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油桐尺蠖单粒包埋核型多角体病毒(BusuNPV) BamHI-H片段的序列分析*

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摘　要　对油桐尺蠖单粒包埋核型多角体病毒(*Buzura suppressaria* single-nucleocapsid nucleopolyhedrovirus, BusuNPV)基因组中BamHI-H片段的序列进行分析，该片段全长2 422 bp，包括三个开放阅读框：*p47*基因(AcMNPV ORF40的同源区)的5′端；完整的组织蛋白酶基因(*cathepsin*)(AcMNPV ORF127的同源区)和*p74*基因(AcMNPV ORF138的同源区)的3′端。序列比较分析表明，BusuNPV的这三个基因与其它杆状病毒的同源基因具有相同的结构保守区。BusuNPV基因组BamHI-H片段上这三个基因的排列顺序完全不同于AcMNPV相应基因的排列顺序。

关键词　油桐尺蠖单粒包埋核型多角体病毒，*p47*基因，组织蛋白酶基因，*p74*基因，序列分析

BusuNPV、BamHI-H片段、基因

杆状病毒是一类能感染节肢动物(主要是鳞翅目昆虫)的病毒。其病毒粒子呈杆状，基因组为双链环状DNA，大小为90～160 kb。杆状病毒科(*Baculoviridae*)分为两个属：核型多角体病毒属(*Nucleopolyhedrovirus*，简称NPV)和颗粒体病毒属(*Granulovirus*，简称GV)[1]。NPV按病毒粒子的包埋形态分为多粒包埋NPV(Multiple-nucleocapsid NPV，简称MNPV)和单粒包埋NPV(Single-nucleocapsid NPV，简称SNPV)。至今已发现的杆状病毒达600多种，目前有四种杆状病毒基因组的全序列已经完成，它们是AcMNPV[2]、BmNPV[3]、OpMNPV[4]和LdMNPV[5]。序列分析表明，AcMNPV、BmNPV、OpMNPV的基因组成和排列较为相近，而LdMNPV的基因组成和排列却与上述三种病毒显著不同。随着更多杆状病毒的基因序列被测定，人们对这些病毒之间的进化关系及杆状病毒多样性的了解将更加深入。

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油桐尺蠖单粒包埋核型多角体病毒于70年代末在我国不同茶区的病死害虫中分离得到[6]，病毒粒子呈单粒包埋型。中国科学院武汉病毒所对该病毒进行了多年的生物学研究，并成功地用于油桐尺蠖的防治[7]。到目前为止，尺蠖科(*Geometridae* family)中已报道的NPV大约有67种[8]。与MNPV相比，SNPV的分子生物学研究还十分贫乏。为此，从80年代末起，我们全面开展了对BusuNPV的分子生物学的研究，构建了该病毒的基因文库、物理图谱，并选择基因组中的不同区域进行了序列分析[9~11]。本文具体报道BusuNPV基因组上一个2 422 bp的BamHI-H片段的序列分析结果，并将该片段上基因的排列顺序与AcMNPV相应基因的排列顺序进行比较。

1 材料和方法

1.1 材料及试剂 油桐尺蠖单粒包埋核型多角体病毒的原始株，是从湖北蒲圻羊楼洞茶场的病死虫中分离获得的[6]；质粒载体pTZ19R及宿主菌$DH_5\alpha$由本实验室保藏；限制性内切酶、连接酶、去磷酸化酶及缺刻平移试剂盒均购自华美生物工程公司。

1.2 病毒DNA的提取及限制性内切酶分析 BusuNPV多角体饲喂感染油桐尺蠖幼虫，虫体死亡后，研磨过滤，多角体经差速离心纯化，显微镜下计数。取3×10^9个多角体离心，加入蛋白酶K(20 mg/mL 5 μL，37 ℃作用30 min；加入500 μL碱解液(0.1 mol/L Na_2CO_3，0.01 mol/L EDTA，0.17 mol/L NaCl，pH>10.8)，37 ℃作用30 min；加入10%SDS至终浓度1%，37 ℃作用30 min；酚、酚：氯仿：异戊醇(25:24:1)及氯仿：异戊醇(24:1)各抽提一次后，0.1×TE(pH8.0)透析48 h，分光光度计测定其纯度及浓度。取1 μg DNA进行内切酶消化，限制性内切酶消化条件按常规方法进行。0.7%琼脂糖凝胶电泳。

