Biological, Serological, and Molecular Variability Suggest Three Distinct Polerovirus Species Infecting Beet or Rape

Sébastien Hauser, Mark Stevens, Christophe Mougél, Helen G. Smith,
Christiane Fritsch, Etienne Herrbach, and Olivier Lemaire

First, sixth, and seventh authors: INRA, URVV, Vection et Lutte Intégrée, 28 rue de Herrlisheim, 68021 Colmar Cedex, France; second and fourth authors: IACR-Broom's Barn, Higham, Bury St. Edmunds, Suffolk, IP28 6 NP United Kingdom; third author: Université Lyon 1, Laboratoire d'Ecologie Microbienne, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France; and fifth author: IBMP (CNRS), 12 rue du Général Zimmer, 67084 Strasbourg Cedex, France.

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ABSTRACT

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Yellowing diseases of sugar beet can be caused by a range of strains classified as *Beet mild yellowing virus* (BMV) or *Beet western yellows virus* (BWV), both belonging to the genus *Polerovirus* of the family *Luteoviridae*. Host range, genomic, and serological studies have shown that isolates of these viruses can be grouped into three distinct species. Within these species, the coat protein amino acid sequences are highly conserved (more than 90% homology), whereas the P0 sequences (open

reading frame, ORF 0) are variable (about 30% homology). Based on these results, we propose a new classification of BMV and BWV into three distinct species. Two of these species are presented for the first time and are not yet recognized by the International Committee on Taxonomy of Viruses. The first species, BMV, infects sugar beet and *Capsella bursa-pastoris*. The second species, *Brassica yellowing virus*, does not infect beet, but infects a large number of plants belonging to the genus *Brassica* within the family *Brassicaceae*. The third species, *Beet chlorosis virus*, infects beet and *Chenopodium capitatum*, but not *Capsella bursa-pastoris*.

Additional keywords: *Beta vulgaris*, *Brassica napus*, host specificity, luteovirus, monoclonal antibody, phylogenetic studies, taxonomy.

Yellowing of beet can be caused by several viruses, especially those belonging to the genus *Polerovirus* of the family *Luteoviridae* (2). The genome of poleroviruses consists of a plus-sense, single-stranded RNA containing six open reading frames (ORFs). All members of the family *Luteoviridae* are phloem limited (25,42) and circulatorily (nonpropagatively) transmitted by several aphid species, including *Myzus persicae*.

The term "beet polerovirus" refers to sugar beet-pathogenic isolates of *Beet mild yellowing virus* (BMV) and *Beet western yellows virus* (BWV). BMV was first reported by Russell (29) in Europe in 1958 as a disease affecting sugar beet. In 1960, Duffus (7) identified in North America a virus that caused yellowing on *Lactuca sativa* (lettuce), *Spinacea oleracea* (spinach), *Raphanus sativus* (radish), and *Beta vulgaris* (sugar beet). Initially named *Radish yellows virus*, the virus was renamed BWV because of its importance in sugar beet. The discovery of BMV in Europe and of BWV in America led Duffus and Russell (9) to search for BWV in Britain. Examination of weeds and crop plants such as lettuce showed that a BWV-like virus was present in hosts previously reported to be immune to BMV. These plants were shown to be infected with a virus resembling the American isolates in transmission characteristics and host range. In 1975, Duffus and Russell (10) conducted host range and serological studies with 12 BMV isolates from English sugar beet crops. All produced a common reaction in certain indicator plants including sugar beet, *Capsella bursa-pastoris* (shepherd's purse), and *Montia perfoliata*. However, BMV isolates failed to infect radish, lettuce, and *Brassica napus* (oilseed rape), which are known to be hosts of BWV (8).

Moreover, a close serological relationship was also shown between the two poleroviruses using polyclonal antisera prepared against BWV and BMV (10,15). These relationships impeded specific detection within plants or aphids and led to overinterpretation of the epidemiology and risk assessment of yellowing diseases due to these two viruses (18). Confusion still exists in the literature between BMV and BWV, and BMV is still considered by some virologists to be a strain of BWV.

