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Study on Plasma Sterilization of Plant Anthracnose Pathogen in Water Mist

Masaru Tominaga [*], Toshiyuki Tanaka[†], Tatsuya Sakoda [‡], Norikazu Mizoguchi [§], Yoshiyuki Kushima [**]

Abstract

In recent years, Miyazaki Prefecture located on the island of Kyushu in Japan promotes agricultural food industry. One of the activities is promotion of agricultural and livestock products. However, Miyazaki Prefecture is very far from large-scale consumption areas such as Tokyo and Osaka cities. To keep freshness of agricultural and livestock products during their transport is a serious problem from a viewing of putrefaction due to outbreak of blight. Apple Mango has been a very famous and representative product in Miyazaki Prefecture and it has successfully continued to make a good profit, however fruit rot disease such as plant anthracnose pathogen after harvest is serious. Therefore, a sterilization technique is strongly required. We here generated the dielectric barrier discharge plasma in water mist which might enable to sterilize the whole of target greengrocery because of its high diffusibility and carried out sterilization. Based on the results, fungicidal activity against *Colletotrichum* sp. was investigated.

Keywords: CT; D-value; plant anthracnose pathogen; plasma sterilization

1 Introduction

Agriculture is a main industry in Miyazaki Prefecture located on the island of Kyushu in Japan, and Miyazaki Prefecture progresses food industry such as food processing for preparation of fresh products for market and manufacture of prepared food products from agricultural and livestock products. However, Miyazaki Prefecture is very far apart from large-scale consumption areas such as Tokyo and Osaka cities. To keep freshness of agricultural and livestock products during their transport is a serious problem from a viewing of putrefaction due to blight outbreak.

[*] University of Miyazaki, Miyazaki, Japan
[†] University of Miyazaki, Miyazaki, Japan
[‡] University of Miyazaki, Miyazaki, Japan
[§] Miyazaki Agricultural Research Institute
[**] Miyazaki Agricultural Research Institute

Incidentally, Apple Mango has been a very famous and representative product in Miyazaki Prefecture and it has successfully continued to make a good profit. However, fruit rot disease such as plant anthracnose pathogen after harvest is serious [1] [2] [3]. It is difficult to choose and select infected mangos from uninfected ones because the Mango anthracnose progress after harvesting, which is one of factors of claim and impairing the reliability [4]. On the other hand, agricultural chemicals use after post-harvest is not allowed in Japan. Therefore, a sterilization technique is strongly required instead of the agricultural chemicals. From this kind of circumstance, we have been developing a sterilization technique using activated species such as OH radicals and H_2O_2 generated by discharge plasmas.

O_3 is generated through reactions between plasma and O_2, and the plasma can also generate OH radicals and H_2O_2 through collisions with H_2O [5] [6] [7] [8]. The oxidization of OH radical is about 10^6 times higher than that of O_3; therefore we focus on an advanced oxidation process with OH radicals and H_2O_2, which is probably superior to a sterilization using only O_3. These activated species are produced through a reaction between O_3 and H_2O even without the plasma. However, the plasma can generate these species much more. Therefore, we generated the dielectric barrier plasma in water mist, which might enable to sterilize the whole of target greengrocery because of its high diffusibility.

In this study, we used *Colletotrichum* sp. as plant anthracnose pathogen and examined fungicidal activity against *Colletotrichum* sp.. We first applied only O_3 gas (hereinafter referred to as "only O_3 use") to *Colletotrichum* sp.. Next, we additionally applied water mist having a particle diameter of 200 nm (hereinafter referred to as "ozone mist"). Finally, the dielectric barrier discharge was generated over *Colletotrichum* sp. in oxygen gas contained the water mist (hereinafter referred to as "plasma mist"). Based on the sterilization results, we compared their sterilization characteristics.

