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Effects of Culture Media, Exposure Time and Temperature on Near-Ultraviolet-Induced Sporulation of *Alternaria alternata*

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ABSTRACT

Effects of culture media, near-ultraviolet exposure time, and temperature on sporulation of *Alternaria alternata* were investigated. Strains RL 671-2 and ATCC 36068 were cultivated on Potato Dextrose Agar (PDA), V8 Juice Agar (V8 Agar) and Mycological Agar (MA). The best culture medium for sporulation of strain RL 671-2 was PDA, followed by V8 agar, with only negligible numbers of spores appearing on MA. Near-UV exposure significantly increased sporulation in strain RL 671-2 on PDA and V8 agar. Significantly higher ($P<0.01$) spore counts were found in PDA cultures of this strain exposed to near-UV at 35 than at 20°C. On V8 agar significantly more spores were observed at 20 than at 35°C. MA was not a satisfactory medium for sporulation of ATCC 36068. Both PDA and V8 agar equally supported sporulation for this strain (ATCC 36068) at all exposure times.

Many *Alternaria* species are plant pathogens. Black spot disease of Japanese pear (*16*), seedling chlorosis of cotton and citrus (*2,16*), tobacco brown spot (*1*), and early blight disease of tomato and potato (*16*) are all caused by *Alternaria* species.

Alternariol, alternariol methyl ether, altenuene and tenuazonic acid are some of the mycotoxins produced by *Alternaria* on infected commodities (*3*). These mycotoxins are toxic to both HeLa and mouse lymphoma cells (*13*) and to *Bacillus mycoides* (*10*). Stinson et al. (*15*) stated that toxic strains of *Alternaria* were found in overwintered grain that caused human fatalities in the USSR.

Fungal spores can be conveniently used for inoculation. In the study of production of mycotoxins by *Alternaria alternata* in synthetic, semisynthetic or rice media, we found it difficult to collect enough spores for inoculation because *A. alternata* sporulated sparsely on mycological agar. Literature research on available techniques to increase fungal sporulation by *Alternaria* species indicated that exposure of the fungus to near-ultraviolet (UV) rays or cultivation of the fungus at high temperature would increase spore production. Sporulation of *Alternaria solani* Maine strain 52 was greatly increased after it was exposed for 20 s to UV light (*9*). The sporulation of *Alternaria dauci* (*7*), *Alternaria tomato* (*4*), *A. solani* (*8*), *Alternaria chrysanthemi* (*5*), and *Alternaria cichorii* Nattras (*17*) were all reported to be induced by near-UV (320-400 nm) irradiation followed by a period of darkness. In addition to near-UV irradiation, high incubation temperatures (27-40°C) also induced sporulation in *A. tomato* and *A. dauci* (*6*). However, sporulation caused by high temperature-induction was less than that induced by near-UV exposure.

Although numerous studies have been conducted to evaluate the effect of light exposure on sporulation in many *Alternaria* species, no study has been performed with *A. alternata*. To facilitate production of large quantities of spores by *A. alternata* for inoculation, the effects of media and near-UV exposure times and temperatures on sporulation of this fungal species were investigated in this study.

MATERIALS AND METHODS

A. alternata RL 671-2 was furnished by F. S. Chu of the Food Research Institute, University of Wisconsin, while *A. alternata* ATCC 36068 was obtained from American Type Culture Collection, Rockville, MD. These isolates were maintained in darkness at room temperature (22 ± 2°C) on Potato Dextrose Agar (PDA, pH 5.7, Difco).

