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Inactivation by Chemical Disinfectants *in vitro* against Tobacco Mosaic Virus

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화학적 제어제에 의한 담배모자이크 바이러스의 불활성화

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Numerous chemicals were tested to show antiviral activity *in vitro* against tobacco mosaic virus (TMV). With a brief exposure of TMV to 1 N HCl or 1-0.1 N NaOH, virions and their encapsidated RNAs were degraded completely and rapidly. When TMV was exposed to 0.1 N HCl, the hydrolysis of viral capsid in 5 min after treatment was observed in the 1% agarose gel. Virions and their encapsidated RNAs were not degraded by 0.01N HCl or 0.01N NaOH. These characteristics indicate that a short exposure to optimal concentration of acid or base is of practical value in eliminating infectious virus. The treatment of 50% isopropanol or UV light did not damage in viral integrity or their encapsidated RNAs. Disinfection of the agricultural tools and laboratory equipments using appropriate disinfectants is necessary to prevent cross contamination in farm and laboratory.

담배모자이크 바이러스에 대한 항바이러스 활성을 검사하기 위해 다양한 화학제를 처리한 결과, 1N HCl과 0.1-1N NaOH에 바이러스와 외피에 보호된 RNA가 완전하고 신속하게 분해되었다. TMV를 0.1N HCl에 처리하였을 때 바이러스의 외피단백질은 산의 가수분해에 의해 부분 분해되었으나, 0.01N HCl 혹은 0.01N NaOH에 처리했을 때는 분해되지 않았다. 위의 결과에서 적절한 산이나 알칼리에 처리하는 것은 바이러스를 제거하는데 효과적인 가치가 있음이 판명되었다. 50% isopropanol과 UV처리는 바이러스와 외피화된 RNA에 어떤 효과도 미치지 않았다. 농장의 농기구와 실험실 기구를 바이러스의 오염으로부터 방지하기 위해서는 적절한 화학제의 적용이 필요하다.

Key words: chemicals, antiviral activity, TMV, virions, encapsidated RNAs, disinfection

I. Introduction

Numerous chemicals have been tested to show antiviral activity either *in vitro* or *in vivo* against a range of bacteriophages (Yahya et al.,

1992; Maillard et al., 1994; Pesaro et al., 1995), animal viruses (Ma et al., 1994; Rivas et al., 1994), and plant viruses (Kaper, 1975; French and Towers, 1992; Malhotra et al., 1996), of which have genetic materials as DNA or RNA. In three dimensional structure of viruses, viral coat proteins and nucleic acids are bound together in a geometric manner by a variety of covalent and noncovalent bonds. The interactions between these structural componenets are included such as protein-protein, protein-nucleic acids, and nucleic acids-nucleic acids.

Among the plant viruses there is a wide range of stabilities. Viruses like turnip yellow mosaic tymovirus (TYMV) with strong protein-protein interactions are the most stable, while others like cucumber mosaic cucumovirus(CMV) predominate with protein-RNA interactions (Kaper, 1975). The effect of pH, ionic strength, kind of ion, temperature, compounds such as phenol and detergents, and hydrogen bond-breaking agents on the stability of viruses have been described (Kaper, 1975). In addition, some divalent metal ions such as Zn++ (Sehnke and Johnson, 1994) and Ca++ (Krüse et al., 1982) may have a marked effect on the stability or infectivity of some viruses. Extreme conditions such as high pH revealed the release of RNA from TYMV particles (Keeling and Matthews, 1982). Alkaline conditions have been used to demonstrate the removal of protein subunits from the TMV rod, beginning at the 5' end, and to reveal intermediates in the stripping process, these being attributed to regions of unusually strong interaction between the protein and RNA (Perham and Wilson, 1978).

The purpose of this study was to expose TMV to a variety of chemical disinfectants for the stability of the virus and to evaluate decontamination procedures currently used in environmental virology to optimize the conditions for each disinfectant's use for equipment and tool decontamination of TMV, which is easily contaminated by man during handling agricultural tools.