1.3 克隆及测序 紫外灯下切取目的带，液氮冻融法回收，与BamHI消化并去磷酸化的载体pTZ19R 14 ℃连接过夜，转化大肠杆菌$DH_5\alpha$。阳性克隆经限制性内切酶分析及Southern blot鉴定。利用通用引物和合成引物以引物延伸法(*Primer walking*)进行序列测定，自动序列测定在加拿大Queen's大学的测序实验中心进行。

1.4 序列分析 序列用UWGCG计算机软件(release 9.0)分析，DNA序列和编码的氨基酸序列用FASTA和BLAST软件与GenBank/EMBl Swissport及PIR数据库进行比较分析，运用GeneDOC软件进行氨基酸序列及同源性分析。

2 结果及讨论

限制性内切酶分析及Southern bolt结果表明，BusuNPV 2.4 kb的BamHI-H片段已被成功地克隆在载体pTZ19R上(图略)。以引物延伸法对阳性克隆中插入片段的两条链进行全序列测定，结果表明，该片段全长为2 422 bp，包括三个开放阅读框：*p47*基因(AcMNPV ORF40的同源区)的5′端，完整的组织蛋白酶(cathepsin)基因(AcMNPV ORF127的同源区)和*p74*基因(AcMNPV ORF138的同源区)的3′端(图1，2)，其结构特征分述如下：

2.1 *p47*基因 BamHI-H片段编码*p47*基因的N端193个氨基酸，该序列与AcMNPV ORF40所编码的氨基酸序列同源。BusuNPV *p47*基因的起始密码子上游第16位有一杆状病毒早期基因转录起始信号CAGT[12]，在－100位和－120位有两个早期启动子组成元件TATA。完整的AcMNPV *p47*基因编码一分子量为47.5 kDa的多肽[13]，据推测为杆状病毒自身RNA聚合酶的亚单位，参与晚期及极晚期基因的转录与表达[14]。到目前为止，有4种杆状病毒的*p47*基因序列已被测定，它们是：AcMNPV[15]、BmNPV[3]、OpMNPV[4]和LdMNPV[5]。

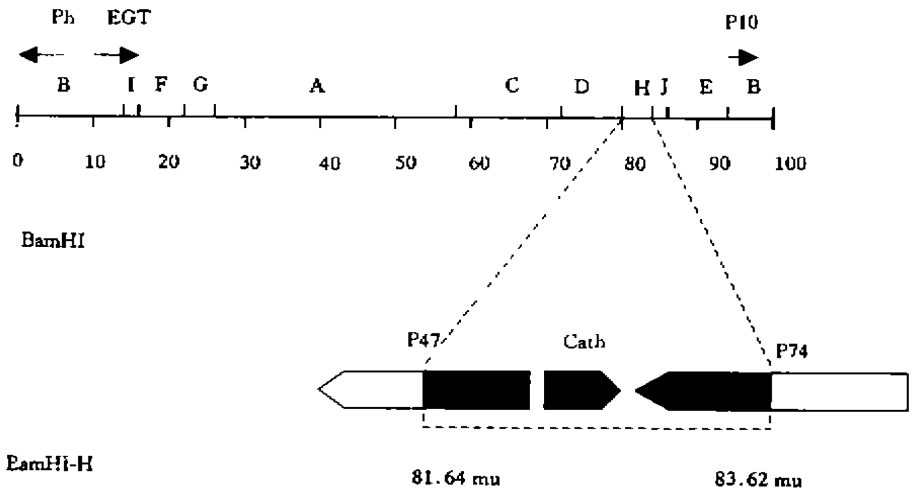


图1　BusuNPV 基因组的 BamH-I 酶切图谱及 BamHI-H 片段的结构

Fig.1　BamHI physical map of the BusuNPV genome and detailed gene organization of the BamHI-H fragment
The arrows represent the ORFs and point to their directions of transcription. *p47* and *p74* have been only partially sequenced (sequenced part were black).

BusuNPV *p47* 基因 N 端 193 个氨基酸序列与所有这些病毒的氨基酸序列分析比较表明,在 BusuNPV Arg110-His164 之间有一段高度保守区,该区含有两个明显的亮氨酸拉链序列(Leucinezipper),这类序列可使肽链形成卷曲的螺旋(coiled-coil)结构,后者在真核生物的转录因子中十分常见[16]。*p47* 亚单位在杆状病毒 RNA 多聚酶中的具体功能尚不清楚,但可以推测,亮氨酸拉链序列与该基因的功能有一定的关系(图3)。

