

微酸性电解水对灰葡萄孢菌杀菌效果与作用机制研究

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摘要: 研究了微酸性电解水对灰葡萄孢菌悬液纯培养及原位培养的杀菌效果, 同时通过扫描电镜和透射电镜初步探究了其对灰葡萄孢菌的杀菌机制。研究表明, 随杀菌时间和有效氯质量浓度的增加, 微酸性电解水对灰葡萄孢的杀菌能力明显增强; 在有效氯质量浓度为 10 mg/L、杀菌处理 15 min, 以及有效氯质量浓度在 30 mg/L、杀菌处理 10 min 时, 对悬液中灰葡萄孢菌的杀菌率均达 99.99%。对用微酸性电解水处理后的灰葡萄孢菌落原位生长状态观察表明, 微酸性电解水可以抑制菌落扩生, 浓度越高抑生长作用越强, 用有效氯质量浓度为 30 mg/L 的微酸性电解水处理菌落 3 d 后, 菌丝逐渐变暗黄、枯萎, 直至死亡, 菌落停止生长, 推测微酸性电解水可杀灭灰葡萄孢的新生孢子并抑制菌丝生长。通过扫描及透射电镜观察, 发现微酸性电解水处理可导致灰葡萄孢子细胞质壁分离, 细胞质溢出, 细胞器溶解, 细胞皱缩, 这可能是引起灰葡萄孢菌无法正常生长繁殖, 最终达到杀菌效果的原因。

关键词: 微酸性电解水; 灰葡萄孢菌; 杀菌率; 杀菌机制

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Investigation on Microbicidal Potential and Action Mechanism for *Botrytis cinerea* of Slightly Acidic Electrolyzed Water

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Abstract: Slightly acidic electrolyzed water is a kind of bactericidal preparation with pH value slightly lower than 7.0 by electrolyzing dilute hydrochloric acid solution or electrolyzing low concentration dilute hydrochloric acid and electrolyte (NaCl/KCl) under non-diaphragm condition. It has a strong ability to kill pathogenic bacteria, but there is little research on the ability and mechanism of killing fungal spores by slightly acidic electrolyzed water. The killing effect of slightly acidic electrolytic water on pure culture spore suspension and in situ culture of *Botrytis cinerea* was studied, and the germicidal mechanism of water on *Botrytis cinerea* was preliminarily studied by scanning electron microscopy and transmission electron microscopy. The results showed that the bactericidal ability of slightly acidic electrolyzed water to *Botrytis cinerea* was increased significantly with the increase of sterilization time and available chlorine concentration (ACC), and bactericidal rate can achieve 99.99% when the available chlorine concentration was not less than 30 mg/L and kill time was 10 min. The growth state of *Botrytis cinerea* colony in situ after treatment with slightly acidic electrolytic water showed that slightly acidic electrolytic water could inhibit colony expansion, and the higher the concentration was, the stronger the inhibition effect was. After 3 d of treatment with slightly acidic electrolytic water with 30 mg/L available chlorine, the mycelium gradually turned yellow and withered until death, and the colony was stopped. It was suggested that microacid electrolyzed water can kill the new spores of *Botrytis cinerea* and inhibit the growth of mycelia. Through microscope observation of scanning and transmission electron, it was found that treatment with acidic electrolyzed water can make separation of membrane and cell wall, cytoplasmic overflow and abnormal lysis of organelles. It was speculated that these may be the reasons which can achieve the bactericidal effect.