II. Materials and Methods

1. Inactivation by acid and base

The following chemicals were tested for their ability to degrade viruses or their encapsidated RNAs. For acid or base inactivation experiments, final concentration of 1-0.01 N HCl and 1-0.01 N NaOH mixed with TMV(final conc. 40 μg), respectively, and exposed for 1-60 min. At selected time intervals, 10 μl of aliquot was neutralized with equal volume of opposite chemical and buffered to pH 7.2 with 10 μl of a 1-0.01 M HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; Sigma, USA).

2. Inactivation by isopropanol and UV

Fifty% of isopropanol (final conc.) was mixed with TMV (final conc. 40 μg) and the resulting mixture exposed for 5-30 min. After the incubation period, 10 μl of aliquot was neutralized by addition of three volumes of electrophoresis sample buffer. UV treatment for TMV inactivation was tested, respectively, for 5-20 min at 5mW/cm2 and for 5-10 min at 7mW/cm2 intensity. All experiments were conducted at room temperature and a minimum of duplicate experiments was performed for each disinfectant evaluated.

3. Electrophoresis

Electrophoresis of virions or encapsidated viral RNAs was performed in 1% agarose in 0.5 X TBE buffer, pH 8.3 at constant 100 V at 4°C. The gel for virion analysis was stained with Coomassie Brilliant Blue, while the gel for encapsidated RNA analysis was stained with ethidium bromide (1 μg/ml).

III. Results and Discussion

The differences in electrophoretic behavior were more distinguished when TMV after

treatment with HCl or NaOH than other treatments. With a brief exposure of TMV to 1 N HCl or 1-0.1 N NaOH virions and their encapsidated RNAs were degraded completely and rapidly (Table 1 and 2).

Table 1. Degradation of TMV and encapsidated RNA by 1-0.01 N HCl

HCl concentration (N)		Exposure time (min)							
		0	5	10	15	20	30	45	60
1	virion	ND[a]	CD[b]	CD	CD	CD	CD	CD	CD
	RNA[d]	ND	CD	CD	CD	CD	CD	CD	CD
0.1	virion	ND	PD[c]	PD	PD	PD	PD	PD	PD
	RNA	ND	PD	PD	PD	PD	PD	PD	PD
0.01	virion	ND	ND	ND	ND	ND	ND	ND	ND
	RNA	ND	ND	ND	ND	ND	ND	ND	ND

[a]ND, no degradation detected
[b]CD, complete degradation
[c]PD, partial degradation
[d]RNA, encapsidated RNA

Results of experiments in which TMV was exposed to either 1 N HCl or 1 N NaOH revealed that maximum less than 5 min was enough to render TMV undetectable by viral and encapsidated RNA analysis. When TMV was exposed to 0.1 N HCl, the hydrolysis of viral capsid in 5 min after treatment was observed (Fig. 1). Exposure to 0.1 N NaOH destroyed the virions and their encapsidated RNAs more efficiently than did exposure to 0.1 N HCl. These results are not surprising since viral RNA is extremely sensitive to degradation by akali (Perham and Wilson, 1976, 1978; Wilson and Perham, 1985; Ma et al., 1994). Analysis by agarose gel electrophoresis revealed that virions exposed at 0.01 N HCl or indicate that a short exposure to optimal concentration of acid 0.01 N NaOH had no detactable degradation. These characteristics or base is of practical value in eliminating infectious virus.

The effect of isopropanol as a disinfectant was also studied. TMV remained structurally stable and no damage in integrity when treated with 50% isopropanol even up to 60 min. No significant breakdown of the encapsidated RNAs was observed (Fig. 2). When electrophoresed in 0.5X TBE buffer, pH 8.3, TMV appeared to be monophoretic and no alteration in electrophoretic behavior. We also evaluated the effect of UV light to degrade viral capsid and nucleic acids, since a number of laboratories frequently have been used UV light to decontaminate surfaces in laminar flow hoods. However, UV light effectively did not harm the viral capsid, and corresponding degradation of nucleic acids was not realized (Fig. 3).