2.2　组织蛋白酶(Cathepsin)基因　BusuNPV *Cathepsin* 基因的起始密码子位于 *p47* 基因的上游 200 bp 处,其转录方向与 *p47* 基因相反(图2)。在起始密码子上游 25 位有强晚期启动子转录起始信号 ATAAG,在其终止密码子下游第 10 位有一 PolyA 终止信号 AAATAA。杆状病毒的 Cathepsin 为一种半胱氨酸蛋白酶[17-19],该酶在 pH 5.0～5.5 时活性最高[18]。在许多杆状病毒基因组中,*Cathepsin* 基因和几丁质酶基因相邻排列。据推测这两个基因共同作用,液化虫体,促进多角体的释放及在环境中的水平传播[20]。已报道 *Cathepsin* 基因的杆状病毒有 7 种,它们是 AcMNPV[18]、BmNPV[19]、OpMNPV[4]、CfMNPV[21]、LdMNPV[5]和 CpGV[22]。BusuNPV 与其它几种杆状病毒 *Cathepsin* 基因氨基酸序列比较分析表明,该蛋白富含 Cys,且这些 Cys 位点高度保守。推测的 AcMNPV 中该蛋白酶的催化活性位点 Cys136、His269 及信号肽的切割位点 Asn98 在这几种 NPV 中都是一致的(图4)。运用 GenDOC 软件对七种杆状病毒 Cathepsin 氨基酸进行同源性分析,Cathepsin 氨基酸同源性最高达 97%(AcMNPV 与 BmNPV),BusuNPV 与 CpGV 同源性最低为 61%(表1)。

