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Short communication

Molecular identification of *Oat mosaic virus* as a *Bymovirus*

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Abstract

A partial sequence of *Oat mosaic virus* (OMV) has been obtained for four isolates of the virus from four European countries. This represents the first available sequence data for this important disease of winter-sown oats. The longest clone of 1699 nucleotides was obtained from infected English oats using a degenerate primer, designed to members of the *Potyviridae* family. Alignment of the predicted amino acid sequence with members of the *Potyviridae* showed closest identity with viruses of the *Bymovirus* genus. The predicted amino acid sequence has one open reading frame corresponding to part of the NIb and capsid protein, with a 3′ untranslated region of 351 nucleotides, followed by a poly(A) tail. PCR primers were designed to the coat protein and NIb gene of members of the *Bymovirus* genus and used to obtain partial sequences of 1441 nucleotides at the 3′ end of infected oats from both Wales and France. A specific primer set designed to the English isolate was used to generate a product of 701 nucleotides from OMV-infected oat leaves from Ireland. All four isolates are highly conserved at the amino acid level.

Oat mosaic virus (OMV) causes a disease of autumn-sown oats (*Avena sativa*) which results in yield reductions of 25–50% in tolerant cultivars and up to 100% in susceptible cultivars (Herbert and Panizo, 1975). Symptoms of the disease appear in early spring as stunted chlorotic plants usually in patches within the field. The symptoms are more pronounced in cool, dull weather and may disappear with warmer brighter weather. OMV was first reported in the United States, (McKinney, 1946) where it occurs in the south-eastern states from Maryland to Florida and Alabama (Hebert and Panizo, 1975). The disease has been identified in England (Macfarlane et al., 1968), Wales (Catherall and Hayes, 1970), Ireland (Kavanagh and Lahert, 1990), Italy (Rubies-Autonell, 1992) and France (Hariri et al., 1996). The identification of this virus in autumn-sown oats relies on electron microscopy and the development of symptoms on host plants (Zheng Jianhua and Kavanagh, 1990).

OMV is a single-stranded positive-sense RNA virus with flexuous filamentous particles which infects *Avena* species and generates pin-wheel inclusion bodies in infected cells (Brunt et al., 1996). OMV is transmitted by *Polymyxa graminis* a common root inhabiting fungus which thrives in wet soils (Anon, 1999). Members of the *Bymovirus* genus are unique in the *Potyviridae* family in that they are bi-partite. *Barley yellow mosaic virus* (BaYMV), *Wheat yellow mosaic virus* (WYMV), *Wheat spindle streak mosaic virus* (WSSMV), *Barley mild mosaic virus* (BaMMV) and *Rice necrosis mosaic virus* (RNMV) have particles of 200–300 and 500–600 nm (Brunt et al., 1989). OMV particle have been found that range in size from 600 to 750 nm in length and 12–14 nm wide (Macfarlane et al., 1968; Catherall and Hayes, 1970; Usugi and Saito, 1981; Kavanagh, 1990), although a second smaller particle for OMV has been reported (Usugi et al., 1989). OMV has also been shown to

have two species of single-stranded RNA, although both RNAs are larger than has been reported for other bymoviruses (Usugi et al., 1989). An antiserum has been raised to partially purified virus particles (Usugi and Saito, 1981). This antiserum does show a serological relationship with three bymoviruses WSSMV, WYMV and BaYMV. Antiserum raised to BaYMV while reacting strongly with WSSMV and WSMV only showed a weak serological relationship with OMV (Usugi et al., 1989). The fungal vector, morphology of particles, host range of the virus and antiserum results suggest that OMV is a member of the *Bymovirus* genus of the family *Potyviridae*.