Key words: slightly acidic electrolyzed water; *Botrytis cinerea*; sterilization rate; sterilization mechanism

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0 引言

微酸性电解水(Slightly acidic electrolyzed water, SAEW)又称微酸性氧化电位水,指在直流电场的作用下,采用无隔膜电解方式电解稀盐酸溶液或稀盐酸和稀盐混合溶液而得到的具有特殊理化及杀菌特性的水溶液,其pH值一般在5.0~7.0之间,接近中性,拥有较低氧化还原电位(Oxidation-reduction potential, ORP)(<800 mV)和一定的有效氯质量浓度^[1~3]。微酸性电解水与强酸性电解水(pH值小于3.0, ORP值大于1 100 mV)具有近似的杀菌效果,但相较于强酸性电解水,其制备方便,对金属及塑料材料的腐蚀性更低^[4~5]。如用于设施农业生产,微酸性电解水对设施设备具有更小的损害。微酸性电解水已在医疗、食品加工及畜禽养殖等领域有了初步的应用研究^[6~10]。

以往利用电解水对真菌及其孢子的杀菌研究多集中在强酸性电解水及中性电解水。如BUCK等^[11]研究发现,使用强酸性电解水处理灰葡萄孢菌及褐腐病菌等,需要30 s或更少的处理时间可杀死其孢子,而厚壁、有色素的真菌(如弯孢菌、蠕虫孢子菌)需要2 min或更长的时间才能显著降低发芽率。ABBASI等^[12]使用强酸性电解水处理了泡囊菌(细菌斑点病原体)、疥疮链霉菌(马铃薯疥疮病原体)和番茄黄萎病镰刀菌(根腐病原体)等植物病原菌繁殖体,2 min可显著减少4~8个对数单位。KE等^[13]研究认为,相比强酸性电解水,中性电解水具有更强的对黄曲霉菌的杀菌效果,OH自由基是其中重要的杀菌因子,是破坏黄曲霉分生孢子细胞结构的重要杀菌因子,它也破坏了细胞正常功能,导致K⁺和Mg²⁺泄漏。在相同的有效氯浓度水平下,中性电解水比强酸性电解水含有更多的OH自由基。HAMZAH等^[14]使用有效氯质量浓度分别为60、120 mg/L的中性电解水对细胞悬液中的蜡样芽孢杆菌和产气荚膜梭菌进行了杀菌处理,5 min可分别达2.46~3.62 lgCFU/mL和2.11~3.03 lgCFU/mL的杀菌效果。目前,利用微酸性电解水对细菌的杀菌研究逐渐增多^[15],但将其用于病原真菌的杀菌效果及机制研究还较少,由此限制了这一新型杀菌制剂在真菌病害防治方面的应用潜力。

灰葡萄孢菌是可以引起多种已知植物灰霉病的坏死营养型病原真菌,具有广寄主性和强致病性,其在空气中广泛分布,且可污染食物和饲料等^[16~18]。本文以灰葡萄孢菌为模型菌,研究微酸性电解水对其纯培养的杀菌效力,在此基础上,通过电镜观察微酸性电解水对灰葡萄孢的微观结构破坏情况,以期对微酸性电解水的杀真菌机制进行初步探究。

1 材料与方法

1.1 微酸性电解水对灰葡萄孢菌的杀菌效力

使用上海富强旺卫生用品有限公司的HD-240L型连续式微酸性电解水机制备微酸性电解水,理化特性见表1。分别设置:相同杀菌时间(5 min)、不同有效氯质量浓度(10、20、30、40 mg/L)的微酸性电解水,进行杀菌处理;相同有效氯质量浓度(10、30 mg/L)、不同杀菌时间(0、1、5、10、15 min)的两组处理,每组处理3次重复,以无菌生理盐水处理为对照,进行杀菌实验,方法如下。

表1 微酸性电解水理化指标

Tab. 1 Physicochemical properties of slightly acidic electrolyzed water

电解水 编号	pH 值	ORP 值/ mV	有效氯质量 浓度/(mg·L ⁻¹)
1	5.9 ± 0.1	760 ± 11	10 ± 1
2	6.1 ± 0.2	767 ± 14	20 ± 1
3	6.2 ± 0.2	774 ± 16	30 ± 2
4	6.5 ± 0.3	782 ± 19	40 ± 2