In the last decade, several attempts have been made to differentiate BMV and BWV based on their host range. Host range studies by Stevens et al. (36) and Graichen and Rabenstein (16) have shown that BMV infects a number of agronomically important European weed species, and its host range is narrower than that of European isolates of BWV. Isolates of BMV do not infect *Brassica* species. Similarly, European isolates of BWV do not infect sugar beet. Based on these observations, Graichen and Rabenstein (16) concluded that BWV and BMV from Europe were two different virus species and proposed that *Brassica*-infecting isolates of BWV should be renamed *Turnip yellows virus* (TuYV) (16).

Serologically, BMV and BWV cannot be distinguished by using either polyclonal antisera (10,15) or most monoclonal antibodies (MAb) (11,26,33). However, MAb BYDV-PAV-IL-1, raised against *Barley yellow dwarf virus*-PAV (BYDV-PAV, genus *Luteovirus*), reacts with BMV, but not with BWV (3), and can be used to distinguish the two viruses in plants and aphids (34). However, Stevens et al. (37) recently showed that this antibody does not react with all isolates of BMV, enabling identification of a second serotype of BMV.

The comparisons of the nucleotide sequences of a BMV isolate (BMV-2ITB) (17) and a BWV European isolate (BWV-FL1) (41) revealed important differences in the 5' part of both genomic RNAs. Seven riboprobes that were synthesized to cover the

Corresponding author: O. Lemaire; E-mail address: lemaire@colmar.inra.fr

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full-length genome of the two beet poleroviruses (19,21) confirmed this heterology within a wide range of BMV and BWV isolates, allowing specific detection of each viral species by nucleic acid hybridization. Recently, by sequence comparison of the RNA 5' ends of 11 TuYV or BMV isolates, Schubert et al. (32) showed that both viruses cannot be considered isolates of the same virus, as they did not share any common sequence motifs in this part of their genome. Moreover, sequence alignment of the 3' end of the polymerase gene (ORF 2), the intergenic region, and the coat protein (CP) gene (ORF 3) of various BMV and BWV isolates from beet, lettuce, and oilseed rape showed the existence of three groups of viruses (4,5).

Studies conducted in several laboratories around the world have shown that beet polerovirus is composed of at least two distinct virus species, each composed of different strains, differing in their biological, serological, and genomic properties. To summarize the differences between these species and strains, we analyzed all available published data. However, these results have been obtained with various isolates studied within different experimental conditions. To clarify and improve the existing knowledge of the beet poleroviruses and to choose the most appropriate biological, serological, and molecular criteria for classification and distinction, we conducted a variability study in similar laboratory conditions and on isolates from our collection using host plants and antisera already described. Furthermore, we conducted phylogenetic studies on well-conserved sequences like ORF 3 (4,5) and on a variable part of the genome-like ORF 0 (25,32) on the same isolates to further understand the large complexity of beet poleroviruses.

This paper compares the biological, serological, and molecular properties of nine BMV and six BWV isolates collected from a number of countries. Based on these comparisons, we propose a new classification of beet poleroviruses in accordance with the recent proposals of D'Arcy and Mayo (2). This new classification will be submitted to the International Committee on Taxonomy of Viruses.

MATERIALS AND METHODS

Virus isolates. In this study, we used nine BMV isolates originating from sugar beet; four BWV isolates from sugar beet, oil-

seed rape, *Vicia faba* (field bean), and lettuce, respectively; two TuYV isolates from oilseed rape; and one BWV-Colorado isolate from sugar beet (Table 1). The two American isolates, BWV-California and BWV-Colorado, were kindly supplied by G. C. Wisler, USDA, Salinas, CA, and recently presented as being a probable new viral species, *Beet chlorosis virus* (BChV) (22,23). The two TuYV isolates were kindly supplied by J. Schubert, Aschersleben, Germany. Isolates were maintained in host plants in a controlled environment at 20 to 25°C and transferred by serial transmissions using *M. persicae* (21).