Experimental cultures were grown on PDA, mycological agar (MA, pH 7.0, Difco), and a medium of 20% V8 juice (Campbell Soup Co.), 0.3% $CaCO_3$ and 1.5% agar (V8 Agar, pH 7.0). Plates were centrally inoculated with 5 or 6 agar plugs of 5-mm diameter taken from the periphery of young and actively growing cultures (8 to 10-d-old) of *A. alternata* grown at room temperature (22 ± 2°C) in the dark. Cultures were incubated at room temperature in continuous darkness for 12 d to allow the fungus to grow over the entire surface of the medium. The cultures were then incubated at 20° or 35°C in a growth chamber (Percival, Boone, IA) and irradiated with near-UV light for 12, 24 or 48 h. The source of near-UV light irradiation was a pair of 60-cm Westinghouse F20T12/BLB fluorescent

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lamps (320-400 nm), 20 W each, held 32 cm above the culture surface. The intensity of near-UV irradiation was measured with a model 751 Optronics recording spectroradiometer (Optronics Lab Inc., Orlando, FL) and was about 2,060 ergs/cm^2/s (2.06 W/m^2). Control cultures were left in darkness at room temperature.

After inductive irradiation, cultures were maintained in the darkness at room temperature for 5 d, a period previously found to yield the highest number of spores on PDA. On the 6th day, spores on each plate were collected by flooding the agar surface twice with 2 ml of 0.1% sodium lauryl sulfate solution and gently scraping the agar surface with a rubber policeman. Spores were counted using a hemacytometer.

Nineteen treatment combinations, including three unexposed cultures (20°C only), were defined using the levels of incubation temperature (20 or 35°C), length of time (12, 24 or 48 h) of near-UV irradiation, and growth medium (PDA, V8 agar or MA). Treatments on PDA and V8 agar of *A. alternata* were replicated twice (Table 1). For MA, sporulation of both strains was tested only once and 10 plates were used for each exposed treatment combination while the unexposed treatments consisted of 20 and 16 plates for RL 671-2 and ATCC 36068, respectively.

Because the variation in the observed spore counts for each plate within each treatment was directly proportional to the average spore count for the treatment, the natural logarithms (ln) of the spore counts (divided by 10^4) were used as data values (*12*). This transformation helped to stabilize the variances within the treatments. Data were subjected to a factorial analysis of variance using the General Linear Models Procedure in the Statistical Analysis System (*11*). The near-UV exposure treatments and the unexposed cultures were compared using Dunnett's test (*14*).

Because sporulation on mycological agar was much less than on PDA and V8 agar, particularly with strain RL 671-2 (Table 1), the data from PDA and V8 agars were analyzed separately from the MA data.

RESULTS AND DISCUSSION

The average values of the original spore counts per plate and the ln (spore count $\times 10^{-4}$) for each of the 19 treatments for both strains on PDA, V8 agar and MA are listed in Table 1. Both the fungal strains and culture media affected the production of spores in *A. alternata*. Strain ATCC 36068, in general, produced significantly higher spore counts on these culture media, even without near-UV exposure, than did strain RL 671-2. Exposure to near-UV light increased sporulation of both strains on both PDA and V8 agar. For strain ATCC 36068, both PDA and V8 agar supported sporulation equally well, while MA was not a satisfactory medium. For strain RL 671-2, the best medium for sporulation was PDA, followed by V8 agar, and then MA. Because strain RL 671-2 produced only negligible numbers of spores on MA, statistical analysis was not performed.

A summary of the analyses of variance for both strains

TABLE 1. *Average spore numbers of* Alternaria alternata *strains RL 671-2 and ATCC 36068 produced on potato dextrose agar, V8 juice agar and mycological agar at each exposure temeprature-time combination.*