将制备好的灰葡萄孢菌孢子悬液(孢子菌体浓度在7.00~7.70 lgCFU/mL之间),在小型漩涡振荡仪上充分振荡分散,分别吸取1 mL悬浮液加入盛有8 mL微酸性电解水的试管中,进行杀菌处理,达到预设时间后,加入1 mL无菌中和剂(0.05% Na₂S₂O₃)终止杀菌^[19],振荡混合均匀后,用生理盐水进行10倍梯度稀释,每稀释度吸取0.1 mL均匀涂抹于PDA(马铃薯葡萄糖琼脂)培养基上,26℃恒温培养72 h待菌落充分生长后,计平板菌落数,然后计算处理前后悬液中菌体浓度(lgCFU/mL)。对照组则将1 mL孢子悬液吸入到盛有9 mL生理盐水的试管中,然后按照实验组的操作方法进行实验。

1.2 灰葡萄孢菌落直径及外观形态的影响观察

在培养基上均匀接种灰葡萄孢菌孢子悬液,制备方法及接种培养方法同1.1节所述,26℃恒温培养2 d,然后每隔24 h喷洒一次不同浓度微酸性电解水(理化指标见表1中编号1和3),以喷洒无菌生理盐水为对照,采用十字交叉法每天测量菌落直径,同时观察菌落形态并记录。

1.3 微观结构观察分析

1.3.1 扫描电镜观察分析

取灰葡萄孢菌落生长均匀的PDA纯培养,无菌操作切取5块正方形(0.5 cm × 0.5 cm)带培养基菌块,转入5个无菌培养皿中,分别取有效氯质量浓度为10 mg/L和30 mg/L微酸性电解水(表1),分别对菌块进行原位杀菌处理5 min和10 min,留一组作

为对照组,之后加入中和剂终止杀菌,倒出液体,留菌块备用。

将处理后菌块放入直径 1.5 cm 的无菌试剂盒,加入 3% 的戊二醛进行固定,为防止孢子漂浮,在真空泵中真空吸附 10~20 min,后固定 2 h;将固定好的菌落块用丙酮进行逐级脱水,采用环氧树脂-618 包埋^[10],制片并用 EVO LS 15 型扫描电镜进行观察分析。

1.3.2 透射电镜观察分析

设置微酸性电解水有效氯质量浓度分别为 10 mg/L 和 30 mg/L(表 1),进行悬液杀菌处理,方法见 1.1 节所述,处理时间分别为 5 min 和 10 min,达到规定时间后加入中和剂终止杀菌备用。

取处理后菌悬液,用磷酸盐缓冲液(0.03 mol/L, pH 值 7.2)在相同条件下离心洗涤 3 次,向沉淀物中加 3% 的戊二醛固定 1 h,使用丙酮溶液逐级脱水,后用环氧树脂进行包埋,制作超薄切片,然后用醋酸铀和枸橼酸铅进行染色^[20],在 JEM-1400 型透

射电镜下观察并分析。

2 结果与分析

2.1 微酸性电解水对灰葡萄孢菌的杀菌效力

由表 2 可知,当固定杀菌时间为 5 min 时,随着有效氯质量浓度的增加,对灰葡萄孢菌的杀菌率逐渐增加,有效氯质量浓度达到 40 mg/L 时,杀菌对数值 3.65 lgCFU/mL,杀菌率达到 99.97%。

由表 3 可知,当固定有效氯质量浓度,随着杀菌时间的延长,杀菌率呈显著性增强,杀菌时间为 1 min 时,杀菌率均较低,有效氯质量浓度为 30 mg/L,杀菌率仅为 54.29%,但杀菌时间为 1 min 和 5 min 时,有效氯质量浓度为 30 mg/L 的微酸性电解水杀菌率均远高于有效氯质量浓度为 10 mg/L 的微酸性电解水,差异显著,结果表明,杀菌时间较短时,随有效氯质量浓度增加,可显著增强杀菌率,但接触时间较短时,杀菌率较低,在有效氯质量浓度 30 mg/L、杀菌 5 min 时,未达到 99.99% 的杀菌率。

表 2 相同时间不同电解水浓度对灰葡萄孢菌的杀菌效力

Tab. 2 Microbicidal potential of slightly acidic electrolyzed water at different concentrations and the same time

