Detection of Cymbidium Mosaic Virus, Odontoglossum Ringspot Virus, Tomato Spotted Wilt Virus, and Potyviruses Infecting Orchids in Hawaii

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ABSTRACT

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Approximately 3,600 orchid plants representing 44 genera from three orchid collections, 22 commercial farms, and six nurseries on the islands of Oahu and Hawaii were tested for Cymbidium mosaic virus (CyMV), Odontoglossum ringspot virus (ORSV), tomato spotted wilt virus (TSWV), and potyviruses with enzyme-linked immunosorbent assay (ELISA). CyMV was detected in 59 samples and ORSV was detected in 23 samples, which represented 61 and 25% of the 44 genera surveyed, respectively. Double infection with both viruses occurred in 20 samples, representing 20% of the genera. CyMV and ORSV were detected in 29 and seven of the 31 sites surveyed, respectively. When 330 cloned orchid samples were tested, CyMV was detected in 45% and ORSV in 9% of the clones. Most commercial *Dendrobium* orchids grown in Hawaii are seed-propagated hybrids produced by the Horticulture Department at the University of Hawaii (UH). Only 4% of the 758 UH *Dendrobium* hybrids less than three years old were infected by CyMV. Of 2,381 UH *Dendrobium* hybrids more than 3 yr old, CyMV was detected in 94% of the samples from some farms, but only 2% from other farms. ORSV was not identified from any commercially grown UH *Dendrobium* hybrids. TSWV-infected *Oncidium* orchids, found in only one nursery in Hawaii, exhibited symptoms ranging from chlorotic ring spots to necrotic lesions 1–2 cm in diameter. The TSWV infection was localized in the ring spots and lesions. Dendrobium mosaic potyvirus was detected in *Dendrobium superbum*.

Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) are the most prevalent and economically important viruses infecting orchids worldwide (13,20). CyMV induces floral (19) and foliar (4) necrosis, and ORSV causes ring spots on leaves and color breaking on flowers (11). Mixed infections of both viruses can cause blossom brown necrotic streak (17). The viruses also reduce plant vigor and lower flower quality, which affects economic value (15). These viruses are very stable and are spread by contaminated tools and pots (13).

Several potyviruses (e.g., bean yellow mosaic virus, turnip mosaic virus, and Dendrobium mosaic virus) infect orchids (10,20). Aphid vectors of potyviruses are occasionally observed on orchids on commercial orchid farms in Hawaii. Tomato spotted wilt virus (TSWV) is the most devastating and widespread plant virus in Hawaii (1). It has a wide host range, infecting at least 192 dicotyledonous species in 33 families and eight monocotyledonous species in five families (2), and causes economic losses in many vegetable and ornamental crops. Two of its vectors, *Frankliniella occidentalis* Pergande and *F. palmi* Karmy, are common insect pests on commercial orchids in Hawaii. Therefore, potyviruses and TSWV were included in this survey.

The Hawaiian orchid industry is expanding rapidly. *Dendrobium* orchid hybrids, one of the most important cut and potted floricultural crops grown in Hawaii, had a wholesale value of $6.3 million in 1990 in Hawaii; and demand is increasing both locally and in neighboring countries such as Japan. CyMV and ORSV infections have been problems in the Hawaii orchid industry for many years. However, since these viruses cause diseases in some orchids but can be symptomless in others (13,20), it was not known how widespread and important the viruses are. Furthermore, it is not known whether other orchid viruses are present in Hawaii. Therefore, this study was undertaken to determine the incidence and distribution of CyMV and ORSV in Hawaii, and the presence of TSWV and potyviruses in orchids in Hawaii. A preliminary report of this work has been published (9).