2 Experimental Setup

A preliminary experiment to probe O_3 contribution to sterilization was carried out by only O_3 use. Figure 1 shows the experimental setup which consists of an ozonizer which is different from a plasma mist reactor which will be described below, a digital flow meter, an O_3 monitor, a glove box ($0.4 \times 0.3 \times 0.4$ m^3), a mist generator with a pump, a showerheads, and a high-frequency power source (6.25 kHz) with a variable transformer. In this experiment, a mist generator pump didn't work. On the other hand, the pump was operated in the case of the ozone mist in Figure 1 was supplied. The OH radicals were obtained through the following reactions of (1) – (6).

$$O_3 + H_2O \rightarrow HO_3^+ + OH^- \qquad (1)$$

$$O_3 + OH^- \rightarrow HO_2^- + O_2 \qquad (2)$$

$$O_3 + HO_2^- \rightarrow O_3^- + HO_2 \qquad (3)$$

$$HO_3 \rightarrow OH + O_2 \qquad (4)$$

$$HO_3^+ + OH^- \rightarrow 2OH_2 \qquad (5)$$

$$O_3 + HO_2 \rightarrow OH + 2O_2 \qquad (6)$$

A surface discharge type ozonizer was arranged at the outside of a glove box, i.e., O_3 generated at the outside of the glove box was supplied from outlet of a shower head with a diameter of about 50 mm. The O_3 concentration was measured by an O_3 monitor before introducing O_3 to the glove box and was set at 5 ppm or 10 ppm, which was controlled by varying applied voltage to the ozonizer. The flow rate was kept at 3.5 L/min.

Cultivated pathogen of plant anthracnose for sterilization examinations is shown in Figure 2. The pathogen of the plant anthracnose was cultivated on Potato Dextrose Agar (PDA) medium for 14 days. A spore of anthracnose which was anticipated to be included in the medium was not apparent as shown in Figure 2(b) because the size of a spore is in the range of about 4 – 6 µm x 12 – 14 µm. The cultivated pathogen on the medium was picked up at any 3 areas from a laboratory dish with 10 mm in diameter. After stirring in hyperpure water, 100 µl of the stirring water was pipetted and coated on another PDA medium. The distance between the PDA medium and the O_3 outlet of the shower head was about 16 mm. After the sterilization, we counted the number of colonies on the medium cultivated for 48 h in an incubator.

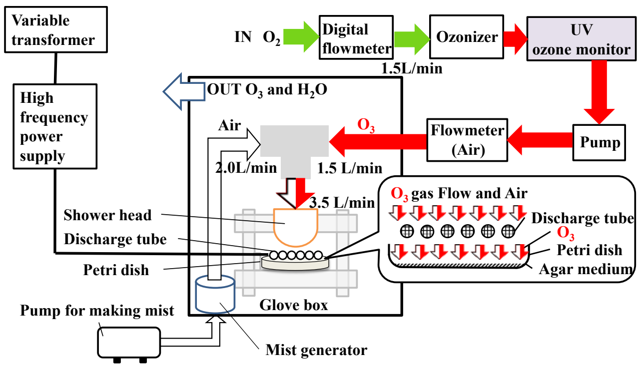
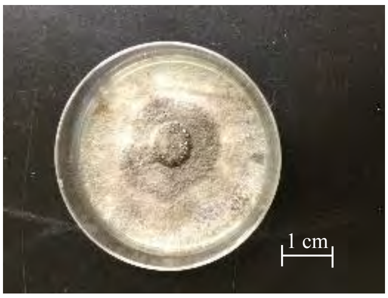
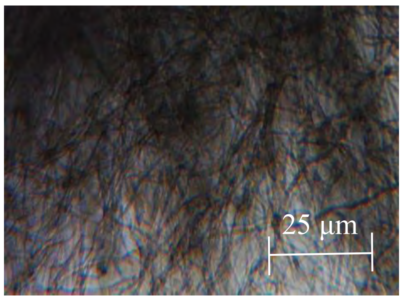


Figure 1: Schematic diagram of experiment setup for only O_3 use



(a) Appearance of medium



(b) Enlarged view

Figure 2: Appearance of pathogen of plant anthracnose

Figure 3 shows the experiment setup in the case where sterilization was carried out by using the dielectric barrier discharge operated in oxygen gas containing water mist. Here, a mist generator was operated, and oxygen gas containing water mist with a particle diameter of about 200 nm was discharged. Temperature and relative humidity in a glove box (0.4×0.3×0.4 m^3) was meas-ured by a thermohygrometer, and then the absolute humidity was calculated. The dielectric bar-rier discharge tubes were arranged in 6 rows, and the distance between PDA medium and the discharge tubes were about 12 mm. Each discharge tube with a length of 100 mm was composed of a copper wire with a diameter of 0.3 mm wound around a glass tube with a diameter of 3 mm. The installation interval of the 6 rows was about 5 mm. The inside of the glass tube were filled with copper powder, to which high voltage was applied. The operating gas with a flow rate of 3.5 L/min was oxygen supplied from a shower head arranged at 16 mm upper part from a PDA medium. Thus, the discharge was produced in oxygen containing water mist. The exhaust O_3 concentration from the glove box was measured by an O_3 monitor.