Host plants. Host range comparisons were performed on key specific host plants, namely beet (*Beta vulgaris* cv. Trestel) and oilseed rape (*Brassica napus* cv. Jet 9). Graichen and Rabenstein (16) showed that these hosts enable distinction of BMV isolates and BWV European isolates, renamed TuYV. *Capsella bursa-pastoris* (shepherd's purse) was chosen for its ability to distinguish between the two strains of BMV (37). *Chenopodium capitatum* was used because the second strain of BMV and the new American strain of BChV, described by Lewellen et al. (22) and Liu et al. (23), induced severe reddening of leaves and because BWV isolates do not infect this species (9,27).

Virus transmission. A cloned population of *M. persicae* was reared on healthy pepper (*Capsicum annum*), which is a plant species known to be immune to BMV and BWV. To inoculate test plants, aphids were given a 24-h acquisition access period on an infected source plant. Ten aphids were transferred to each healthy test plant for an inoculation access period of 4 days; the plants were then sprayed with an insecticide (4 ml of mevinphos per liter). Infection of test plants was monitored 3 and 6 weeks later by triple-antibody sandwich (TAS)-enzyme-linked immunosorbent assay (ELISA) using MAFF 24 (raised against BMV-1 isolates from the United Kingdom), which is routinely used for detecting BMV and BWV, although it does not discriminate between them (33).

TAS-ELISA. TAS-ELISA was performed as previously described (28,33). Leaves were ground in PBM buffer, composed of phosphate-buffered saline-Tween-polyvinylpyrrolidone with 0.1% dry milk, at 1/5 (wt/vol) dilution. MAFF 24 was diluted at 1 µg/ml in PBM, and anti-rat alkaline phosphatase conjugate was used at 1/1,000 dilution. Optical density (OD) readings at 405 nm were

TABLE 1. Summary of the field, host range, serological, and sequence data for virus isolates used in this study

Virus isolates	Isolate origin ^a				Host range ^c				Serology ^d			Cluster ^e		Accession number ^f	
	Plant host	Region, country	Year	Biotype ^b	Beet	Rape	Caps	Chen	MAb1	MAb2	MAb3	CP	P0	ORF 3	ORF 0
BMV-2ITB	Sugar beet	Eure et Loire, F	1980	BSP	+	–	+	–	+	–	+	5	1	X83110	X83110
BMV-1	Sugar beet	Suffolk, GB	1997	BSP	+	–	+	–	+	–	+	5	1	AF167474	AF168598
BMV-L	Sugar beet	Haut-Rhin, F	1981	BSP	+	–	+	–	+	–	+	5	1	AF167481	AF168604
BMV-J	Sugar beet	Haut-Rhin, F	1990	BSP	+	–	+	–	+	–	+	5	1	AF167480	AF168603
BMV-Iran	Sugar beet	Iran	1992	BSP	+	–	+	–	+	–	+	5	1	AF167479	AF168602
BMV-56r	Sugar beet	Suffolk, GB	1996	BSP	+	–	+	–	+	–	+	5	1	AF167482	AF168599
BMV-Colorado*	Sugar beet	Colorado, USA	1997	BnSP	+	–	–	+	–	–	+	3	3	AF167483	AF168607
BMV-California*	Sugar beet	California, USA	1996	BnSP	+	–	–	+	–	–	+	3	3	AF167485	AF168609
BMV-56	Sugar beet	Suffolk, GB	1994	BnSP	+	–	–	+	–	–	+	4	3	AF167476	AF168605
BMV-2a	Sugar beet	Suffolk, GB	1997	BnSP	+	–	–	+	–	–	+	4	3	AF167475	AF168610
BWV-FL1	<i>Lactuca sativa</i>	Vaucluse, F	1977	Und.	Und.	Und.	+	–	–	–	+	1	2b	X13063	X13063
BWV-Col	Oilseed rape	Haut-Rhin, F	1986	Br	–	+	+	–	–	–	+	2	2a	AF167477	AF168600
BWV-Fev	Field bean	Haut-Rhin, F	1986	Br	–	+	+	–	–	–	+	1	2a	AF167478	AF168601
TuYV-BN5**	Oilseed rape	Gatersleben, D	nd	Br	–	+	+	nd	–	+	+	1	2a	AF167484	AF168606
TuYV-GB**	Oilseed rape	Norwich, GB	nd	Br	–	+	+	–	–	+	+	1	2a	AF167486	AF168608

^a Isolate origin indicates (from left to right) the plant host species where the virus was originally isolated from, the geographic region, and the year in which the original field host was collected. For countries, D = Germany, F = France, GB = Great Britain, and USA = United States.