MATERIALS AND METHODS

Antisera production. CyMV and ORSV were purified from infected *Dendrobium* orchid leaves, CyMV by the method of Frowd and Tremaine (5), and ORSV by the method of Paul et al (16). Purified virus preparations were injected into New Zealand white rabbits at multiple sites on the back, weekly for three consecutive weeks (8). The first immunization consisted of 1 ml of purified virus (1 mg) mixed with 1 ml of Freund's complete adjuvant. The two subsequent injections consisted of 0.5 mg of purified virus mixed with 1 ml of Freund's incomplete adjuvant. The rabbits were bled 1 wk after the third injection and every week thereafter. Ouchterlony double-diffusion assay was used to check the specificity of the antisera (18). Immunoglobulin G (IgG) was purified by protein A column chromatography (6). The purified IgG was conjugated to alkaline phosphatase type VII (Sigma, St. Louis, MO) for use in enzyme-linked immunosorbent assay (ELISA) (3).

ELISA. The standard 2-day procedure of double antibody sandwich (DAS) ELISA was used for detection of CyMV, ORSV, and TSWV (3,7). An antibody specific to TSWV lettuce strain was obtained from D. Gonsalves, Cornell University. A simple ELISA procedure was developed to rapidly check samples for CyMV and ORSV. Briefly, ELISA plates were coated with 2 μg/ml of purified IgG and stored at 4 C. Virus preparations of 50 μl (1:10 in ELISA extraction buffer) and 50-μl virus-specific antibodies conjugated with alkaline phosphatase (1:500 in enzyme buffer) were mixed in ELISA plate wells and incubated at 37 C for 2 hr. Indirect ELISA by a monoclonal antibody against a shared epitope of potyviruses (MAb-PTY 1, supplied by Ramon L. Jordan, USDA-ARS, Beltsville) was used for the detection of potyviruses (12). A_{405nm} was measured with a Model 450 Microplate Reader about 60 min after the addition of the substrate. In all steps, 100 μl of plant extracts was placed in each ELISA plate well. Controls in all ELISA tests included virus extraction buffer, healthy orchid, and known infected samples. A reaction was considered positive only if the absorbance was at least four times higher than the background range of healthy control.

Electron microscopy. Negative staining of viruses, leaf dip, and immunosorbent electron microscopy (ISEM) tests were done as described by Hu et al (8). Grids were stained with 2% uranyl acetate for 1 min and examined with a Phillips electron microscope.

Sampling and testing. Orchid samples (3,602) were collected from three orchid collections and 28 commercial farms and nurseries on the islands of Oahu and Hawaii. Most commercial *Dendrobium*

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orchids grown in Hawaii are seed-propagated hybrids produced at the University of Hawaii (UH *Dendrobium* hybrids). In each farm or nursery, UH *Dendrobium* hybrids of different age groups (less than 3 yr old or more than 3 yr old) were tested. From each hybrid (or age group), 10 leaf samples were collected and tested individually. For clonal materials, five leaves per clone were combined and tested as a single sample. Some samples were tested for CyMV by both ELISA and bioassay on indicator plants (*Cassia occidentalis* L.) to compare the reliability of the two assays. After demonstrating the reliability of ELISA, most of the orchid samples were tested for CyMV, ORSV, TSWV, and potyviruses only by ELISA.

RESULTS

Development of detection assays. The specific antisera produced against CyMV and ORSV in New Zealand white rabbits had high titers to homologous viruses but no reaction to healthy orchid samples or to heterologous viruses (Table 1). In ELISA, the monoclonal antibody against potyviruses (MAb-PTY 1) reacted with all potyviruses tested, including potato virus Y, papaya ringspot virus, watermelon mosaic virus II, zucchini yellow mosaic virus, tobacco etch virus, and turnip mosaic virus; it did not react with cucumber mosaic virus, CyMV, ORSV, or TSWV. CyMV and ORSV were reliably detected with the simple direct ELISA procedure, which provided results in 3–4 hr (*unpublished*). Dilution end point (DEP) determinations were done to compare the sensitivity of ELISA, ISEM, and bioassay for the detection of CyMV and ORSV. Results from this test indicated that ELISA was more sensitive than ISEM and that ISEM was more sensitive than the bioassay for the detection of both CyMV and ORSV from plant sap (Table 2). The DEP of ELISA was 10 times higher than that of ISEM. Further effort was made to compare the reliability of ELISA to that of bioassay for the detection of CyMV from field samples. Fifty unknown samples were tested by both ELISA and bioassay. The results of ELISA and bioassay for detecting CyMV from field samples were similar (Table 3).