有效氯质量浓度/(mg·L ⁻¹)	电解水特性		悬液活菌菌体浓度/(lgCFU·mL ⁻¹)		杀菌率/%
	pH 值	ORP 值/mV	初始	残留	
0(对照)	7.06 ± 0.2	280 ± 17	7.23 ± 0.19	7.23 ± 0.19	0
10 ± 1	5.90 ± 0.1	760 ± 11	7.23 ± 0.19	6.47 ± 0.17	82.62
20 ± 1	6.10 ± 0.2	767 ± 14	7.23 ± 0.19	5.39 ± 0.15	98.55
30 ± 2	6.20 ± 0.2	774 ± 16	7.23 ± 0.19	4.46 ± 0.15	99.83
40 ± 2	6.50 ± 0.3	782 ± 19	7.23 ± 0.19	3.58 ± 0.11	99.97

表 3 不同时间相同电解水浓度对灰葡萄孢菌的杀菌效力

Tab. 3 Microbicidal potential of slightly acidic electrolyzed water at the same concentrations and different times

杀菌时间/min	悬液活菌浓度/(lgCFU·mL ⁻¹)		杀菌率/%	杀菌时间/min	悬液活菌浓度/(lgCFU·mL ⁻¹)		杀菌率/%
	初始	残留			初始	残留	
0	7.23 ± 0.19	7.23 ± 0.19	0	0	7.23 ± 0.19	7.23 ± 0.19	0
1	7.23 ± 0.19	7.02 ± 0.15	38.34	1	7.23 ± 0.19	6.89 ± 0.17	54.29
5	7.23 ± 0.19	6.47 ± 0.17	82.62	5	7.23 ± 0.19	4.46 ± 0.15	99.83
10	7.23 ± 0.19	4.21 ± 0.13	99.90	10	7.23 ± 0.19	3.21 ± 0.16	99.99
15	7.23 ± 0.19	3.19 ± 0.14	99.99	15	7.23 ± 0.19	3.02 ± 0.11	99.99

比较表 2 和表 3 可以看出,有效氯质量浓度为 10 mg/L 时,处理时间为 10 min 时的杀菌率高于有效氯质量浓度为 30 mg/L 时,处理时间为 5 min 时,结果表明,充分的杀菌时间是保证杀菌效力的重要因素。有效氯质量浓度为 30 mg/L,杀菌时间为 10 min 时,以及有效氯质量浓度 10 mg/L,杀菌时间 15 min,对灰葡萄孢的杀菌率均达到 99.99%。

2.2 灰葡萄孢菌落直径及外观形态

由菌落直径测定数据可以看出(表 4),未经微酸性电解水处理的灰葡萄孢菌落直径,逐日增长明

显,经微酸性电解水处理 2 d 后,菌落直径与未处理组对比出现显著差异,有效氯质量浓度越高,对菌落生长抑制越强,有效氯质量浓度为 30 mg/L 的微酸性电解水,经过 3 d 的处理菌落停止生长,有效氯质量浓度为 10 mg/L 时,处理 3 d 后,虽还有生长但生长缓慢。结果表明,微酸性电解水可以在体外有效抑制灰葡萄孢菌落的生长。推测认为微酸性电解水可以使菌丝及新生孢子死亡,导致灰葡萄孢无法产生新的孢子,抑制其繁殖,进一步观察发现菌丝逐渐变暗黄,枯萎,直至死亡。

表4 灰葡萄孢菌PDA纯培养经微酸性电解水处理后菌落直径

Tab. 4 Colony diameter after being treated by slightly acidic electrolyzed water

处理分组	处理时间/d					cm
	0	1	2	3	4	
SAEW1(有效氯质量浓度10 mg/L)	1.13 ± 0.09	1.42 ± 0.07	1.52 ± 0.08	1.58 ± 0.06	1.60 ± 0.07	
SAEW2(有效氯质量浓度30 mg/L)	1.14 ± 0.08	1.34 ± 0.08	1.42 ± 0.07	1.43 ± 0.06	1.43 ± 0.06	
对照组(生理盐水)	1.13 ± 0.10	1.47 ± 0.09	1.64 ± 0.08	1.91 ± 0.07	2.17 ± 0.07	