^b Biotype refers to the classification of isolates based on their host range.

^c Host range results on fodder beet (beet), oilseed rape (rape), *Capsella bursa-pastoris* (caps), and *Chenopodium capitatum* (chen). + and – indicates a positive or negative reaction on an inoculated test plant by triple-antibody sandwich-enzyme-linked immunosorbent assay using monoclonal antibody MAFF 24 (28, 33). Undefined (Und.) means that BWV-FL1M was previously described as being able to infect oilseed rape and beet (E. Herrbach and O. Lemaire, *personal communication*), but in this test, no infected oilseed rape was observed and few beets were infected with low optical density values. nd = Not determined.

^d Positive (+) or negative (–) reactions have been assessed on isolates using monoclonal antibodies BYDV-PAV-IL-1 (MAb1), G4C10 (MAb2), and 3C2 (MAb3).

^e Cluster indicates organization of coat protein (CP) and P0 amino acid sequences as presented in Figures 1 and 2.

^f Accession number of GenBank for ORF 3 and ORF 0, except for BMV-2ITB and BWV-FL1 whose genomic RNA sequence has been published (17,40).

* and ** indicate isolates provided by G. Wisler and J. Schubert, respectively.

taken after 0.5, 1, and 2 h. Threshold values were deduced by calculating the mean of the OD values of six different healthy plants plus three times the standard deviation of these values. MAb BYDV-PAV-IL1 (3) and MAbs G4C10 (28) and 3C2 (12), provided by C. J. D'Arcy and F. Rabenstein, respectively, were used to determine the serological profile of the different isolates.

RNA extraction, cDNA synthesis, and polymerase chain reaction (PCR). Fresh leaf material (100 mg) was ground in liquid nitrogen, and total RNA was isolated using PUREscript kit (Gentra Systems, Inc., Minneapolis, MN) according to the manufacturer's instructions. cDNA was synthesized from purified RNA, as described by Kotewicz et al. (20), and amplified by PCR (30). PCR reactions were carried out using a Hybaid Touchdown thermal cycler (Hybaid, Ltd., Teddington, Middlesex, England): 30 cycles of 30 s at 95°C, 45 s at 50°C, and 60 s at 72°C; followed by one cycle of 10 min at 72°C. The primers used to amplify ORF 3 and ORF 0 of both beet polerovirus isolates are listed in Table 2.

Cloning, sequencing, and phylogenetic analyses. Purified reverse-transcription (RT)-PCR products were cloned in pSK(+) Bluescript plasmid from PCR-Script Amp Cloning Kit (Stratagene Inc., La Jolla, CA), following the manufacturer's instructions. The clones were sequenced in both directions using the SequiTherm EXCEL II Long-Read DNA Sequencing Kit-LC (TEBU, le Perray en Yvelines, France) with IRD41-labelled primers (M13 reverse and M13 forward). The sequences were analyzed by the LI-COR DNA sequencer 4000 L (LI-COR, Lincoln, NE). Three clones for each ORF from each isolate were sequenced to ensure consistent and reliable sequence data.

Sequences were compared and analyzed using the ClustalX program (38) and PHYLIP 3.572 (13), and phylogenetic trees were drawn using neighbor-joining methods (31) or the PileUp program (6). To verify the statistical validity of the clusters obtained on the neighbor-joining tree, a bootstrap value was calculated on 1,000 trials. The nucleotide sequences were deposited in the EMBL nucleotide sequence database. The accession numbers are listed in Table 1.