Detection of CyMV and ORSV. Of the 132 samples assayed, CyMV was detected in 59 samples and ORSV was detected in 23 samples, representing 61 and 25% of the 44 orchid genera surveyed, respectively (Table 4). Double infection by both CyMV and ORSV occurred in 20 samples, representing 20% of the genera tested (Table 4). CyMV and ORSV were detected at 29 and seven of the 31 sites surveyed, respectively. When 330 cloned orchid samples from 22 commercial farms and six nurseries were tested, CyMV was detected in 45% of the clones and ORSV in 9% (Table 5). CyMV was detected from cloned orchid samples at the early stage of plant development, i.e., when the orchid shoots were in the original flasks. Of 758 UH *Dendrobium* hybrids less than 3 yr old, only 33 (4%) were found to be infected with CyMV (Table 6). Of 2,381 UH *Dendrobium* hybrids more than 3 yr old, incidence of CyMV was 94% on some farms, but only 2% on others (Table 6). ORSV was not identified from any commercially grown UH *Dendrobium* hybrids.

To address the possibility of seed transmission of CyMV, extensive ELISAs were done for three orchid farms from which orchid samples were virus free in the initial survey (Table 7). On two farms (Farm I and II), we did not detect any virus in 464 UH *Dendrobium* hybrids. However, on Farm III, CyMV infection was detected in four of 911 samples tested (Table 7).

Detection of TSWV. TSWV-infected orchids (*Oncidium* cultivar Gower Ramsey) exhibiting symptoms ranging from chlorotic ring spots to necrotic lesions 1–2 cm in diameter were found in a nursery on Oahu (cover, April 1992 PLANT DISEASE). The orchid plants were about 4 yr old and were being grown adjacent to chrysanthemums which were severely infected with TSWV. TSWV was detected by ELISA in ring spots and lesions with an antibody specific to TSWV. Samples from symptomless areas of infected leaves, new growth on infected

Table 1. Development of enzyme-linked immunosorbent assays (ELISAs) for detection of viruses from orchids[a]

Viruses	A_{405nm} in ELISA with antibodies against viruses[b]			
	CyMV	ORSV	TSWV	Potyviruses
CyMV	0.807	0.067	0.044	0.063
ORSV	0.017	1.357	0.042	0.055
TSWV	0.007	0.034	1.234	0.126
Potyviruses[c]	0.060	0.043	0.011	1.372
Healthy orchid	0.023	0.016	0.029	0.053

[a] Double antibody sandwich (DAS) ELISA was used for detection of Cymbidium mosaic virus (CyMV), Odontoglossum ringspot virus (ORSV), and tomato spotted wilt virus (TSWV) with specific polyclonal antibodies. Coating antibodies (1 μg/ml) and conjugated antibodies (1:1,000) were used for all three antibodies. An indirect ELISA was used for detection of potyviruses, in which a monoclonal antibody (1:2,000) against a universal epitope of potyviruses (MAb-PTY 1, supplied by Ramon L. Jordan, USDA-ARS, Beltsville) was used.

[b] The absorbance readings were means from several tests. in each ELISA, homologous and heterologous controls were included.

[c] Potyviruses tested were potato virus Y, papaya ringspot virus, watermelon mosaic virus II, and zucchini yellow mosaic virus, which were in separate herbaceous plants. Absorbance readings for all the potyviruses tested were high in several tests. The absorbance reading given here is a mean for all the potyviruses tested and averaged over several tests.

Table 2. Comparison of dilution end points of Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) in three assays[a]

Sap dilutions	CyMV			ORSV		
	ELISA	ISEM	Bioassay	ELISA	ISEM	Bioassay
1:10	+	+	+	+	+	+
1:100	+	+	+	+	+	+
1:1,000	+	+	+	+	+	+
1:10,000	+	+	−	+	+	−
1:100,000	+	+	−	+	+	−
1:1,000,000	+	−	−	+	−	−
1:10,000,000	−	−	−	−	−	−

[a] Specific polyclonal antibodies were used in double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and immunosorbent electron microscopy (ISEM). In ELISA, positive results (+) reflect absorbance readings at least four times higher than healthy orchid. In ISEM, at least ten EM fields were counted. In the bioassay, *Cassia occidentalis* and *Chenopodium amaranticolor* were the indicator plants for CyMV and ORSV, respectively. The comparison tests were repeated three times.