The English isolate of OMV

OMV infected oat leaves were obtained from IACR-Rothamsted, UK, in 1995, that originated from oats grown in infected soil in Cranbook, Kent, England. The leaves were snap frozen in liquid nitrogen and stored in the −70 °C freezer. RNA was extracted using the RNeasy Plant Mini kit (Qiagen). cDNA was generated with the first-strand RT-PCR kit (Stratagene) following manufacturer's instructions, with 7 μg of total RNA in a 50 μl volume and using the primer oligo d(T). Amplification from cDNA was performed in a MJ Research mini-cycler™. The primer oligo d(T)-Not1 (5′-AATTCG CGG CCG $C(T)_{15}$-3′) was used with the universal *Potyviridae* degenerate primer PV2 (5′-ACC ACA GGA TCC GGB AAY AAY AGY GGD CAR CC-3′) (Gibbs and Mackenzie, 1996). This primer was designed to a conserved region within the NIb coding region found in members of the *Potyviridae*. The 20 μl PCR reaction mix contained 80 ng of each primer, 1.8 μl of 11× buffer (494.4 mM Tris·Cl pH 8.8, 123 mM $(NH_4)_2$ SO_4, 49.6 mM $MgCl_2$, 75 mM 2-mercaptoethanol, 50 μM EDTA pH 8.0, 11.1 mM each dNTP, 1.26 mg ml^{-1} BSA), 1 μl of cDNA and 0.5U Taq polymerase (Promega). The PCR thermal cycling conditions were: 94 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min 30 s repeated for 30 cycles and then held at 72 °C for 10 min. Products were separated on a 1% agarose gel containing ethidium bromide and viewed under UV light. A product of the expected size was amplified. This was purified from the gel using the Gene Clean III kit (BIO 101) and cloned using the TOPO TA cloning vector and One Shot INVαF′ competent cells (Invitrogen). Two clones were sequenced in both the forward and reverse direction by Lark Technologies Inc. Computer analysis of the sequence, using GeneJockey II for Apple Macintosh (BioSoft), revealed a product of 1699 nucleotides (excluding primers), with a single open reading frame (ORF) of 1348 nucleotides (448 amino acids) open at the 5′ end. This ORF terminates with a UAA at position 1346 and is followed by a 351 nucleotide untranslated region (UTR) and a poly (A) tail. The nucleotide and predicted amino acid sequence of this English isolate can be found at GenBank accession No. AF314536. A Blast search of the databases found a match with members of the *Potyviridae* family, the greatest identity was found with RNA 1 of members of the *Bymovirus* genus, BaMMV, BaYMV, WSSMV, WYMV and RNMV. The sequence has conserved motifs that are typical of bymoviruses. Part of the conserved motif (S or T)GX_3-TX_3-N(S or T)$X_{18–37}$GDD proposed to be the active site of the RNA dependent RNA polymerase is seen at position 2 of the predicted amino acids, TX_3-NTX_{32}GDD (Koonin, 1991). The rest of the motif is within the PV2 primer used to generate the product. OMV does have the LQA motif at position 187–189 of the predicted amino acid sequence (AF14536). The LQA motif common to bymoviruses is thought to be the cleavage site between the NIb and capsid proteins (Dinant et al., 1991). Using the LQ/A site, the predicted molecular weight of the coat protein is 28,781 Da, this is consistent with the predicted weight of 30 and 29 kDa from SDS-PAGE of purified virus particles (Usugi, 1981; Elliott and Lommel, 1989).

A phylogenetic tree of the putative coat protein of OMV with members of the *Bymovirus* genus WSSMV (accession No. X73883), WYMV (accession No. AF067124), BaYMV (accession No. X69757), RNMV (accession No. U95205) and BaMMV (accession No. X69204) and representative members from other genera of the *Potyviridae*, *Potyvirus*, *Potato virus Y* (PVY, accession No. D00441), *Ipomovirus*, *Sweet potato mild mottle virus* (SPMMV, accession No. Z73124), *Tritimovirus*, *Brome streak mosaic virus* (BStV accession No. Z48506); *Macluravirus*, *Maclura mosaic virus* (MacMV, accession No. U58771) and *Rymovirus*, *Ryegrass mosaic virus* (RGMV, accession No. U27383) was constructed (Figure 1). The tree was generated with DNAMAN for Windows (Lynnon BioSoft), which uses the Neighbor-joining method (Saitou and Nei, 1987), 100 replicates were used for bootstrapping, all default parameters were used. OMV appears on the same branch of the tree as the bymoviruses, further more, the coat proteins of the bymoviruses WYMV, WSSMV and BaYMV are