RESULTS

Biological variability of BMV and BWV isolates. A standardized host range test, using key plant species previously shown to discriminate between BMV and BWV strains, was performed to study the biological variability of beet poleroviruses. Results are summarized in Table 1. The two American isolates, BWV-California and BWV-Colorado, were known to induce severe reddening of *C. capitatum* leaves and interveinal yellowing of beet leaves with vein greening (22). Similar symptoms appeared when using the same hosts inoculated with BMV-2a and BMV-56 isolates from England. In our controlled conditions, BMV-2ITB, BMV-1, BMV-L, BMV-J, BMV-Iran, and BMV-56r isolates did not induce any symptoms on beet or *C. capitatum*.

The hosts range results revealed three distinct biotypes of virus as shown on Table 1. The first two biotypes (beet and shepherd's purse [BSP] and beet but not shepherd's purse [BnSP]) consist of virus isolates that do infect beet but not oilseed rape, hence corre-

sponding to BMV isolates. The third biotype (brassica specific [Br]) consists of isolates that infect oilseed rape but not beet, including the BWV European isolates and TuYV isolates. The results obtained in our host range study are consistent with those presented by Graichen and Rabenstein (16).

Biotype BSP includes isolates that infect shepherd's purse, but not *C. capitatum*, and corresponds to isolates formerly described as BMV. Conversely, biotype BnSP is composed of isolates that are unable to infect shepherd's purse, but can infect *C. capitatum*; this biotype corresponds to BMV European isolates (second strain) and the American isolates (BWV-California and BWV-Colorado) recently described, respectively, by Stevens et al. (37) and by Lewellen et al. (22) and Liu et al. (23).

Isolate BWV-FL1 was previously described as being able to multiply weakly in beet and oilseed rape (E. Herrbach and O. Lemaire, *personal communication*). Under our experimental conditions, no oilseed rape and only 25% of inoculated beet (each having low absorbance values) became infected by BWV-FL1. This was the only isolate exhibiting such a particular host range.

Serological properties. We attempted to correlate the biological and serological properties of the virus isolates using a panel of well-characterized MAbs.

MAb BYDV-PAV-IL-1 (3), raised against BYDV-PAV, recognized all BMV isolates from sugar beet able to infect shepherd's purse. As could be expected from previous studies (37), no positive reactions were recorded with BWV isolates.

MAb G4C10 (28), raised against BWV, reacted here only with the German isolates of TuYV, thereby confirming previous results obtained with this MAb specific to the "second serotype" of BWV from rape in Germany and France (28).

MAb 3C2, raised against BYDV-PAV, reacted with all beet polerovirus isolates under study, even with those previously reported as not recognizable by this MAb, i.e., those detected using MAb G4C10 (12).

Molecular variability within ORF 0 and ORF 3. We sequenced two distinct parts of the genome to estimate the molecular variability of beet polerovirus isolates: ORF 3, which encodes the CP, and ORF 0, which encodes a protein that could be involved in host range specificity (24,40) and symptom expression (39). Alignments of the deduced amino acid sequences and phylogenetic trees (Figs. 1 and 2) were performed using the DNA analysis programs mentioned above. Reliability of the cluster organization was supported by bootstrap values, ranging from 91.4 to 100% on each cluster node in Figures 1 and 2. But for the CP amino acid sequence phylogenetic tree, the relationship between the clusters is not statistically significant (bootstrap value lower than 80%). Except for the BWV-FL1 CP amino acid sequence, we obtained the same cluster organization with each DNA alignment program, although there were differences in the relationship of isolates within clusters. This was due to the different ways the DNA alignment programs assess sequence information. Consequently, we observed only the composition of clusters and not the phylogenetical relation between isolates.

CP amino acid sequence variability was previously reported by de Miranda et al. (4), who characterized three groups of viruses.