Table 2. Degradation of TMV and encapsidated RNA by 1-0.01 N NaOH

NaOH concentration (N)		Exposure time (min)					
		0	1	5	10	15	30
1	virion	ND[a]	CD[b]	CD	CD	CD	CD
	RNA[c]	ND	CD	CD	CD	CD	CD
0.1	virion	ND	CD	CD	CD	CD	CD
	RNA	ND	CD	CD	CD	CD	CD
0.01	virion	ND	ND	ND	ND	ND	ND
	RNA	ND	ND	ND	ND	ND	ND

[a]ND, no degradation detected
[b]CD, complete degradation
[c]RNA, encapsidated RNA

Similar results have been observed for poliovirus (Ma et al., 1994) and bacterial (Josephson et al., 1993). Several other tests were conducted to establish if additional differences existed in the morphology of the electrophoretically migrated virions.

These procedures effectively inactivate virions, however with application of gene amplification by polymerase chain reaction techniques, there is the possibility that viral nucleic acids may

Fig. 1. Agarose gel (1%) electrophoresis of tobacco mosaic virus in 0.5 X TBE buffer at constant 10 V for 1 hr at 4oC. The gel was stained with Coomassie Brilliant Blue. Partial degradation of TMV by acid hydrolysis was observed after 0.1 N HCl exposure of 0(A), 5 (B), 10 (C), 15 (D), 20 (E), 30 (F), 45 (G), and 60 (H) min.

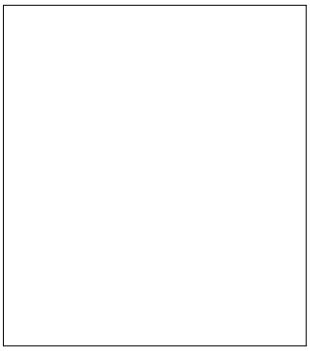

Fig. 2. No degradation of TMV treated with 50% is opropanol after 0 (A), 5 (B), 10 (C), 20 (D) and 30 (E) min exposure. Agarose gel (1%) was electrophoresed in 0.5 X TBE buffer at constsnt 100V for 1 hr at 4oC and stained with Coomassie Brilliant Blue.

not be destroyed by these disinfectants. It will be tested whether TMV is kept the infectivity or lost it by local lesion assay. Although isopropanol and UV light are recognized as potent virucidal agents with a limited range of application, they are not effective for inactivating plant viruses. Therefore, they are not recommendable agents as disinfectants
to be applied in tobacco farm. Disinfection of

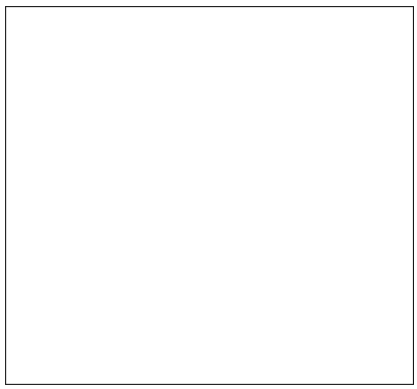

Fig. 3. Effect of UV light to encapsidated RNAs of TMV. (A) no exposure, (B) 5, (C) 10, (D) 2 0 min exposure at 5 mW/cm2, (E) 5, (F) 10 min exposure at 7 mW/cm2. Agarose gel (1%) electrophoresis of the encapsidated RNAs of TMV was run in 0.5 X TBE buffer at constant 100 V for 1 hr at 4oC and stained with ethidium bromide (1 µg/ml). No degradation could be observed.

the agricultural tools, plant debris, soil, irrigated water and other equipments. between cultivation, is necessary to prevent cross contamination in the field. This experiment is useful even to deal with plant viruses that may be present in laboratory equipments.

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