2.3 灰葡萄孢菌经微酸性电解水处理后的微观结构观察及分析

由微酸性电解水处理前后灰葡萄孢原位扫描电镜结果(图1,放大倍数1 000),可以看出经电解水杀菌处理前后灰葡萄孢菌丝和孢子形态的变化。未

经处理的灰葡萄孢菌,在电镜下结构清晰,菌丝表面基本光滑,孢子形态未出现明显皱缩(图1a)。经微酸性电解水原位杀菌处理后,菌丝伸展不规则,有不同程度的折叠扭曲现象,孢子开始皱缩(图1b~1e,2b~2e),随电解水有效氯质量浓度和处理时间增

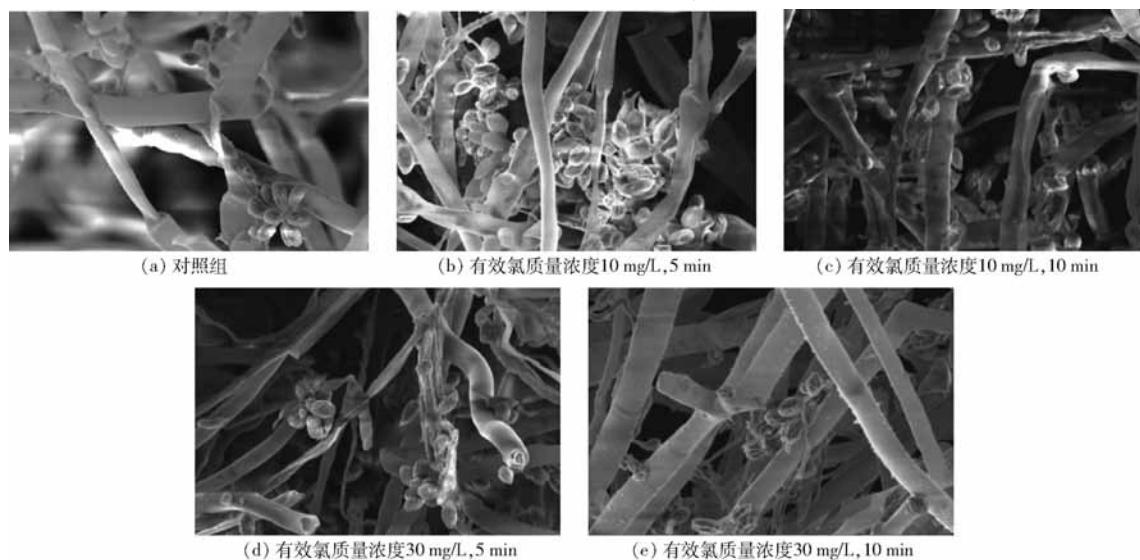


图1 微酸性电解水对灰葡萄孢处理前后的扫描电镜图

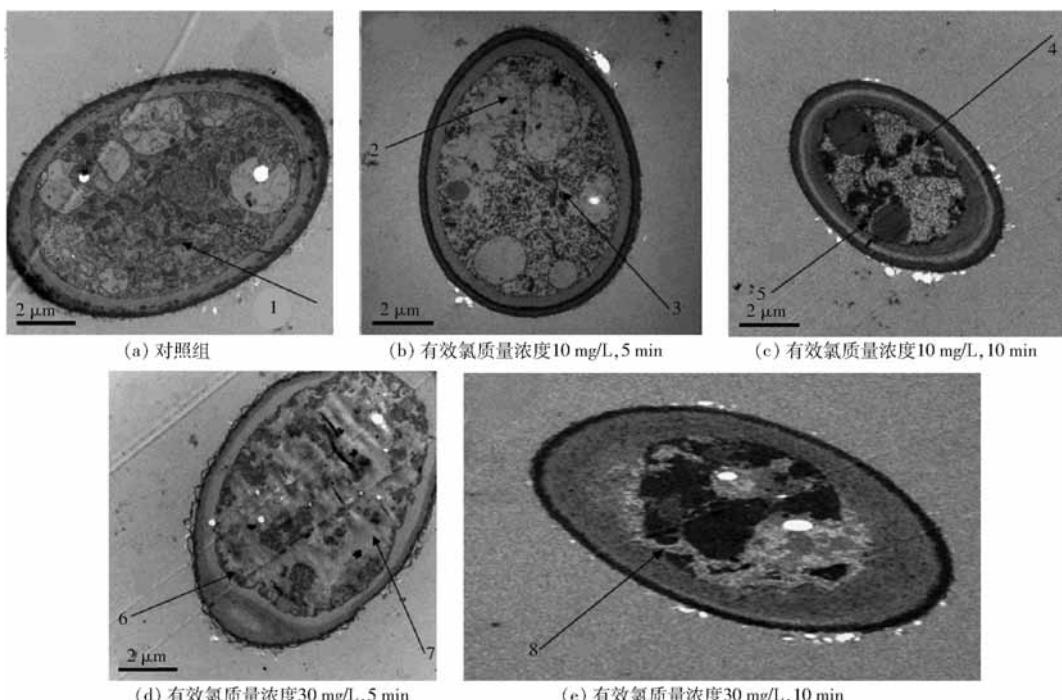
Fig. 1 SEM photos of *Botrytis cinerea* before and after slightly acidic electrolyzed water treatment

图2 微酸性电解水对灰葡萄孢处理前后的透射电镜图

Fig. 2 TEM photos of *Botrytis cinerea* before and after slightly acidic electrolyzed water treatment

加,孢子皱缩明显,大量孢子从菌丝脱落。灰葡萄孢菌依靠菌丝释放孢子囊,进而吐出孢子进行繁殖,推测微酸性电解水可同时通过破坏菌丝以及孢子的结构,从而抑制孢子的繁殖和生长,起到杀菌的作用。

