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Experiment Study on Solid Culture Medium by Microwave Sterilization

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Abstract: In order to identify the relationship between the microwave and the sterilization effect and apply microwave to sterilize in fungus production process, the microwave sterilization craft on the solid culture medium in fungus growth is studied, the results show that microwave not only can be used to sterilize the micro-organisms which is useless for fungus growth in the solid culture medium, but also has positive effect on fungus growth; and the sterilization process is featured with shorter time and higher efficiency compared with the traditional method.

Keywords: Microwave, solid culture medium, sterilization

INTRODUCTION

Since microwave was discovered by Raytheon in 1947, applications have been reported (Metaxas ad Meredith, 2003; Adam, 2003) more and more. Especially in recent years, the applications in the chemical (Nuchter *et al*., 2004; Roy *et al*., 1999), metallurgical (Cho *et al*., 1990) and food-drying (Gunasekaran, 1989) have aroused great interest. However, there are few research reports about the sterilization the medium of cultivating edible fungi using microwave. At present, the method of sterilization the medium of cultivating edible fungi is compelled to use traditional methods. Traditional sterilization is to place the medium in an autoclave within which the temperature reaches 121°C or higher and usually is to hold more than 2 h during sterilizing process. Coal as an energy source using traditional sterilization methods, Coal consumption is significantly and the environment is polluted seriously during sterilizing process. But Electricity as an energy source using microwave sterilization, the rate of utilization is higher and no contamination generates, so using microwave sterilization the medium for edible fungi is one of trends (Wang, 1998).

Sterilization effect to which the microwave power, the size between the microwave source and the sterilized objects, the volume of sterilized objects is first introduced, then to identify the relationship among these factors and provides a theoretical basis for the application of microwave in fungus production process.

MATERIALS AND METHODS

Materials: Cotton Seed Hull, Polyethylene plastic bags.

The main instruments: Experimental microwave ovens (2450 MHz), sterilization workbench, Autoclave, Constant temperature incubator, Digital thermometer (Infrared Thermometer).

Microwave sterilization test: One hundred gram Cotton Seed Hull and 125 g water was mixed uniformly; and then the mixture was loaded into a φ 75×240 mm polyethylene plastic bag which had plastic seals at both ends. Next, the bag was put into a microwave oven and temperature of medium was being measured. Then, the sterilized medium was multi-point sampled and the number of micro-organisms was counted. At the same time, the number of micro-organisms was also counted before sterilization. First, the microwave power (400, 600 and 850 W, respectively) and influence of duration time on the effect of sterilization are studied in the test; and the impacts of different distances from the microwave generator (50, 150 and 250 mm, respectively) and the different volumes (φ 75×240 mm and φ 96×330 mm) on the effects of sterilization are studied.

Count methods and the fatality rate of microbiology: The total number of bacteria is measured by reference to GB4789.2-94(2012), the total number of mold and yeast is measured by reference to GB4789.15-94(2012), the fatality rate of microbiology = (The total number of bacteria before sterilization - The total number of bacteria after sterilization) ×100%/ The total number of bacteria before sterilization.

RESULTS AND ANALYSIS

Microwave power and time on the effect of sterilization: The solid culture medium of

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Table 1: The sterilized consequent effect of microwave power and processing time

		Fatality rate of microorganism /%			
Microwave power/W	Processing time/min	Bacterium	Mold	Yeast	Actinomycetes
400	1	0	93.20	93.20	95.53
	2	21.28	99.12	99.12	100
	3	99.90	100	100	100
	4	100			
600	1.5	20.98			100
	3.5	100	100	100	
850	1	95.27	99.31	99.31	100
	2	99.97	100	100	
	2.5	100			

Table 2: The sterilized consequent effect of different distances

			Fatality rate of microorganism /%			
Microwave power/W	Processing time/min	Distance/mm	Bacterium	Mold	Yeast	Actinomycetes
		50	100	100	100	100
850	1.5	150	99.97	100	100	100
		250	99.93	100	100	100

Table 3: The sterilizing consequence of different solid culture medium

The bags' dimension/mm^3	Microwave power/W	Sterilization time/min	Fatality rate of microorganism/%
ϕ75×240	600	3.5	100
	850	2	100
ϕ 96×330	600	15	100
	850	9	100

φ 75×240 mm was sterilized under different microwave power (400, 600 and 850 W, respectively) and within a certain time, The fatality rates is shown in Table 1.

