链区域^[8], 比如在二级结构 500—550 位置之 间, Archaea (Ha. B2) 和 Eucarya 的 16S rRNA 都有一个由 6 对核苷酸侧突环; 而 Bacteria 中这 一结构为 5 对核苷酸构成的梗以及在此基础上 由 6 个核苷酸构成的梗以及在此基础上由 7 个 核苷酸组成的侧突环; 而 Bacteria 中这一结构为 5 对核苷酸构成的梗和由 6 个核苷酸组成的侧 突环^[7,16]。另外, Achaea (Ha. B2) 的二级结构 也表现出不同于其它两类的特性即在 180—197 之间以及 405—498 之间的特殊结构^[7,16]。

用 Ha. B2 的二级结构与嗜盐菌其它三属的 二级结构^[8-10]相比也可看出存在一些差别, 比 如在 150—250 位置之间的发夹环结构这四属 之间的差别就很明显。

总起来说, 分子序列和分子结构比经典的 表型特征能更好地揭示生物间的进化关系, 也 只有在这种分子的水平上才能更好地看清生物

世界的这种三分叉的关系^[1], 而不是经典的五分类关系。Ha. B2 的分类地位通过序列分 析, 能够很明确地放在 Archaea 类、Euryarchaeota 界中, 并进一步证明了以前的论点^[3], 即 Ha. B2 应归于嗜盐菌目, 嗜盐菌科, 嗜盐小盒菌属中的一新种 *Haloarculaaidinensis* strain B2。

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NUCLEOTIDE SEQUENCE OF THE 16S rRNA FROM AN ARCHAEA, *HALOARCUA* *AIDINENSIS* STRAIN B2

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Abstract The sequence of the 16S rRNA from an Archaea, *Haloarcula aidinensis* strain B2 (Ha. B2) has been determined by PCR-based sequencing method. The sequence of the archaeal rRNA indicates typical archaeal features. Although Ha. B2 is closer in sequence to bacterial 16S rRNA than to the eucaryal 16S-like rRNA, its sequence shows certain specific similarities to eucaryal counterpart. In sequence and structure, the similarities of the Archaea (Ha. B2) with Bacteria or Eucarya are higher than the similarities between Bacteria and Eucarya. The comparison of this archaeal 16S rRNA sequence with those from other species of various halobacterial genera strongly supports the previous conclusion that the Ha. B2 should belong to a new species of Genus *Haloarcula*.

Key words 16S rRNA, Archaea, *Haloarcula aidinensis* strain B2