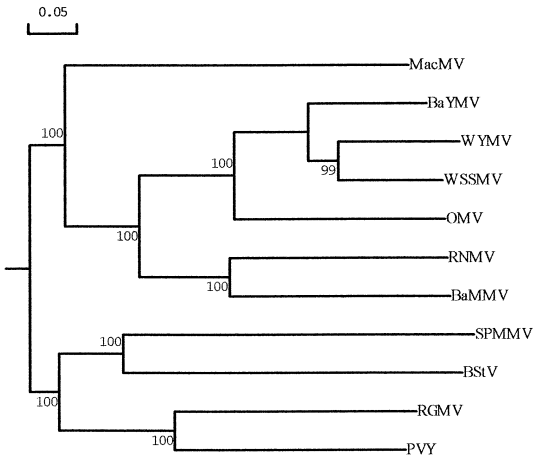

Figure 1. Phylogenetic tree generated from the alignment of the putative coat protein of OMV with other members of the *Potyviridae* family (see text). The tree was produced using DNAMAN (Lynnon BioSoft), which uses the Neighbor-joining method with all default parameters. The numbers at each branch indicate the percentage of 100 bootstrap analysis which support the grouping at that node.

more closely related to that of OMV than to BaMMV and RNMV.

Taking the LQ/A motif as the cleavage site between the NIb and capsid proteins. An alignment of the OMV sequence with other members of the *Bymovirus* genus is shown in Figure 2a,b. Clustal W (http://www.clustalw.genome.ad.jp) and the boxshade server (http://www.ch.embnet.org/software/BOX_form.html) were used to create the shading of aligned sequences, (using the default parameters with the output in PICT format). The alignment of the partial NIb proteins show large blocks of identity between all members which would be expected within this highly conserved protein. The C-terminal of the NIb protein retains a high degree of conserved residues between OMV, WYMV, WSSMV and BaYMV and the LQ/A cleavage site is at the same position. BaMMV and RNMV are more variable in this region, with additional amino acids before the cleavage site (Figure 2a). An alignment of the coat proteins of the six bymoviruses shows that the N-terminal is well conserved between WSSMV, WYMV and BaYMV, but the N-terminal of OMV, RNMV and BaMMV is shorter and not conserved. At the C-terminal region of the coat protein, OMV shares blocks of identity with WSSMV, WYMV and BaYMV (Figure 2b).

A pairwise comparison between the coat proteins of the bymoviruses and OMV, taken from the A residue of the LQA motif, finds OMV to share a high degree of identity with WSSMV, WYMV and BaYMV (50% or more) and to a lesser extent with BaMMV (29.9%) and RNMV (33.8%) (Table 1). The pair comparison data was generated with the Clustal option on the multiple alignment command of GeneJockey (BioSoft), this is based on The Wilbur–Lipman algorithm (using the default parameters). From the phylogenetic tree, multiple alignment and table, OMV appears to have a closer relationship with WSSMV, WYMV and BaYMV than the other bymoviruses RNMV and BaMMV and *vice versa*.

A previous report found that antiserum raised to OMV particles did cross-react with the bymoviruses WSSMV, BaYMV and WYMV (Usugi and Saito, 1981). This relationship is not surprising since OMV shares a high degree of homology, 50% or more at the amino acid level, with the coat proteins of these viruses (Table 1). The serological relationship between WSSMV, BaYMV and WYMV is stronger than that found with these viruses and OMV (Usugi et al., 1989). The sequence data supports this finding; WSSMV, BaYMV and WYMV are closer to each other than to OMV (Figure 1, Table 1).

The Welsh and French isolates of OMV

Oat leaves showing symptoms of OMV infection were obtained from Llanishen, Monmouthshire, Wales and from Preaux, Indre, France. RNA was extracted and cDNA generated according to Clover and Henry (1999). Two primers were designed using alignments to the published sequences of members of the *Bymovirus* genus; BYMO16.2F within the NIb gene (5′-GCG AAA ATC CCT ACA TGT C-3′) and BYMO17.3F within the capsid protein (5′-AAC CAA CCG CAG GGT TCT GG-3′). These primers were used with the primer Oligo d(T) Not1 and the Expand™ high fidelity PCR system (Roche Molecular Biochemicals) to produce products of 1441 and 403 nucleotides respectively (excluding primers), at the 3′ end of the virus genome of both isolates. The PCR thermal cycling conditions were 94 °C for 2 min, then 10 cycles of 94 °C for 45 s, 55 °C for 30 s and 72 °C for 1 min; followed by 20 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 3 min finished by 72 °C for 7 min. Amplified products were observed after electrophoresis in an ethidium bromide-stained 1.2% agarose gel. Cloning was performed with the pGEM-T vector and competent JM109 *Escherichia coli* (Promega). Recombinant