Table 3. Detection of Cymbidium mosaic virus (CyMV) from orchid samples in two assays[a]

Dendrobium hybrids	No. of positive samples/ no. of samples tested	
	ELISA	Bioassay
UH 44	2/10	2/10
UH 306	4/20	3/20
UH 503	1/10	1/10
UH 507	1/10	1/10
Total	8/50	7/50

[a] Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used for detection of CyMV. *Cassia occidentalis* was the indicator host in bioassay for detection of CyMV. Unknown samples were ground in phosphate-buffered saline at 1:10 ratio for bioassay. The samples were then diluted 1:2 with ELISA sample extraction buffer and used in ELISA.

plants, and roots were free of TSWV. At this one location, 50–100% of the *Oncidium* plants had the chlorotic ring spots to necrotic lesions. Typical TSWV virions of 80–100 nm in diameter were purified from the infected *Oncidium* orchids (*unpublished*). No TSWV was detected by ELISA in healthy *Oncidium* control plants. CyMV, ORSV, and potyviruses were not found in the infected *Oncidium* orchids. *Dendrobium* orchids (109 plants of hybrids UH 232 and 44) grown adjacent to TSWV-infected chrysanthemum were TSWV-free.

Detection of potyviruses. Of all the samples tested, only samples of *Dendrobium superbum* Reichb. (known in Hawaii as the honohono orchid) were positive in ELISA to MAb-PTY 1. Chlorotic mosaic and distortion symptoms were observed on leaves of the honohono orchids. Diseased honohono orchids also showed symptoms of color breaking and distortion of the flowers. Flexuous rod-shaped, potyvirus-like particles were observed in leaf samples examined by electron microscopy with negative staining. Samples collected from 10 symptomless and 18 symptomatic honohono *Dendrobium* orchids were tested by double antibody sandwich direct ELISA for CyMV, and by indirect ELISA for potyviruses. Of 28 samples tested, 26 were positive for CyMV, and all 18 symptomatic samples were positive for potyviruses. A single sample from a symptomatic plant was CyMV negative but potyvirus positive. When potyvirus-free honohono orchids were mechanically inoculated with the potyvirus, the plants became infected and similar symptoms occurred. Samples from diseased honohono orchids were tested by ELISA for different potyviruses by Agdia, Inc., and they were negative for potyviruses that included bean common mosaic virus, potato virus Y, papaya ringspot virus, watermelon mosaic virus II, tobacco etch virus, zucchini yellow mosaic virus, soybean mosaic virus, potato virus A, and dasheen mosaic virus.

DISCUSSION

CyMV and ORSV are widespread in Hawaii, with CyMV being prevalent. About 45% of cloned orchids were infected by CyMV. Because of the level of incidence, it is necessary to index orchid materials before vegetatively propagating plants. Orchids from other countries should be tested with rapid and sensitive assays before their introduction into the United States. ELISA is a more rapid method for detecting CyMV and ORSV than mechanical inoculation bioassay, and it may replace bioassay in regular indexing programs. ISEM is also a sensitive and rapid assay, but it is not convenient for indexing many samples.

The orchid industry in Hawaii is unique in that most commercial orchids (>75%) are *Dendrobium* orchids, which are seed-propagated hybrids produced at the University of Hawaii (UH *Dendrobium* hybrids). A previous study showed that a low proportion of seed transmission of CyMV (one seedling out of 123) was observed in *Dendrobium* (19). In our survey, we tested 1,375 samples from three orchid farms where CyMV was not detected during an initial screening. Our hypothesis was that if we detected no

Table 4. Detection of Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) in cultivated orchids in Hawaii[a]