In this case, not only O_3 but also radicals such as O and OH may be produced. The OH radicals were obtained through the following reactions of (7) – (9). It is said that oxidation power of OH is higher than that of O_3 and that sterilization power is much higher than that of O_3. Additionally, unstable H_2O_2 formed through reaction (9) reacts with another species, and then radicals such as OH and HO_2 are formed. Therefore, effective sterilization is expected when the dielectric barrier discharge is produced in oxygen containing water mist. Phots of treatment area and dielectric barrier discharge are shown in Figure 4.

$$H_2O + e\ (> 6.4\ \text{eV}) \rightarrow H + OH + e \tag{7}$$

$$O + H_2O \rightarrow OH + OH \tag{8}$$

$$OH + OH \rightarrow H_2O_2 \tag{9}$$

$$O + H_2O_2 \rightarrow OH + HO_2 \tag{10}$$

$$H_2O_2 + OH \rightarrow H_2O + HO_2 \tag{11}$$

$$H_2O_2 + HO_2 \rightarrow H_2O + O_2 + OH \tag{12}$$

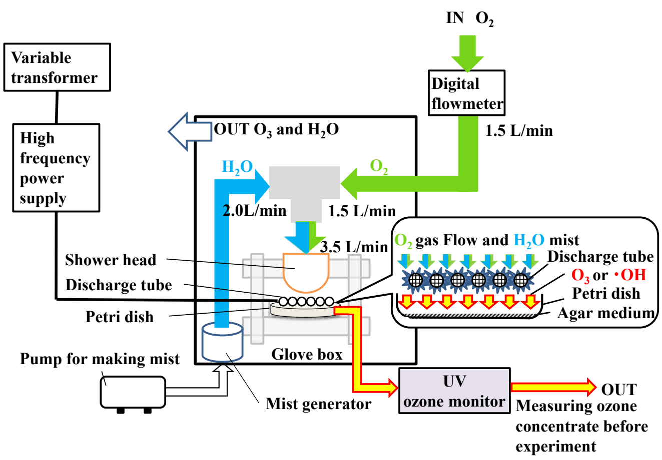
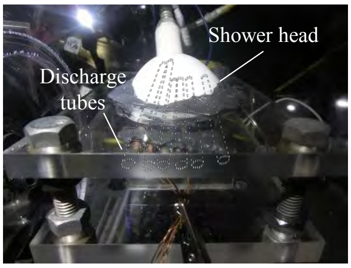
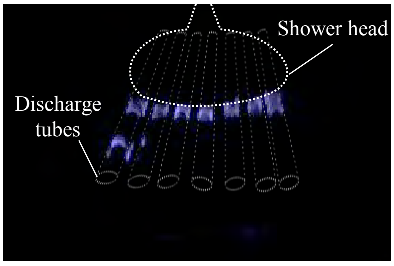


Figure 3: Schematic diagram of experimental setup for the plasma mist having a particle diameter of 200 nm

We executed sterilizations in which CT (product of O_3 concentration and sterilization time) was varied until 40 ppm·min at intervals of 4 ppm·min. It is possible to compare sterilization effect related to O_3 and radicals because CT value represents the absolute amount of O_3. After sterilization experiment, we counted the number of colonies on the medium cultivated for 48 h in an incubator. We carried out 2-times experiments for each experimental condi-tion.