2.3　*p74* 基因　BusuNPV BamHI-H 片段中 *Cathepsin* 基因的下游,编码 *p74* 基因的 C 端 196 个氨基酸,与 *Cathepsin* 基因方向相反。在 AcMNPV[23]、OpMNPV[24]和 CfMNPV[25]等基因组中,*p74* 基因总是以相反的方向位于 *p10* 基因的下游。这一点与 BusuNPV 显著不同[26]。据推测 *p74* 基因位于多角体包埋型病毒粒子(polyhedra-derived virus,简称 PDV)囊膜

```
        BamH-I
1       GGATCCTATTGAATTGATAAATTTTTCAAAATGTACACTGTCCCAGCCGCGAGTCGGAGT
         S  G  I  S  N  I  F  K  E  F  H  V  S  D  W  G  R  T  P  T
61      GCTTTCGTCTTCGCGACCAGTGTGATGCTTGAAATCGAGACCGCTTTGGATCAGTTTAGT
         S  E  D  E  R  G  T  H  H  K  F  D  L  G  S  Q  I  L  K  T
121     GGTTATGCGAACGCTTAATTGCTGTCCCAGTGTGTAGTGGTTTTCGTAACCGCGCTCCCA
         T  I  R  V  S  L  Q  Q  G  L  T  Y  H  N  E  Y  G  R  E  W
181     CATTGTGTTACATGCAAACCCGACGAGTGCCTTGACGTCGCGTCGCGTTAATATTTTGCG
         M  T  N  C  A  F  G  V  L  A  K  V  D  R  R  T  L  I  K  R
241     CAAACGGACAAAGTCGCCTTTGTGCCAGCGGTCACGCGCCAATAATTTAAGCAATTTGAT
         L  R  Y  F  D  G  K  H  W  R  D  R  A  L  L  K  L  L  K  I
301     TTCATGATCAGTAAGCATAGTTTTGCGCGTCGCGTCAATGTAATGCTCGATTTCGACGGG
         E  H  D  T  L  M  T  K  R  T  A  D  I  Y  H  E  I  E  V  P
361     CGTCGAATTGCGCAGGTTTTCCATATCGAAGACCACGTTTAAATAATATAATTGCACAAA
         T  S  N  R  L  N  E  M  D  F  V  V  N  L  Y  Y  L  Q  V  F
421     TCCCTCGTCATTGACGGATACGTTGTGTTCCATGACGTGAAACGAGTTTGAAACGTTATT
         G  E  D  N  V  S  V  N  H  E  M  V  H  F  S  N  S  V  N  N
481     CAGGTACATATTGTACATGTTGATTGGTTCACTCAAATATTTGCAACATTGGGGTAGCCA
         L  Y  M  N  Y  M  N  I  P  E  S  L  Y  K  C ·C  Q  P  L  W
541     TTGTGTGCGAGTTTCAACGTAACGTGCAAACGCCATGATGCTTGTTAAAATACTGAAATA
                                         GTA               TGAC
         Q  T  R  T  E  V  Y  R  A  F  A  M  < P47
601     AAATAGTAAAATTATCAAAGTGCTTAAATCAATTGTAGATTATTATTGATTTTGTGAAAA

661     ATGTTTTTAAATTTGTATACAAAATGGGTCGAAATATATAAATTTGTGTTAATGTATTTG
                           ATAT                  ATAT
721     TAAAAAAAACAATTTGTTTTTGAGTAATAAGTAAATTTAATTTAGTGTATGAGCTATGAA
                                                   Cathepsin `  M  K
781     AAAATTAGTAATATGCATAATACTGAATTTGATCGTGGCGAAAAATTATGCCTTCGCTTA
         K  L  V  I  C  I  I  L  N  L  I  V  A  K  N  Y  A  F  A  Y
841     CCGATCTGTTGAAAGCGGGCGATTATTTCGAAACTTTTCTTGCCAATTACAATAAAATGT
         D  L  L  K  A  G  D  Y  F  E  T  F  L  A  N  Y  N  K  M  Y
901     CAATGACACATCCGAAAAGGAGCGACGTTTCAGTATATTTCAACAAACTTTAGAAGAAAT
         N  D  T  S  E  K  E  R  R  F  S  I  F  Q  Q  T  L  E  E  I
961     TAACTATAAGAATCGGTTAAACGACAGCGCCGTTTATCAGATTAATAAATTTGCCGATTT
         N  Y  K  N  R  L  N  D  S  A  V  Y  Q  I  N  K  F  A  D  L
1021    GAGTAAAAATGAAATTATATCAAAATACACCGGGTTAAATATGCCCGTACAAACGACAAA
         S  K  N  E  I  I  S  K  Y  T  G  L  N  M  P  V  Q  T  T  N
1081    TTTTTGCAAAACGATCGTCATCGATCAGCCGCCGGGCAAGGGACCGCTTAATTTCGATTG
         F  C  K  T  I  V  I  D  Q  P  P  G  K  G  P  L  N  F  D  W