As can be seen from Table 1, microwave has the ability of sterilizing bacterium, mould, yeast and actinomycetes. The greater microwave power has higher fatality rate during the same processing time. In other words, higher power and longer processing time led to better experimenting results. From relation between sterilization and the micro-organisms, we can know that microwave can be used to sterilize bacteria, mould, yeast and actinomycetes. When the bacteria are sterilized completely, mould, yeast and actinomycetes have been sterilized completely already and when the mould, yeast and actinomycetes are sterilized completely, bacteria have not been sterilized completely. So, microwave sterilization effect on the bacteria can be used as measurable indictors on the all microorganisms.

Distance on the effect of sterilization: The solid culture medium of φ 75×240 mm was sterilized at the microwave power of 850 W, the time of 1.5 min and the different distance (50, 150 and 250 mm, respectively) the effect is shown in Table 2.

As can be seen from Table 2, microwave has a significant role in the sterilization of mould, yeast and actinomycetes; Due to a lot of bacteria in the solid culture medium, there is less difference among fatality rates according to the different distance. However, from the number of residual bacteria, it can be seen that effect of the distance on the microwave sterilization is quite obvious. This is because the microwave power density is weakened as increasing the distance.

The effect of microwave sterilization on different volumes: There are two kind plastic bags of φ 75×240 mm and φ 96×330 mm in the actual production of edible fungi. Microwave sterilization was carried out using the each bag respectively. The results of the complete microwave sterilization are shown in Table 3.

As can be seen from Table 3, the duration time of the microwave sterilization process was increasing with the increase of solid culture medium volume under a certain power. The Polyethylene plastic bag of φ 75×240 mm was commonly used in practical occasions than the other sizes; therefore the microwave power of 850 W and the time of 2.5 min can be more suitably applied in the actual production of edible fungi.

Comparison test: Thirty (30) plastic bags of φ 75×240 mm was fetched, with each loaded with 500 g solid culture medium; then they were divided into 3 groups, namely 10 bags each group. The first group was not sterilized; the second was sterilized using traditional methods; and the third was sterilized under microwave power of 850 W and the sterilizing time was 2.5 min. Then, the 30 plastic bags were placed at room temperature. After 7 days, the plastic bags in first group were all covered with mold and the rest did not have any microorganism growth. This shows that the microwave can be applied to obtain complete sterilization the solid culture medium.

Microwave sterilization law on the bacteria: Various microwave powers were used to sterilize the solid culture medium of φ 75×240 mm and after a certain time, the curve of temperature versus duration time is

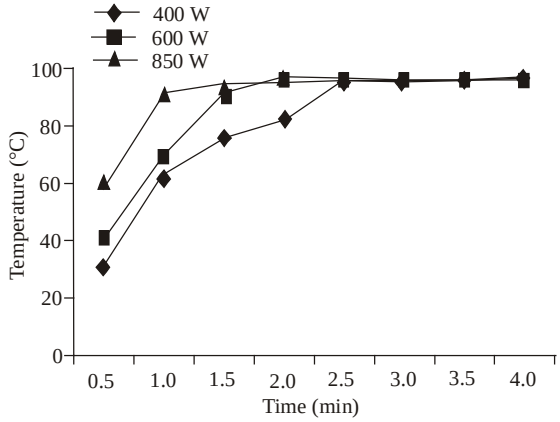

Fig. 1: The relation of temperature and time

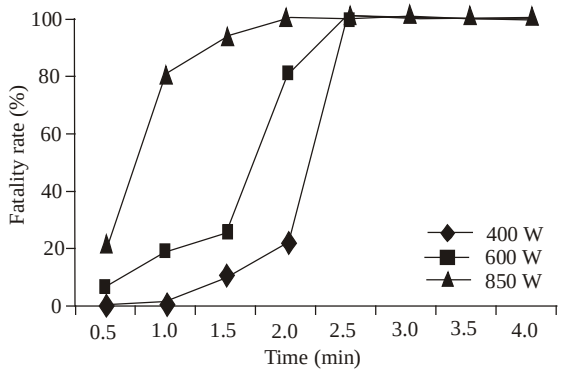

Fig. 2: The relation of bacterium mortality rate and time

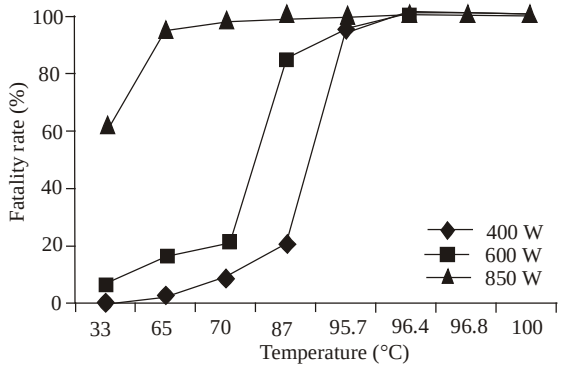

Fig. 3: The relation of temperature and bacterium fatality rate

shown in Fig. 1. The curve of fatality rate of bacteria as a function of various duration times is shown in Fig. 2 and the effect of temperature on fatality rate is shown in Fig. 3.