(a) Appearance of discharge area



(b) Photo of dielectric barrier discharge

Figure 4: Phots of treatment area and dielectric barrier discharge

Results and Discussions

Table 1 shows the averaged number of colonies in the case of only O_3 use and ozone mist. CT dependence of colony survival rate for sterilization of pathogen of plant anthracnose for only O_3 use and ozone mist are shown in Figures 5 and 6, respectively. Additionally, Tables 2 and 3 show approximation for only O_3 use and ozone mist, and those experimental conditions of temperature, relative humidity, absolute humidity, and O_3 concentration. As shown in Table 3, the absolute humidity for two experiments were the almost same in the case of only O_3 use. On the other hand, the absolute humidity for the case of ozone mist became higher because a mist generator operated here although those for two experiments were the almost same. The horizontal axis is CT value while the vertical axis is the logarithmic function on colony survival rate. We evaluated sterilization effect using D-value which is time to reduce the number of colonies to one - tenth of that of preprocessing colonies. Therefore, the smaller the D-value, the higher the sterilization effect becomes.

Circles in Figures 5 and 6 denote results obtained for only O_3 use with concentration of 5 ppm. The applied voltage to generate O_3 of 5 ppm was 1.82 kV. Circles denote ones obtained for only O_3 use with concentration of 10 ppm. In this case, the applied voltage was 1.85 kV. These colony survival rate was anticipated to decrease exponentially from CT = 0 ppm･min. However, there was a period of time during which sterilization didn't advance until a certain CT (hereinafter referred to as "induction period"). Such CT exists at around 16 ppm･min in Figures 5 and 6. The induction period is probably due to the septal wall of mycelia *Colletotrichum* sp. which has tol-erant against O_3. To approximate accurately, an approximately line was drawn exponentially as shown in Table 2 in which CT-value shown as "*x*", which referred colony survival rate after induction period. From these approximately lines, D-values in the cases of 5 ppm and 10 ppm for only O_3 use are 41 ppm･min and 26 ppm･min, respectively. Similarly, D-values in the cases of 5 ppm and 10 ppm for ozone mist are 48 ppm･min and 29 ppm･min, respectively. Thus, the sterilization effect at 10 ppm was higher. This sterilization effect is due to the large amount of O_3 per unit time.

Table 1: Number of colony for only O_3 use and ozone mist

CT value [ppm·min]	Only O_3 use		Ozone mist	
	5 ppm	10 ppm	5 ppm	10 ppm
0	308	67	308	78
4	351	62	335	82
8	323	53	307	64
12	307	51	353	64
16	346	44	287	46
20	259	19	137	17
24	202	12	172	24
28	85	6	167	4
32	42	3	84	3
36	48	1	0	2
40	44	1	47	10

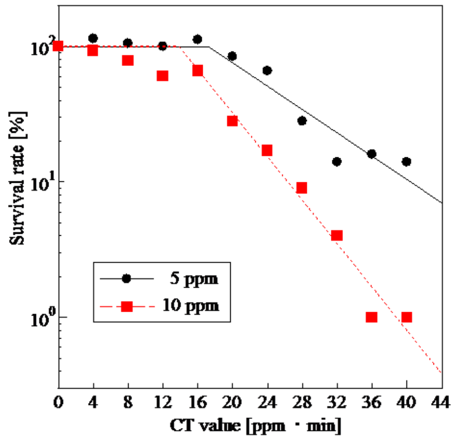


Figure 5: CT dependence of colony survival rate for sterilization for only O_3 use

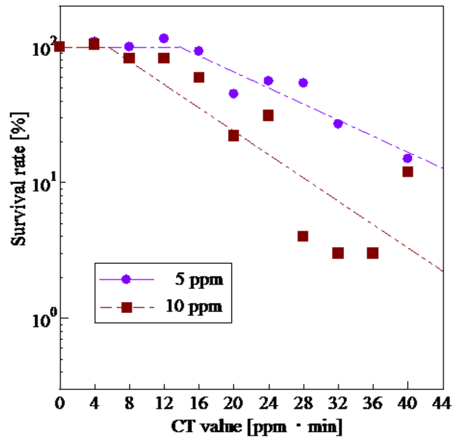


Figure 6: CT dependence of colony survival rate for sterilization for ozone mist

Table 2: Approximation for only O_3 use and ozone mist

Processing method	5 ppm	10 ppm
Only O_3 use	$e^{(-0.10)x+6.3}$	$e^{(-0.18)x+7.2}$
Ozone mist	$e^{(-0.07)x+5.5}$	$e^{(-0.10)x+5.2}$