1141    GCGCCAACAAAACAAAGTGACTTCGATTAAAAATCAAAAAGCGTGTGGCGCTTGCTGGGC
         R  Q  Q  N  K  V  T  S  I  K  N  Q  K  A  C  G  A  C  W  A
1201    GTTCGCCACTTTGGCCAGCATCGAGAGTCAGTACGCGATAAAAAACAACGTGCACATCGA
         F  A  T  L  A  S  I  E  S  Q  Y  A  I  K  N  N  V  H  I  D
1261    TCTGTCCGAGCAGCAGATGATTGATTGCGACTATGTGGACATGGGCTGCGACGGCGGTTT
         L  S  E  Q  Q  M  I  D  C  D  Y  V  D  M  G  C  D  G  G  L
```

```
1321  ATTGCACACGGCATTTGAACAAATGATCCAAATGGGAGAATTGGTACAAGAGCACGAATA
       L  H  T  A  F  E  Q  M  I  Q  M  G  E  L  V  Q  E  H  E  Y
1381  CCCGTACGCGGGTGTCAATAAACCGTGCGAACTACGCGGCGACGAAACTGGCGTAGTCAA
       P  Y  A  G  V  N  K  P  C  E  L  R  G  D  E  T  G  V  V  K
1441  AGTAAAGGGTTGTTATCGGTACGTCGTGTTTCGCGAGGAAAAACTAAAAGATTTGCTGCG
       V  K  G  C  Y  R  Y  V  V  F  R  E  E  K  L  K  D  L  L  R
1501  CGCCGTCGGACCCATTCCGATGGCCATTGACGCATCCGGAATCGTTAATTACCATCATGG
       A  V  G  P  I  P  M  A  I  D  A  S  G  I  V  N  Y  H  H  G
1561  CATTATACATTATTGCGAAAACTACGGATTAAACCATGCTGTGCTTTTAGTGGGATACGG
       I  I  H  Y  C  E  N  Y  G  L  N  H  A  V  L  L  V  G  Y  G
1621  GGTTGAGAATAACGTACCTTTTTGGACGTTTAAAAACACTTGGGGCAAAGATTGGGGTGA
       V  E  N  N  V  P  F  W  T  F  K  N  T  W  G  K  D  W  G  E
1681  AGAAGGTTATTTTAGAGTTAGACAAAATGTAGACGCTTGCGGAATGACTAACGAATTGGC
       E  G  Y  F  R  V  R  Q  N  V  D  A  C  G  M  T  N  E  L  A
1741  TTCATCCGCAGTCATCGATTGGGACGCTTAAAAATTAAAAAAATAATCATTTAGTTTTTC
       S  S  A  V  I  D  W  D  A  *
1801  GTATGTATAGAATGAATTCCTACGCGACAATCATTCGTACAAGTTTTTGTACCATTTTGT
                                            *  E  Y  L  N  K  Y  W  K  T
1861  TTGTGCAGACGCCGTGACTTGTCTTAGTTTAAAATAATATAACAAGGAATTTTGAATAAT
       Q  A  S  A  T  V  Q  R  L  K  F  Y  Y  L  S  S  N  Q  I  I
1921  CAAGTAAACGGTGCAAATGACAAACACCACAAACAAAATTGTTAACTTGATACTGTTAGC
       L  Y  V  T  C  I  V  F  V  V  F  L  I  T  L  K  I  S  N  A
1981  GTTGTATAATACGATTATTAGCGCACCCAACCCGAATAAATTAGCCATTAGCAGGTTAGT
       N  Y  L  V  I  I  L  A  G  L  G  F  L  N  A  M  L  L  N  T
2041  GTAAGTCGTATCATTGGCAAATAAAATAGCATTATGTCGCTGCGCGTATAGTTCAAAATC
       Y  T  T  D  N  A  F  L  I  A  N  H  R  Q  A  Y  L  E  F  D
2101  CAATCGAGTGTACAAAGAACTAGATGCTAGCGCGTTTCCTACCAACGTTGCTTCGTCAAA
       L  R  T  Y  L  S  S  S  A  L  A  N  G  V  L  T  A  E  D  F
2161  ATCATCAATTTCTGCACTTTCTTCAAAATTTAATAACTGACCATTCGAATTCACTTCTAG
       D  D  I  E  A  S  E  E  F  N  L  L  Q  G  N  S  H  V  E  I
2221  TTCGCTAACGTAATCGAGTAAGTATAGAAACGACTCAAACATGGCCACATCAGTATTTTC
       E  S  V  Y  D  L  L  Y  L  F  S  E  F  M  A  V  D  T  N  F
2281  TATTAAATAGTCAAAAAACTCCGGCAGCATTTCGAGTATGTCGCGCGTCTCGCCAAACGA
       I  L  Y  D  F  F  E  P  L  M  E  L  I  D  R  T  E  G  F  S
2341  ATTAAAATATCCAATTATAAACGAAAAAGCCAAATCATCTGGAAACTGGCGGGGAAACAT
       N  I  Y  G  I  I  F  S  F  A  L  D  D  P  I  Q  R  P  F  M
                   BamH I
2401  GTTACTGTAACCAAACGGATCC
       N  S  Y  G  F  R  I
```