Genus	No. of samples infected/no. of samples tested		
	CyMV	ORSV	Both
Angraecum	1/1	0/1	0/1
Ansellia	1/1	0/1	0/1
Aranda	3/3	0/3	0/1
Brassavola	4/6	4/6	4/6
Brassolaeliocattleya	1/2	0/2	0/1
Brassidium	2/2	2/2	2/2
Broughtonia	1/1	0/1	0/1
Calanthe	0/1	0/1	0/1
Catasetum	1/2	2/2	1/2
Cattleya	18/36	4/36	3/36
Caularthron	1/1	1/1	1/1
Cymbidium	1/4	1/4	1/4
Dendrobium	16/25	1/25	1/25
Doritis	1/1	0/1	0/1
Encyclia	1/2	0/2	0/1
Epidendrum	2/4	1/4	1/4
Epiphronitis	0/3	0/3	0/3
Gongora	1/1	0/1	0/1
Grammatophyllum	1/1	0/1	0/1
Haemaria	0/1	1/1	0/1
Laelia	0/1	0/1	0/1
Laeliocattleya	1/2	0/2	0/2
Maxillaria	0/1	0/1	0/1
Miltonia	1/2	0/2	0/2
Neofinetia	1/2	0/2	0/2
Oncidium	5/9	2/9	2/9
Paphiopedilum	0/2	0/2	0/2
Phalaenopsis	5/8	4/8	4/8
Rhynchostylis	0/1	0/1	0/1
Spathoglottis	1/1	0/1	0/1
Trichoglottis	1/1	0/1	0/1
Vanda	1/2	0/2	0/2
Vandopsis	1/1	0/1	0/1
Vanilla	1/1	0/1	0/1
Total plants	59/132	23/132	20/132
	45%	17%	15%
Total genera	27/44	11/44	10/44
	61%	25%	20%

[a] Orchid samples were collected from Foster Botanical Garden, Lyon Aboretum, H & R Nursery, and the Department of Horticulture, University of Hawaii. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to detect CyMV, ORSV, and tomato spotted wilt virus (TSWV), and indirect ELISA was used for potyviruses. TSWV and potyviruses were not found in these samples.

Table 5. Detection of viruses from cloned orchids by enzyme-linked immunosorbent assay (ELISA)[a]

Island	Area (no. of sites)	No. of positive samples/no. of samples tested			
		CyMV	ORSV	TSWV	Potyvirus
Oahu	Honolulu (3)	58/82	20/82	0/82	1/82
	Kahaluu (5)	18/20	0/20	0/20	0/20
	Mililani (1)	0/2	0/2	1/2	0/2
	Waianae (4)	5/15	3/15	0/15	0/15
	Waimanalo (8)	34/129	5/129	0/129	0/129
Hawaii	Hilo (6)	15/27	0/27	0/27	0/27
	Kona (4)	17/56	0/56	0/56	0/56
	Totals	149/331	28/331	1/331	1/331
		45%	9%	0.3%	0.3%

[a] Different orchids (*Dendrobium, Cattleya*, etc.) were collected from Oahu and Hawaii islands and tested in double antibody sandwich (DAS) ELISA for Cymbidium mosaic virus (CyMV), Odontoglossum ringspot virus (ORSV), and tomato spotted wilt virus (TSWV); potyviruses were tested in indirect ELISA. Usually five leaves from different plants were combined as one sample for each clone. In reconstitution experiments, the viruses were detected from one infected in five healthy leaf disk samples.

CyMV infection in 1,375 plants, all of which were more than 5 yr old, then CyMV was not likely to be seed transmitted. From Farm III, four of 911 plants were found to be infected with CyMV. Because there were no other CyMV sources in that nursery, it is possible that the virus originated from infected seeds. However, since some seedlings were obtained in community pots from a nursery which had many CyMV-infected orchids, it is also possible that a few UH *Dendrobium* hybrid seedlings were previously contaminated. It is still not known whether the seeds were virus infected or the infection arose from contamination on the farms. The possibility of seed transmission needs to be more carefully examined.

TSWV-infected *Oncidium* orchids from a nursery in Hawaii were apparently an isolated case, because TSWV was not detected in about 3,600 other orchid samples in this survey. The 8,000 *Oncidium* orchids were being grown adjacent to chrysanthemums severely infected with TSWV. When the chrysanthemums were removed, it is possible that viruliferous thrips may have moved to the *Oncidium* orchids and transmitted TSWV to them. However, the TSWV infection was found to be localized in the symptomatic tissues, and the growers were able to remove the infected leaves and thereby reduce economic losses. Because *Dendrobium* plants from the same portion of this nursery were not infected by TSWV, TSWV probably does not infect *Dendrobium* orchids.