经微酸性电解水处理前后灰葡萄孢透射电镜结果(图2)观察可知,未经电解水处理的孢子中线粒体(箭头1)较为明显,形状规则,且细胞质分布均匀致密(图2a)。经电解水处理后,灰葡萄孢菌孢子中线粒体明显有分裂的现象(箭头3),且细胞质(箭头2)变得疏松(图2b);随电解水浓度增加,还可见孢子质壁分离明显,细胞膜收缩,且边缘变得不规则,细胞质中的线粒体等出现不同程度的溶解(箭头4)。随电解水有效氯质量浓度增加和处理时间延长,孢子细胞内容物出现不同程度的溶解,细胞膜严重皱缩,细胞质中各种细胞器分别不够明显(图2c~2e)。

推测微酸性电解水首先通过细胞壁进入孢子

内,导致质壁分离,与膜上物质反应,可改变细胞膜通透性,导致膜内容物溢出,细胞膜收缩而变得不规则,及部分细胞器溶解,最终导致灰葡萄孢菌孢子的死亡。

3 结束语

微酸性电解水对灰葡萄孢纯培养具有良好的杀菌作用,在有效氯质量浓度为10 mg/L、杀菌处理时间为15 min,以及在有效氯质量浓度为30 mg/L、杀菌时间为10 min时,对其孢子悬液均达到99.99%的杀菌效果,相较于单纯提高浓度,延长处理时间可获得更强的杀菌效力。微酸性电解水对灰葡萄孢具有原位抑菌作用,喷洒微酸性电解水可抑制灰葡萄孢菌落的生长。对微酸性电解水处理前后灰葡萄孢微观结构进行观察,发现微酸性电解水进入灰葡萄孢孢子细胞壁后,导致细胞质壁分离,细胞膜结构被破坏,细胞质外溢,部分细胞器溶解,细胞发生皱缩,这可能是细菌孢子失去活性的原因。

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