图 2　BusuNPV BamHI-H 片段序列分析

Fig. 2　Nucleotide sequence of the BamHI-H fragment

The predicted amino acids are indicated by one-letter code designations below the nucleotide sequence. The genes, their direction of transcription and the BamHI restriction enzyme sites are indicated, putative transcription signals such as TATA and CAGT motifs for early transcription initiation; TAAG for late baculovirus transcription initiation and AATAAA for polyadenylation are bolded. Signals on the complementary strand of the DNA are underlined and their complementary sequence are shown.

的外侧，在 PDV 的吸附和融合过程中具有重要作用，是多角体感染虫体所必需基因。已报道 *p74* 基因的杆状病毒有 5 种，它们是：AcMNPV[23]、OpMNPV[24]、BmNPV[3]、CfMNPV[25]和

LdMNPV[5]，将 BusuNPV P74C 端 196 个氨基酸序列与这些 P74 蛋白比较分析表明，该多肽与其它杆状病毒 P74 高度同源，并具有 Adam 等人推测的典型的病毒吸附蛋白特征[26]。

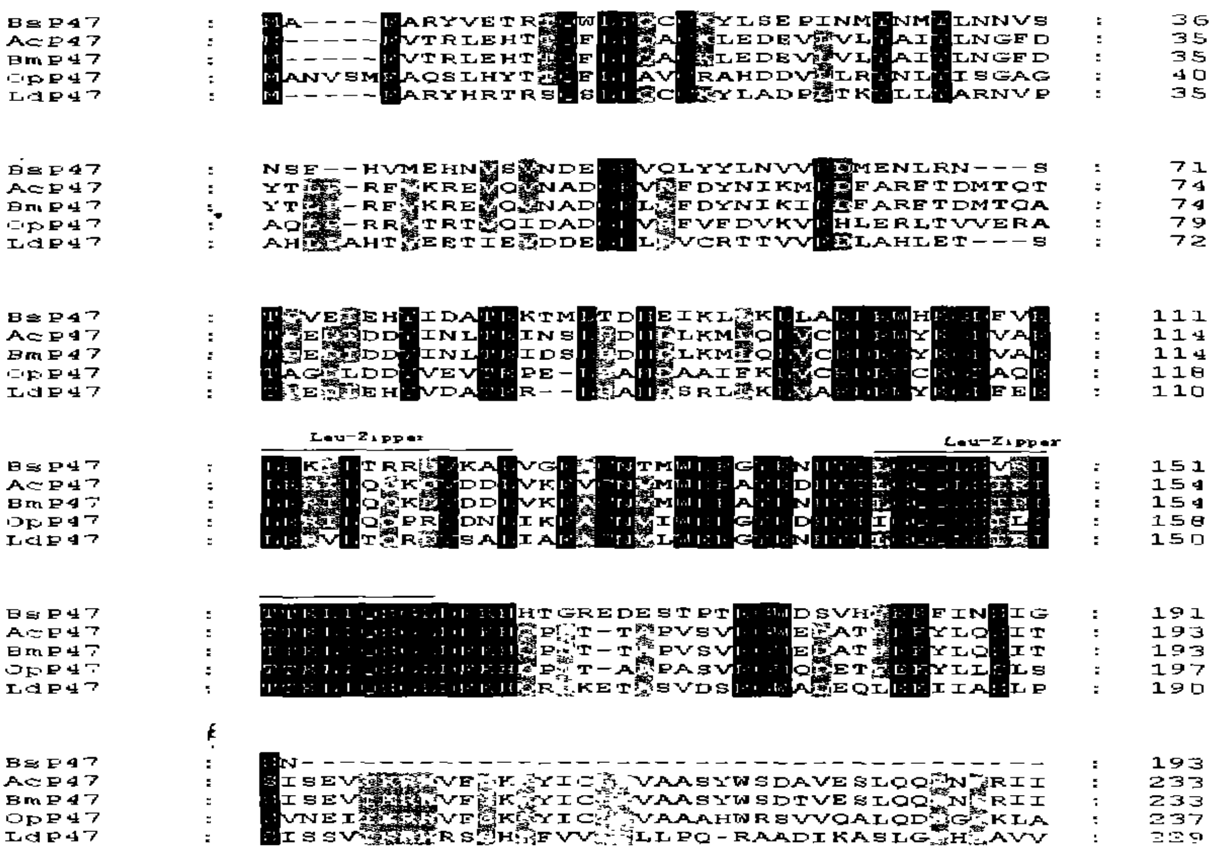


图 3　杆状病毒 P47 氨基酸序列同源性比较

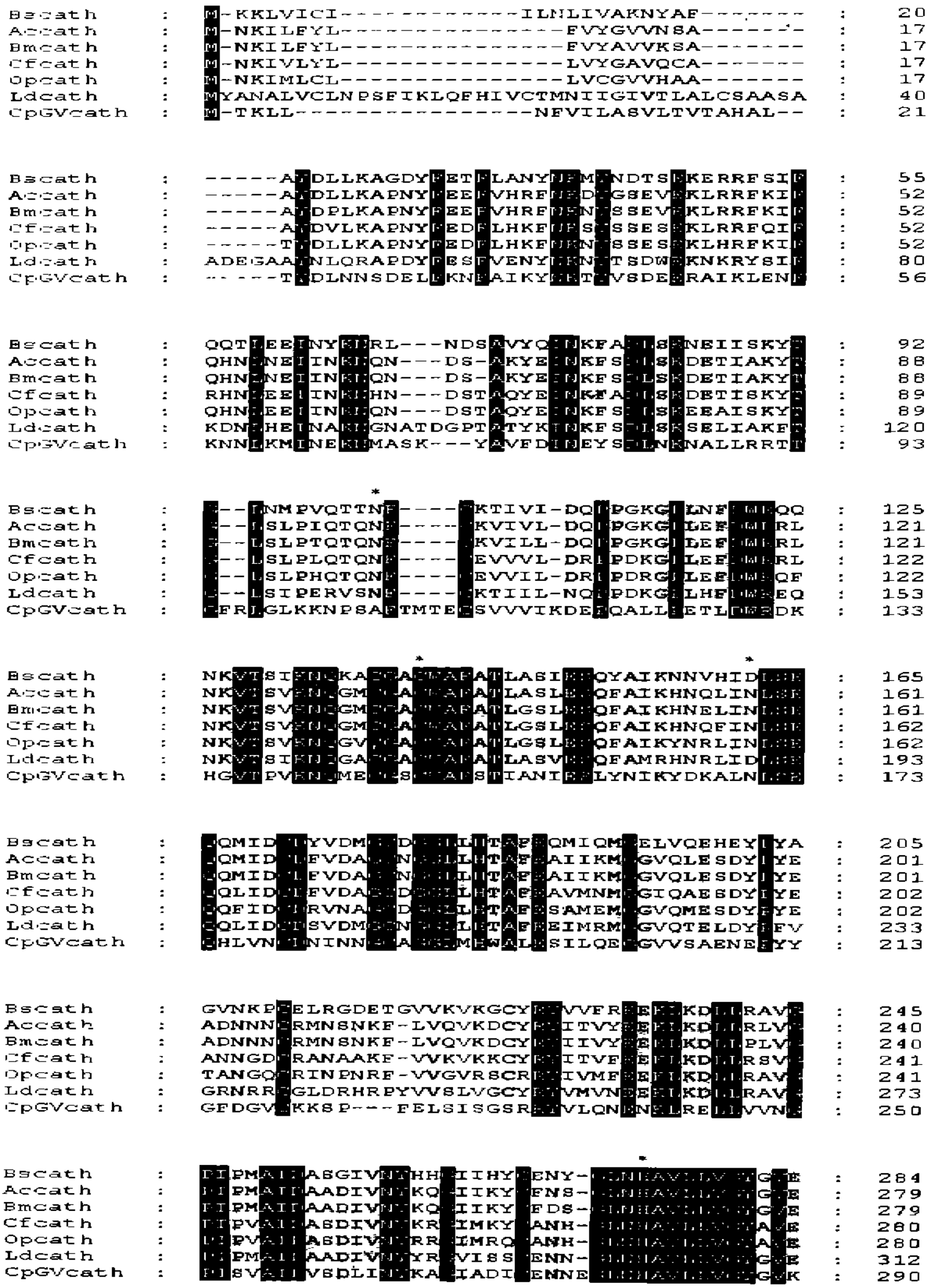
Fig.3　Alignment of baculovirus P47

The location of leucine zipper motifs are shown. The sources of the sequences are Oelling *et al*. (1987)[14] for AcMNPV, Gombart *et al* (1989)[15] for OpMNPV, S. Maeda *et al*[3] (Genbank accession number L33180) for BmNPV, Kuzio J *et al* (1999)[5] for LdMNPV. GeneDoc software was used for homology shading. Two shading levels were set: black for 100% identity and grey for 80% identity.

表 1　7 种杆状病毒组织蛋白酶氨基酸同源性比较(%)

Table 1　Amino acid sequence identity (%) between baculovirus cathepsin proteins

	BusuNPV	AcMNPV	BmNPV	CfNPV	OpMNPV	LdMNPV	CpGV
BusuNPV		78	77	76	75	73	61
AcMNPV			97	91	89	73	64
BmNPV				91	89	73	64
CfMNPV					91	73	64
OpMNPV						73	64
LdMNPV							62