Honohono *Dendrobium* orchids were found to be infected by a potyvirus in this survey. Our results suggest that the mosaic and distortion symptoms on honohono orchids are caused by the potyvirus. The potyvirus was found only in honohono *Dendrobium* orchids, and not in any others surveyed. Forty years ago, Murakishi described a mosaic virus disease on honohono orchids in Hawaii (14). In 1973, Inouye characterized a potyvirus associated with Dendrobium mosaic disease in Japan, but he did not compare the Dendrobium mosaic virus serologically with other potyviruses (10). Based on our preliminary information, the honohono Dendrobium mosaic potyvirus is not serologically related to bean common mosaic virus, potato virus Y, papaya ringspot virus, watermelon mosaic virus II, tobacco etch virus, zucchini yellow mosaic virus, soybean mosaic virus, potato virus A, and dasheen mosaic virus. Further characterization of the Dendrobium mosaic potyvirus is in progress.

It has been suggested that CyMV could be controlled by using virus-free orchid materials and good sanitation practices (18). In this survey, we observed that on many farms on which growers had collections of diverse orchid species, some of which were 8–12 yr old, the CyMV infection incidence in UH *Dendrobium* hybrids 4–8 yr old was more than 90%. However, on young orchid farms or nurseries, or ones with fewer orchid varieties, whose growers paid more attention to virus control, the CyMV incidence was very low (on average, 2%). The CyMV incidence was zero in two nurseries that employed strict sanitation measures (18). These findings demonstrate that successful CyMV control could be obtained with strict sanitation practice. However, because CyMV is stable and is spread easily, once the virus is introduced into a nursery either through seeds or contaminated plants, widespread infection can occur rapidly. Therefore, because of the great stability of the virus, the possibility of seed transmission, the existence of symptomless hosts, and the continued manipulation of mature plants during harvesting procedures, cultural practices alone may not be sufficient to control CyMV's spread in the Hawaiian orchid industry. An integrated approach may provide more complete control of CyMV in orchid.

Table 6. Detection of CyMV in UH *Dendrobium* hybrids from commercial farms[a]

Dendrobium hybrids	No. of positive samples/no. of samples tested		
		> 3 Yr old	
	1-3 Yr old	Group I[b]	Group II
UH 44	3/80	67/75	2/240
UH 232	3/144	92/97	7/614
UH 306	7/222	140/148	26/513
UH 503	6/50	121/130	5/50
UH 507	12/90	60/60	4/432
UH 630	3/30	20/22	...
UH 800	3/80	...	...
UH 919	5/32	...	...
UH 955	2/30	...	...
Totals	33/758	500/532	20/1,849
	4%	94%	2%

[a] UH *Dendrobium* hybrid samples were collected from 28 orchid farms and nurseries on the islands of Oahu and Hawaii. The samples were tested in double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for Cymbidium mosaic virus (CyMV), Odontoglossum ringspot virus (ORSV), tomato spotted wilt virus (TSWV), and potyviruses.

[b] In group I farms, growers had diverse orchid species, and many of the orchids were 8–12 years old. In group II farms, growers cultivated fewer orchid varieties and had young orchid farms or nurseries.

Table 7. Detection of CyMV in *Dendrobium* orchid hybrids from three farms[a]

Dendrobium hybrids	No. of positive samples/no. of samples tested		
	Farm I	Farm II	Farm III
UH 44	...	...	0/180
UH 232	0/120	0/113	1/290[b]
UH 306	0/120	...	0/180
UH 507	...	0/111	3/261[b]
Total	0/240	0/224	4/911

[a] Orchid plants were more than 5 yr old. Cymbidium mosaic virus (CyMV) was detected in double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

[b] Typical CyMV lesions on leaves were observed. CyMV infection was confirmed in ELISA, bioassay, and electron microscopy.

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