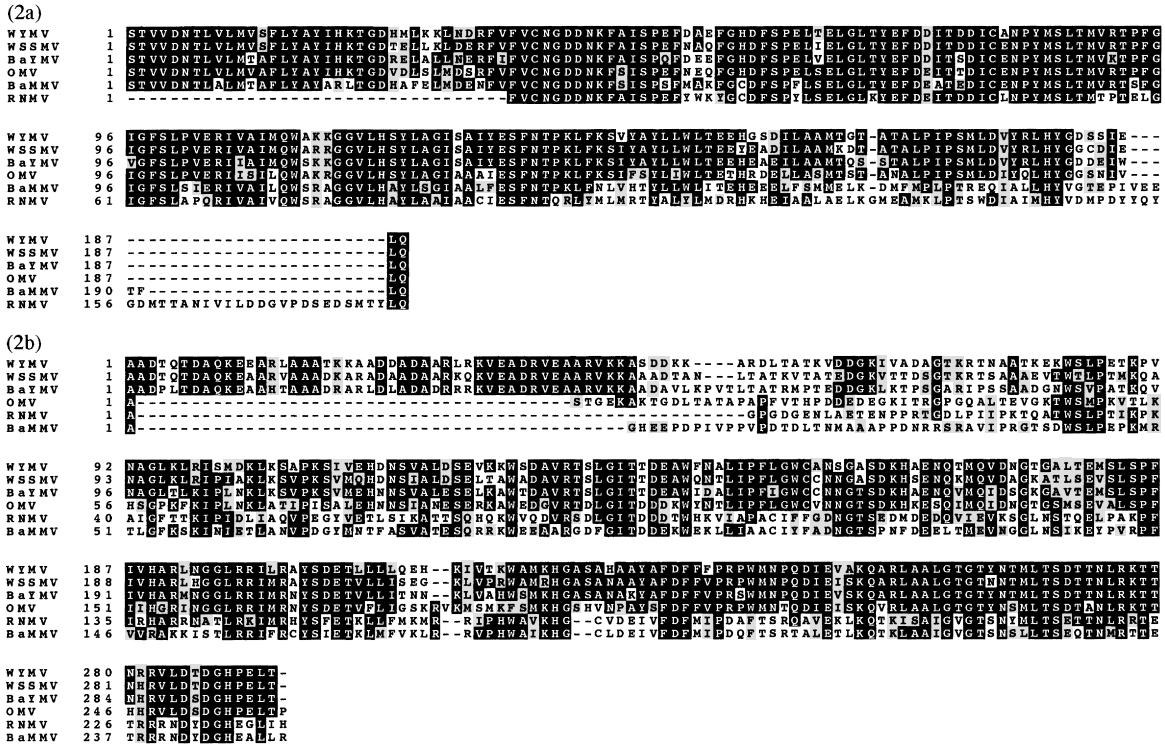

Figure 2. (a) Multiple alignment of the C-terminal amino acid residues of the NIb coding region of the bymoviruses; BaMMV (X69204), BaYMV (X69757), WSSMV (X73883), WYMV (AF067124) and RNMV (U95205) with OMV (AF314536). (b) A multiple alignment of the coat proteins of the bymoviruses with OMV, the N-terminal of the proteins were taken as the A residue of the LQA motif. The alignments was generated with Clustal W and the boxshade server (see text). (www.clustalw.genome.ad.jp and www.ch.embnet.org/software/BOX_form.html). Identical amino acids found with the majority of sequences, are boxed on a black background, chemically similar amino acids are on a grey background.