			RL 671-2			ATCC 36068		
Medium	Exposure temperature (°C)	Exposure time (h)	Pooled plate no.	No. of spores ($\times 10^4$)	Ln (# spores/10^4)	Pooled plate no.	No. of spores ($\times 10^{-4}$)	Ln (# spores/10^4)
Potato dextrose agar[a]	20	Unexposed	30	12.82	2.20	40	266.99	5.56
		12	17	52.61	3.67	20	895.34	6.72
		24	20	73.73	4.15	20	994.91	6.79
		48	20	88.80	4.39	20	1253.60	7.03
	35	12	19	93.33	4.44	20	807.95	6.66
		24	22	119.85	4.70	20	956.06	6.83
V8 juice agar[a]	20	Unexposed	40	4.69	1.35	36	536.39	6.04
		12	20	16.39	2.54	20	788.99	6.61
		24	20	16.84	2.66	20	1002.16	6.89
		48	20	24.64	3.06	20	1315.20	7.14
	35	12	20	4.20	1.28	20	747.32	6.53
		24	18	6.78	1.80	20	841.40	6.71
Mycological agar[b]	20	Unexposed	20	0.85	−0.6215	16	28.89	3.32
		12	10	0.085	−0.7601	10	23.16	2.76
		24	10	0.04	−0.9786	10	34.48	3.51
		48	10	0.02	−0.8294	10	44.4	3.78
	35	12	10	0.28	−0.7459	10	7.84	1.95
		24	10	0.68	−0.0073	10	10.82	2.08
		48	--	--	--	10	4.96	1.50

[a]Data from potato dextrose agar and V8 juice agar are from two replications of the first 12 treatments.

[b]Data from mycological agar are from one replication of the final 7 treatments. Average values are listed in original and natural logarithmic units.

on PDA and V8 agar is presented in Table 2. Of particular interest is the significance ($P<0.0001$) of the AGAR × TEMP interaction for strain RL 671-2. The interpretation of the AGAR × TEMP interaction with strain RL 671-2 is illustrated in Table 3. With strain RL 671-2 grown on PDA, the average ln (spore count) at 35°C was significantly ($P<0.01$) higher than the average ln (spore count) at 20°C as determined by Tukey's honestly significant difference procedure (*11*). However, when RL 671-2 was grown on V8 agar, the opposite effect was observed; the average ln (spore count) at 35°C was significantly ($P<0.01$) lower than the average ln (spore count) at 20°C.

A difference between the culture media at each temperature was also observed (Table 3). At 20 and 35°C the average ln (spore count) for PDA was significantly ($P<0.01$) higher than the average ln (spore count) for the V8 agar. However, at the higher temperature the difference in the average ln (spore count) between PDA and V8 agar was approximately 2.5 times greater than the difference at the lower temperature. For PDA the average ln (spore count $\times 10^{-4}$) for the unexposed plates was significantly ($P<0.01$) lower than the average ln (spore count $\times 10^{-4}$) at each of the five exposed treatments (Table 1). With V8 agar, the average ln (spore count $\times 10^{-4}$) for the unexposed plates was only significantly lower than the exposed treatments at 20°C as determined by Dunnett's test (*14*).

With strain ATCC 36068 cultivated on PDA, the temperature at which the fungal culture was exposed to near-UV had no effect on the average ln (spore count) at any of the exposure times. Culture medium did not demonstrate a significant effect. Exposure time was the only factor with a significant effect ($P<0.0001$, Table 2). It can be seen from data in Table 1 that with both media, the number of spores increased with increased intervals of near-UV exposure at both temperatures. On each culture medium, all of the near-UV exposed treatments had significantly ($P<0.01$) higher spore counts than the unexposed plates.

The average of the ln spore count of the seven treatments (13 -19 in Table 1) of ATCC 36068 cultivated on MA are listed in Table 4. The summary of the analysis of variance of these seven treatments is presented in Table 5. The TIME × TEMP interaction was highly significant ($P<0.0001$). For this strain, sporulation at 20°C was significantly higher than at 35°C for 12, 24 and 48 h of near-UV exposure, respectively. In fact the difference between sporulation at 20 and 35°C increased with near-UV exposure time (Table 4). Furthermore, four of the treatment means were significantly ($P<0.05$) lower than the means of the unexposed plates, indicating that the

TABLE 2. *Analysis of variance table for* Alternaria alternata *strains RL 671-2 and ATCC 36068 on potato dextrose and V8 juice agar.*

	RL 671-2				ATCC 36068			
Source	d.f.	Mean square	F value[a]	Significance level	d.f.	Mean square	F value[a]	Significance level
Medium	1	206.472	503.6	0.0001	1	0.042		
Temperature	1	1.118			1	0.194		
Medium × temperature	1	30.319	73.9	0.0001	1	0.123		
Time	2	5.406	13.2	0.0001	2	2.122	13.3	0.0001
Medium × time	2	0.022			2	0.106		
Temperature × time	1	0.028			1	0.001		
Medium × temp × time	1	1.170			1	0.100		
Unexposed vs others	2	72.895	177.8	0.0001	2	30.513	191.9	0.0001
Error	254	0.410			264	0.159		

[a]Only significant F values are listed.