TABLE 2. Sequences of primers used for reverse-transcription-polymerase chain reaction amplification

Primer abbreviation	Sequence 5' - 3'	Amplified sequence ^a	Origin of sequence
CP+	ATG AAT ACG GTC GTG GGT AGG AG	ORF 3 of beet polerovirus isolates	BMV-2ITB ^b
CP-	CCA GCT ATC GAT GAA GAA CCA TTG	ORF 3 of beet polerovirus isolates	
BMORF0+	ATG CAA TTT CAG CTT AAA ACA AAC A	ORF 0 of BMV polerovirus isolates	BMV-2ITB ^b
BMORF0-	TCA CAA ACG TAG CTC TTT CTG GAT	ORF 0 of BMV polerovirus isolates	
BWORF0+	ATG CAA TTT CTC GCT CAC GCA AAC A	ORF 0 of BWV and TuYV isolates	BWV-FL1 ^c
BWORF0-	TCA TAC AAA CAT TTC GGT GAT GAC	ORF 0 of BWV and TuYV isolates	
2aORF0+	ATG AAC TTT GAG ATT TGC TTC	ORF 0 of BMV-2a and BMV-56 isolates	BMV-2a
2aORF0-	TTA GGT CTC TCT ATG CAA ACT C	ORF 0 of BMV-2a and BMV-56 isolates	

^a ORF = open reading frame, BMV = *Beet mild yellowing virus*, BWV = *Beet western yellows virus*, and TuYV = *Turnip yellows virus*.

^b Deduced from work by Guilley et al. (17).

^c Deduced from work by Veidt et al. (41).

However, the host range and serological data were incomplete, preventing exploration of the relationships between sequences and biological or serological properties. The CP sequence of the BMV type member (BMV-2ITB), for which the full genomic sequence was determined by Guilley et al. (17), was not included in study by de Miranda et al. Integration of the BMV-2ITB CP sequence within the phylogenetic tree of de Miranda et al. (4) showed that this sequence did not belong to any cluster described in this paper. Contrary to the conclusions of de Miranda et al. (4), BMV isolates should be classified into a distinct cluster. We built an additional phylogenetic tree with the CP amino acid sequences from our study and the consensus sequences determined for the three groups of de Miranda et al. (4) (Fig. 1, *Grp 1*, *Grp 2*, and *Grp 3*). The CP amino acid sequence of beet poleroviruses appears to be highly conserved; the weakest percentage of similarity amongst all CP sequences was 89.8% (ClustalX program). Five different clusters containing isolates sharing a minimum of 95% homology were observed within the phylogenetic tree (Fig. 1). According to our host range studies, all clusters corresponded to a single biotype of virus. The isolates belonging to the Br biotype were found in two different clusters: cluster 1 containing BWYV-Fev and TuYV isolates, and cluster 2 containing BWYV-Col isolates.

The BWYV-FL1 CP amino acid sequence was closer to cluster 2 when the analysis was performed by the PileUp program, as shown by de Miranda et al. (4,5). However, when comparisons were made by the ClustalX program, BWYV-FL1 was closer to cluster 1. Extensive analyses of the sequence homologies showed that the percentage of homology between BWYV-FL1 and *Grp 1* or *Grp 2* was identical (95.2%). When the nucleotides were compared, the BWYV-FL1 5' end of ORF 3 was closer to *Grp 1* and the ORF 3 3' end was closer to *Grp 2*. Although the association of BWYV-FL1 with cluster 1 or 2 mainly depends on the method used for multiple alignment, it is clear that the BWYV-FL1 amino acid sequence is divergent from other BWYV isolates. It is noteworthy that, in both cases, BWYV-FL1 belongs to a cluster containing only Br biotype isolates.

Isolates of the BnSP biotype were divided into two clusters: cluster 3 earlier defined by de Miranda et al. (5) and containing the European isolates (BMV-2a and BMV-56) and a new cluster (cluster 4) containing the American isolates (BWYV-California and BWYV-Colorado). BMV isolates from the BSP biotype formed a new cluster (cluster 5).

Phylogenetic analysis of the P20 amino acid sequence encoded by ORF 4 and overlapping with ORF 3 showed a similar cluster organization with few differences in the relationships between isolates. This reflects a similar evolution of the two proteins, with the location of ORF 4 within ORF 3 providing a significant constraint on the changes allowed within the CP gene. This selection pressure would reduce the ability of the viral CP to evolve and could explain the high rates of similarity between clusters of different biotypes.