Table 1. Pair alignments of the putative coat protein of OMV (AF314536) and members of the *Bymovirus* genus RNMV (U95205); BaMMV (X69204); WYMV (AF067124); WSSMV (X73883) and BaYMV (X69757). Alignment data was generated using the Clustal program of GeneJockey II (BioSoft), which uses the Wilbur–Lipman algorithm and all default parameters. The N-terminal of the proteins were taken as the A residue of the LQA motif

	OMV	BaYMV	WSSMV	WYMV	RNMV
BaYMV	56.5				
WSSMV	53.5	72.4			
WYMV	50.0	68.6	73.0		
RNMV	33.8	34.2	32.9	34.2	
BaMMV	29.9	31.9	30.7	33.1	54.5

plasmid DNA was purified using the Wizard Plus DNA purification system (Promega) and sequenced.

Each isolate has an ORF of 1090 nucleotides (362 amino acids) followed by a 3′ UTR of 351 nucleotides, the sequences are available at GenBank, AF318180 (Welsh) and AF318181 (French). The LQA motif of the Welsh and French isolates can be found at position 101–103 of the predicted amino acid sequence. The French and Welsh isolates were well conserved with only 20 nucleotides, 2 amino acid differences found between them. When the French and Welsh isolates were compared against the same region of the English isolate they were found to differ by 20 and 22 nucleotides, 1 and 3 amino acids respectively.

The Irish isolate of OMV

OMV-infected oat leaves were obtained from Cappoquin in County Waterford Ireland. Specific primers designed to the English isolate of OMV (accession No. AF314536), designated OMVA (5′-GCA AGA CGG ACC TGC TTT G-3′) at nucleotide position 1233–1215 and OMVB

(5′-GCT TTG CCA ATA CCA TC-3′) at position 497–513, were used to amplify a region of 701 nucleotides (excluding primers) of the virus genome. The RT-PCR product was generated, purified and cloned as described for the English isolate. The OMVA primer was also tried with the oligo d(T)-Not1 primer but no product was obtained. The Irish isolate may have a shorter poly A tail.

The RT-PCR product represents the C-terminal end of the NIb gene and most of the capsid protein. The nucleotide and predicted amino acid sequences of the Irish isolate of OMV can be found at GenBank, accession No. AF314537. An alignment of the Irish isolate with the other three isolates in this region found that the Irish isolate differs in nucleotide sequence more than the others; from the English isolate by 47 nucleotides; from the Welsh isolate by 42 nucleotides and from the French isolate by 45 nucleotides (Table 2). The nucleotide differences found amongst the four isolates are concentrated at the 5′ end of the overlapping sequences, this corresponds to the C-terminal region of the NIb gene and the N-terminal region of the coat protein. However, most of these changes are silent, not affecting the predicted amino acid sequence. All four isolates share a high degree of identity at the amino acid level, of at least 97.9% (Table 2).

All other members of the *Bymovirus* genus have been wholly or partially sequenced; this paper describes the first sequence data available for OMV. Four isolates of the virus have been partially sequenced from England, Wales, Ireland and France. Despite known differences in the size of particles and RNA between OMV and members of the genus, the partial sequence is consistent with OMV being a member of the *Bymovirus* genus.

The present detection method for OMV relies on electron microscopy of the virus particles and the development of symptoms on a secondary host plant (Zheng Jianhua and Kavanagh, 1975). The virus has been found to be readily amplified from oat leaves with both specific and degenerate primers. Together with the conserved nature of the virus sequence found with isolates from four countries, OMV would appear an ideal candidate for the development of a RT-PCR diagnostic test.

Table 2. Pair alignments of the nucleotide and putative amino acid sequences of the four isolates of OMV; English (AF314536), Irish (AF314537), Welsh (AF318180) and French (AF318181). The region of alignment is the 701 nucleotides between the specific primers OMVA and OMVB (see text). Alignments was performed using the Clustal program of GeneJockey II (BioSoft), which uses the Wilbur–Lipman algorithm and all default parameters

	RNA			Amino acid		
	Ireland	Wales	France	Ireland	Wales	France
England	92.9	98.4	99.1	98.3	98.7	99.6
Ireland		93.9	93.3		97.9	97.9
Wales			98.7			99.1

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