As can be seen from Fig. 1, the temperature of solid culture medium is up forward with time increasing in the process of sterilization. Temperature was rising much more rapidly when time is less than 2.5 min and then temperature almost remain same after 90°C. The final temperature maintained between 96~98°C. The high temperature is an important reason why

Table 4: The flammulina's growth contrast of microwave sterilization and traditional

Technical index	Steam sterilization	Microwave sterilization
Primordium growth time	2004.10.2	2004.10.2
Mushroom cap diameter (cm)	1.0	1.1
Mushroom cap thickness (cm)	0.5	0.5
Mushroom cap color	Milky white	Milky white
Mushroom stalk length (cm)	7.8	7.9
Mushroom stalk diameter (cm)	0.4	0.6
Mushroom stalk color	Light yellow	Light yellow

Table 5: The pleurotus' (clavate) growth contrast of microwave sterilization and traditional

Technical index	Steam sterilization	Microwave sterilization
Primordium growth time	2004.10.1	2004.9.30
Mushroom cap diameter (cm)	2.9	3.0
Mushroom cap thickness (cm)	1.3	1.2
Mushroom cap color	Light yellow	Light yellow
Mushroom stalk length (cm)	7	7
Mushroom stalk diameter (cm)	2.2	2.3
Mushroom stalk color	Light yellow	Light yellow

Table 6: The pleurotus nerbrodensis' growth contrast of microwave sterilization and traditional

Technical index	Steam sterilization	Microwave sterilization
Primordium growth time	2004.9.28	2004.9.28
Mushroom cap diameter (cm)	8.0	8.2
Mushroom cap thickness (cm)	1.3	1.2
Mushroom cap color	White	White
Mushroom stalk length (cm)	4.1	4.2
Mushroom stalk diameter (cm)	1.5	1.4
Mushroom stalk color	White	White

microwave can sterilize the microorganism. At the same time, we can see that the greater microwave power led to faster rising of temperature.

As can be seen from Fig. 2, the fatality rate of bacteria increased slowly at the beginning of the sterilizing process under the microwave power of 400 and 600 W, respectively. However, after 1~2 min the fatality rate of bacteria showed a steep upward trend; the fatality rate of bacteria was up from 20 to 80% within 0.5 min; and then reached a platform. The reason is that the change of the bacteria fatality rate was less obvious with time further increasing. The fatality rate of bacteria reached 100% when the time was extended to 2.5 min. The fatality rate of bacteria curve has shown a rapid upward trend in 0~1 min under the microwave power of 850 W; however after 1 min the fatality rate of bacteria curve rose slowly; at this point the fatality rate of bacteria is lifted to 95%. At 2.5 min, it climbed up to 100%. These laws coincide with the sterilization conditions gotten before.

As can be seen from Fig. 3, temperature rise of the solid culture medium is the key factor in microwave sterilizing; and the higher temperature, the higher fatality rate. When the temperature exceeded 90°C, the fatality rate reached 99% or higher. The temperature needed to completely sterilize bacteria is 97, 97.4 and 96°C, respectively, under the microwave power of 400, 600 and 850 W, respectively. These three temperatures

are not the same when sterilized completely but they were not to reach 100°C, this shows that temperature is one but not exclusive of the important factors in microwave sterilizing.

Comparison of the edible fungus growth effects between the microwave sterilization and the traditional: Fifteen (15) plastic bags of solid culture medium were sterilized by applying microwave sterilization and by the traditional respectively; and then cultivate Flammulina, Plearotus (clavate), Pleurotus Nerbrodensis; their growth is shown in Table 4, 5 and 6.

As can be seen from Table 4, 5 and 6, there is no difference in the edible fungus growth under by using the two different sterilizing methods, microwave sterilization and traditional methods. Therefore, microwave sterilization to solid culture medium is entirely feasible.

CONCLUSION

- Microwave has a significant role in the solid culture medium sterilization.
- Temperature and mortality rates increased with the extension of the time in the process of sterilizing. The final temperature reaches 96~98°C, bacterial fatality rate reaches 100%; the higher temperature, the higher fatality rate; The greater microwave power, the faster temperature, the faster sterilization.

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