Sequence variability on the 5' ends of RNA of BMV and TuYV isolates was conducted in a previous study by Schubert et al. (32), showing that these two virus species were split between two different clusters with faint sequence homology. Similar results were obtained by Guilley et al. (17) using dot-plot methods (6). We performed phylogenetic analyses of the P0 amino acid sequence using our collection of isolates to associate the variability of the 5' end sequences with host range data and CP clustering (Fig. 2). Schubert et al.'s (32) consensus sequences did not entirely cover ORF 0; thus, it was not possible to incorporate them in our study, but we analyzed identical isolates (TuYV-BN5, TuYV-GB, BMV-2ITB, and BWYV-FL1). Our P0 sequence data showed three clusters, corresponding to the three biotypes (BSP, BnSP, and Br) described above. However, the similarity within the P0 cluster (less than 25% identity) was significantly less than the similarity between the CP clusters (more than 89.8%). Furthermore, the amino acid sequences were highly conserved within each P0 cluster (more than

90% identity). Analysis of the amino acid consensus sequence between different P0 clusters showed that the three clusters correspond to three different proteins with no amino acid sequence motifs conserved between clusters. We found that cluster 1 contained isolates of BMV from the BSP biotype and that cluster 2 contained BWYV and TuYV isolates from the Br biotype that did not infect beet. BWYV-FL1 differs from members of other clusters by about 20% within P0. Cluster 3 contained American and European isolates of the BnSP biotype. Our phylogenetic tree (Fig. 2) showed, for the first time, a close relationship between the sequence of P0 and the host range of the virus.

Similar results were obtained when the P1 N-terminal amino acid sequence encoded by part of ORF 1 overlapping with ORF 0 was compared with the P0 sequence (data not shown), reflecting a similar evolutionary pathway for P0 and P1.

DISCUSSION

The use of key beet polerovirus indicator species such as *Cap-sella bursa-pastoris* and *C. capitatum* allowed us to identify three distinct virus biotypes. Each biotype was found to correspond to one of the three clusters deduced from the amino acid sequence of P0. Amino acid sequencing of the CP gene (ORF 3) allowed identification of five clusters, each attributable to a single biotype of virus. MAb BYDV-PAV-IL-1 is commonly used to distinguish between BMV and BWYV, although it does not detect the BnSP biotype. The BnSP biotype represents more than 10% of the BMV iso-

Fig. 1. Phylogenetic tree reconstructed by the neighbor-joining method (31) from the multiple alignment of 15 beet polerovirus coat protein amino acid sequences. *Grp 1*, *Grp 2*, and *Grp 3* represent consensus sequences obtained by de Miranda et al. (4). The scale bar indicates distance in Kimura units, and bootstrap values are given on each cluster node. The five clusters are supported by bootstrap values (in bold), but relationships between clusters are not supported by bootstrap values. Within each cluster, isolates share more than 95% amino acid sequence homology. Minimum similarity observed is 90.4%.

lates found in the English sugar beet crop (37) and has been found in France and other European countries (35). As the three biotypes have less than 30% homology in the P0 sequence and have different serological properties, we propose to distinguish three distinct virus species within the BMV isolates, TuYV and BWYV European isolates, and BWYV American isolates. The proposed species are as follows.

BMV isolates from the BSP biotype are related when compared by their P0 (cluster 1) and CP (cluster 5) amino acid sequences. This BMV species is also distinguishable from other beet poleroviruses using MAb BYDV-PAV-IL-1.

The name *Brassica yellowing virus* (BrYV) could be used to describe European isolates of BWYV, which does not infect beet but infects a large number of plant species belonging to the family *Brassicaceae* (16). TuYV, described by Graichen and Rabenstein (16), can be classified within the BrYV viral species because comparison of the CP and P0 sequences showed a close relationship to other BrYV isolates. However, MAb G4C10 distinguished TuYV from other BrYV, showing that TuYV should be considered a distinct serotype of BrYV, but not a different viral species. BWYV-FL1 appears to be a particular strain differing within the P0 amino acid sequence by only 15 to 20% from other BrYV isolates and is able to infect fodder beet at a low rate when using *M. persicae*.