Table 3: Experimental conditions for only O_3 use and ozone mist

Processing method	O_3 [ppm]	Temperature [°C]	Relative Humidity [%]	Absolute Humidity [g/m^3]
Only O_3 use	5	14.8	30	3.8
	10	14.1	26	3.1
Ozone mist	5	15.2	60	6.4
	10	14.3	55	7.2

Table 4 shows the number of colonies in the case of the plasma mist. Figure 7 shows CT dependence of colony survival rate for sterilization of pathogen of plant anthracnose in which the dielectric barrier discharge was produced in oxygen containing water mist. In addition, Table 5 shows the experimental conditions. The absolute humidity for two experiments is high as those for experiments using ozone mist because a mist generator also operated here. Circles in Figure 7 denote results obtained by the plasma mist with exhaust O_3 concentration of 5 ppm. In this case, the applied voltage was 1.64 kV. Square denote ones obtained by plasma mist with exhaust O_3 concentration of 10 ppm. In this case, the applied voltage was 1.76 kV. Any induction period, as shown in Figures 5 and 6, were not recognized for two cases. Thus, approximation for 5 ppm and 10 ppm were drawn exponentially as $e^{-0.05x+4.6}$ and $e^{-0.11x+4.6}$, in which a survival rate at 100 % is matched with CT = 0 ppm·min. From these approximately lines, D-values in the cases of 5 ppm and 10 ppm of O_3 in Figure 7 are 45 ppm·min and 20 ppm·min, respectively. As in the case of only O_3 use, the sterilization effect for O_3 concentration of 10 ppm is higher. Here, as mentioned above, it should be noted that there is no induction period. Active species such as O, OH, H_2O and so on probably destructs septal wall of mycelia of *Colletotrichum* sp..

In addition, to confirm superiority of the plasma mist, we compared the sterilizations obtained by the plasma mist, only O_3 use and ozone mist. The comparison results by three processing methods at O_3 concentration of 5 ppm is shown in Figure 8. Table 6 summary of D-values at O_3 concentration of 5 ppm and 10 ppm. From Figure 8 and Table 6, the different sterilization effect among only O_3 use, ozone mist and plasma mist was not apparent at 5 ppm. Figure 9 shows the comparison result by three processing methods at O_3 concentration of 10 ppm. From Figures 8 and 9, active species such as O, OH, H_2O_2 are not probably generated as they could contribute the sterilization because the D-values of the ozone mist for 5 ppm and 10 ppm are the almost same as that of only O_3 use. The sterilization of the plasma mist is more effective than those of only O_3 use and ozone mist. D-value, shown in Table 6, of the plasma mist is about 0.71 times smaller than that of only O_3 use. Additionally, the plasma mist also is about 0.69 times smaller than the ozone mist.

Thus, in a high humid environment produced by water mist having a particle diameter of 200 nm, not only O_3 but also active species such as O, OH, H_2O and so on are produced by applying relatively high voltage to generate the dielectric barrier discharge. That is, the increase of applied voltage promote the increases of O_3 and active species formed through reactions between H_2O and O_3. When the applied voltage was set to generate O_3 with concentration of 10 ppm, not only O_3 but also active species such as O, OH, H_2O and so on were produced and they contributed to sterilization effectively. In contrast, the amount of active species was less when O_3 concentration was 5 ppm. The produced active species probably destructed septal wall of mycelia of *Colletotrichum* sp.; therefore, the survival rate began to decrease from CT = 0 ppm･min. However, obvious superiority was not seen in comparison with other two processing methods.

Table 4: The number of colonies for plasma mist

CT value [ppm･min]	5 ppm	10 ppm
0	251	65
4	247	80
8	207	39
12	131	25
16	198	20
20	114	5
24	112	0
28	19	1
32	62	1
36	64	0
40	17	3

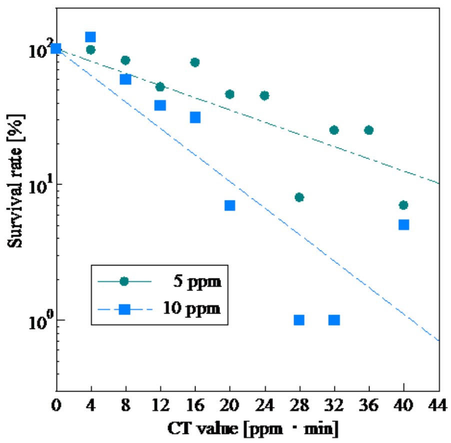


Figure 7: CT dependence of colony survival rate for sterilization in the case of the plasma mist