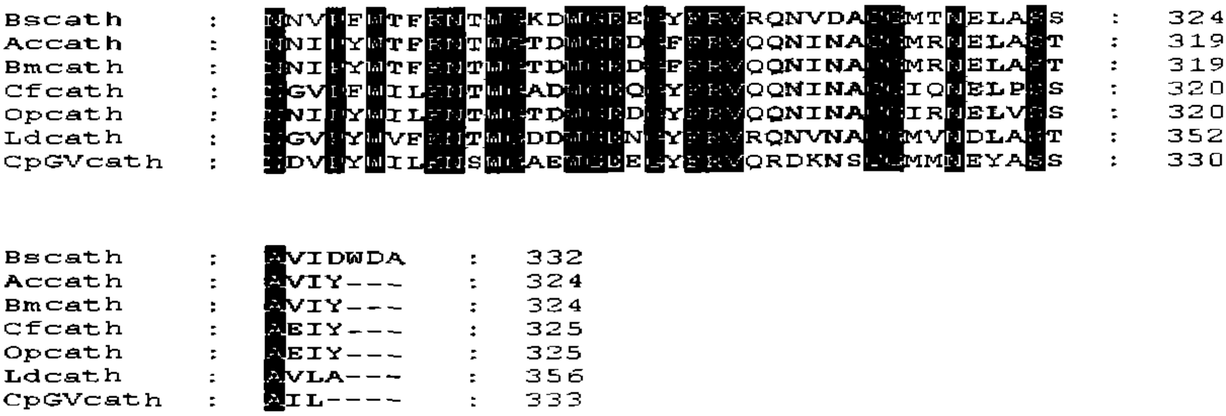


图4 杆状病毒组织蛋白酶基因氨基酸序列同源性比较

Fig.4 Alignment of baculovirus cathepsins, predicted N-linked glycosylated site Asn158, the signal sequence cleavage site Asn97, catalytic Cys136 and His 269 residues are indicated by * above the sequence. The sources of the sequence are Ayres *et al*(1994)[18] for AcMNPV, Hill *et al*(1995)[21] for CfMNPV, Ohkawa *et al*(1994)[19] for BmNPV, Ahrens *et al*(1997)[4] for OpMNPV, Kuzio J *et al*(1999)[5] for LdMNPV, Kang W. *et al*(1999)[22] for CpGV. GeneDoc software was used for homology shading. Two shading levels were set: black for 100% identity and grey for 80% identity.

2.4 基因组排列 BusuNPV和AcMNPV基因组排列比较表明,BusuNPV BamHI-H片段上相邻的三个基因 *p47*、*Cathepsin*、*p74* 在AcMNPV基因组上相隔很远(图5)。在AcMNPV、OpMNPV、BmNPV等病毒中,*p26*、*p10*、*p74* 基因均相邻排列,但在BusuNPV中,*p74* 基因与 *p26*、*p10* 基因却位于不同区域[27]。在AcMNPV基因上,*Cathepsin* 与几丁质酶基因相邻排列,但转录方向相反;在BusuNPV中,它们虽然转录方向相反,在基因组上的位置相距将近23个图距单位。BusuNPV基因组中基因分布的特异性已被该病毒基因组中其它区域的序列所证实[28],说明BusuNPV属于一个独立的进化分支。

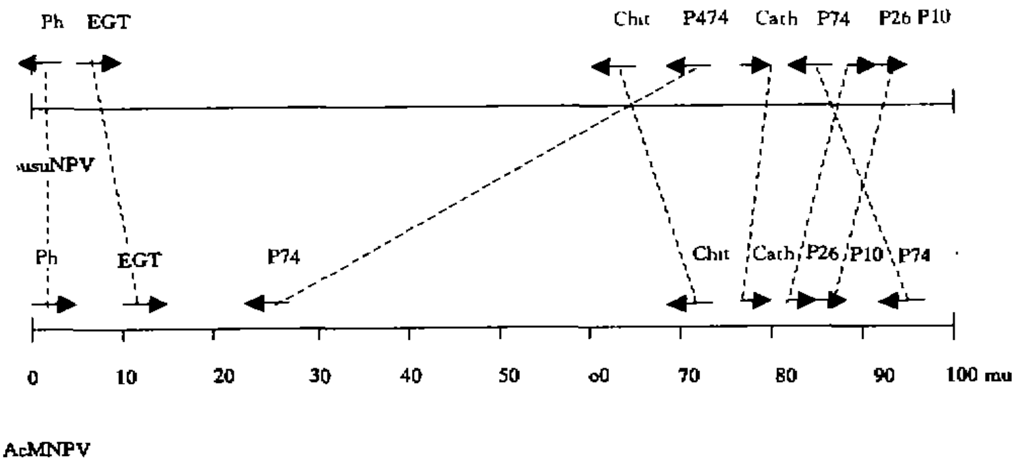


图5 BusuNPV与AcMNPV基因排列比较

Fig.5 Comparision of the gene organization between the genomes of AcMNPV and BusuNPV

The arrows represent the ORFs and their directions of transcription. Gene homologues are linked by a line.

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Genetic Organization of the BamHI-H Region of the Single Nucleocapsid Nucleopolyhedrovirus of *Buzura suppressaria* (BusuNPV)

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Abstract In order to investigate the genomic organization of the single-nucleocapsid nucleopolyhedrovirus (SNPV) of *Buzura suppressaria* (BusuNPV), the BamHI-H fragment located at map units (mu)81.6~83.6 of the viral genome was sequenced. The fragment contained two partial and one complete open reading frames (ORFs) representing the 5′ end of a *p47* gene, a *cathepsin* gene and the 3′ end of a *p74* gene. Sequence analysis futher revealed that these ORFs have the same conserved motifs and gene structure as those observed in their homologues from other baculoviruses. The genomic arrangement of the ORFs in the BusuNPV BamHI-H fragment is very different from the arrangement of their homologues in the genome of *Autographa californica* mutiple nucleocapsid (M) NPV.

Key words Single-nucleocapsid nucleopolyhedrovirus of *Buzura suppressaria* (BusuNPV), *p47* gene, *Cathepsin* gene, *p74* gene, Sequence analysis