Although sequence and host range differences between BWYV-FL1 and other BrYV isolates are sufficient to consider it a new species (2), we propose that BWYV-FL1 be considered a strain of BrYV. Most likely, BWYV-FL1 isolated from lettuce in 1977 and then maintained on *Physalis floridana* in Colmar (France) has evolved as a variant, losing its ability to infect or move into oil-seed rape. Interestingly, the variability of CP amino acid sequences within members of BrYV is greater than that within BMV members. This probably reflects the adaptation of isolates to different plant species and vectors, resulting in a broader host range of this viral species. This variability in the CP sequence may also explain the difficulties in raising a common MAb, which would cross-react with all BrYV and BWYV isolates.

The term BChV, suggested by Liu et al. (23) based on symptoms observed in infected beet in California, could be used to describe the American and European isolates from the BnSP biotype. Comparison of CP amino acid sequences showed that this species was composed of two clusters (Fig. 1): cluster 3, previously described by de Miranda et al. (4,5) and corresponding to the European strain of BChV, and cluster 4, corresponding to the American strain. The differences between the two strain clusters could be explained by the evolution of the viruses on different continents and in different environments. This pattern of geographically associated

Fig. 2. Phylogenetic tree reconstructed by the neighbor-joining method (31) from the multiple alignment of 15 beet polerovirus P0 amino acid sequences. The scale bar indicates distance in Kimura units, and bootstrap values are given on each cluster node. Within each cluster, isolates share more than 90% amino acid sequence similarity. Minimum similarity observed is 15.9%.

variation reflects the spatial and temporal isolation of the two viruses and their adaptation to different vector species and host plants. To date, no host range or vector specificity differences were found for these two strains of the BnSP biotype.

Determining the biological, serological, and molecular properties of different isolates under constant conditions has enabled us to better describe the key features of the beet polerovirus group. It would be of great interest to perform similar experiments with more American isolates of beet polerovirus to determine if different BWYV species exist and whether BMVYV occurs in sugar beet crops in the United States.

Knowledge of the sequence diversity among isolates of BMVYV and BWYV within P0 and P3 can be exploited to discriminate between the virus species by using specific primers for RT-PCR or restriction fragment length polymorphism procedures. The accurate and reliable characterization of these species could greatly improve epidemiological and risk assessment studies such as those conducted in the United States (18).

BWYV-FL1 is the only BWYV that has been fully sequenced (41), and an infectious clone was synthesized by Veidt et al. (40). However, this work has shown that BWYV-FL1 should be considered an atypical strain, as its biological and molecular properties were different from those of other BrYV isolates. There is a need to obtain a new full-length genomic RNA sequence from another well-characterized BrYV isolate to estimate these differences. This conclusion is important, since numerous studies are performed using the BWYV-FL1 strain.

The P0 amino acid sequence is highly variable among the three beet polerovirus species. Conversely, there is more than 90% homology within the CP amino acid sequences we studied, and we observed three clusters of the P0 sequence associated to five clusters of the CP sequence. Such incongruent lineage of two parts of the same genome suggests that beet poleroviruses appeared during evolution of the family *Luteoviridae* by recombination of RNA ancestors (1,14). It has been suggested that members of the family *Luteoviridae* share a common ancestor in the 3' region, but different ancestors in the 5' part of the genome (1,14). Variability within the 5' region of the viral RNA is strongly correlated to the different biotypes, indicating that the protein domain involved in controlling host range could be located in the 5' region of the luteoviral genome. This hypothesis is supported by results obtained with BWYV-FL1, since the CP amino acid sequence of BWYV-FL1 is similar to that of BrYV-GB (more than 95% homology), whereas only 81% homology was found for the P0 amino acid sequence; these two isolates show distinct biological properties in beet and oilseed rape.

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