Table 5: Temperature, humidity and absolute humidity in the case of plasma mist

O_3 [ppm]	Temperature [°C]	Relative Humidity [%]	Absolute Humidity [g/m^3]
5	15.4	48	6.3
10	14.6	48	6.0

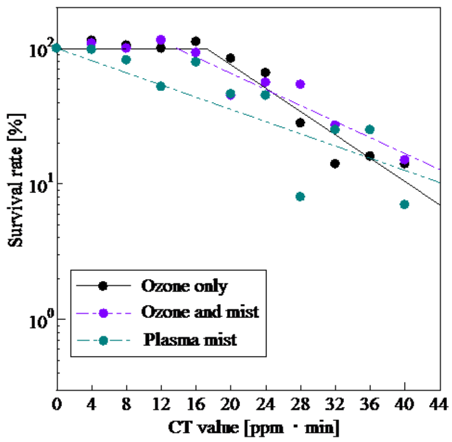


Figure 8: Comparison of sterilization between the only O_3 use, ozone mist and the plasma mist for O_3 concentration of 5 ppm

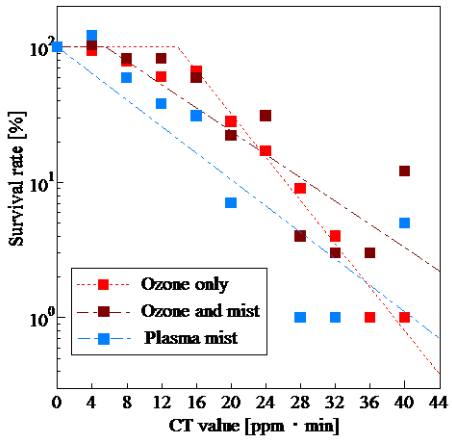


Figure 9: Comparison of sterilization between the only O_3 use, ozone mist and the plasma mist for O_3 concentration of 10 ppm

Table 6: Comparison of D-values between three methods

O_3 [ppm]	The only O_3 use [ppm·min]	The ozone mist [ppm·min]	The Plasma mist [ppm·min]
5	41	48	45
10	28	29	20

3 Conclusion

To reduce fruit rot disease of Apple mango which is very famous and representative product in Miyazaki Prefecture, we examined to sterilize plant anthracnose pathogen by using O_3 or active species formed by the dielectric barrier discharge in oxygen gas containing water mist having a particle diameter of about 200 nm. Here, we used *Colletotrichum* sp. as plant anthracnose pathogen.

As a preliminary experiment to probe the contribution of O_3 to sterilization, only O_3 gas generated at the outside of a processing chamber was applied. O_3 with 10 ppm was more effective to the sterilization than that with 5 ppm, and a large amount of O_3 per unit time contributed to the sterilization of *Colletotrichum* sp.. Additionally, for only O_3 use, the induction period during which sterilization didn't advance until a certain CT existed. The induction period is probably due to the septal wall of mycelia *Colletotrichum* sp. which has tolerant against O_3. In the case of the ozone mist, the similar trend to only O_3 use was apparent. The sterilization effect by the ozone mist with O_3 with 5 ppm and 10 ppm was the almost same as that of only O_3 use. Therefore, active species such as OH radicals and H_2O_2 and so on were not generated enough to contribute the sterilization.

Incidentally, any induction period was not recognized for sterilization by using the plasma mist. When the dielectric barrier discharge is produced in oxygen containing water mist, not only O_3 but also OH radical may be produced through reactions between H_2O, electron and O. Furthermore, radicals such as OH and HO_2 are formed through between unstable H_2O_2 and another species. Therefore, effective sterilization is expected when the dielectric barrier discharge is produced in oxygen containing water mist. Septal wall of mycelia might be destructed by the active species. The superiority of the plasma mist could be confirmed when exhaust O_3 concentration was 10 ppm. Thus, not only O_3 but also active species such as O, OH, H_2O and so on are produced and they contributed to sterilization effectively when the applied voltage was set to generate O_3 with concentration of 10 ppm in a high humid environment.

4 Reference

4.1.1 Article in a journal or magazine

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