TABLE 3. *Interpretation of the MEDIUM × TEMP interaction with strain RL 671-2 using the average ln (spore count × 10^{-4}) values at the two near UV exposure temperatures with both culture medium. The averages were calculated using the data at times 12 and 24 h only.*

	Temperature		Temperature Effect
Medium	20°C	35°C	35°C-20°C
Potato dextrose	3.93	4.58	0.65[a]
V8 Juice	2.60	1.56	−1.04[a]
Medium effect PDA-V8	1.33[a]	3.02[a]	

[a]Denotes difference is highly significant ($P<0.01$) as determined by Tukey's honestly significant difference procedure.

TABLE 4. *Treatement means and differences of* Alternaria alternata *strain ATCC 36068 on mycological agar. Averages are in units of ln (spore count × 10^{-4}).*

		Exposure Time (h)		
Unexposed	Temperature	12	24	48
3.32	20°C	2.76[a]	3.51	3.78
	35°C	1.95[a]	2.08[a]	1.50[a]
	Temperature effect 20°C-35°C	0.81[b] (0.21)[c]	1.43[b] (0.21)[c]	2.28[b] (0.21)[c]

[a]Denotes treatment mean which is significantly ($P<0.05$) lower than unexposed mean as determined by Dunnett's test.
[b]Denotes temperature effect is significantly ($P<0.01$) greater than zero.
[c]Denotes standard error of the temperature effect.

TABLE 5. *Analysis of variance table for* Alternaria alternata *strain ATCC 30608 on mycological agar.*

Source	D.F.	Mean square	F value	Significance level
Temperature	1	34.08	146.4	0.0001
Time	2	0.98	4.2	0.02
Time × temperature	2	2.70	11.6	0.0001
Unexposed vs others	1	6.57	28.2	0.00001
Error	69	0.23		

near-UV exposure reduced sporulation of ATCC 36068 on MA.

Thus, as with other *Alternaria* species (*4,5,7,8,9,17*), exposure to near-UV light increased sporulation of both *A. alternata* strains RL 671-2 and ATCC 36068 on both PDA and V8 agar. For strain RL 671-2, near-UV induction of sporulation was more noticeable on PDA than on V8 agar at both 20 and 35°C. However, this is not true for strain ATCC 36068. Both culture media supported sporulation of this fungal strain to the same degree, while exposure temperatures had no effect on sporulation. Although high cultivation temperature induced sporulation of *A. tomato* and *A. dauci (6),* it was shown in this study that the higher exposure temperature during near-UV exposure enhanced sporulation of *A. alternata* RL 671-2 significantly on PDA but not on the V8 agar. In this instance, the lower temperature produced significantly higher numbers of spores. This temperature-related enhancement in spore counts did not occur in strain ATCC 36068 on PDA or V8 agar. On MA, only exposure to near-UV at 20°C for 24 and 48 h enhanced sporulation. The MA medium is not suitable for sporulation of strain RL 671-2. The fungus produced negligible numbers of spores on this medium even after near-UV induction for 48 h and 24 h at 20 or 35°C, respectively.

With the recognition of *Alternaria* mycotoxin toxicities and their effects on human and animal health, a better understanding of growth and toxin production by the mold is needed to control these problems. This study on enhanced spore production by *A. alternata* hopefully will provide an additional tool to aid in these research works.

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REFERENCES

1. Fargacs, J., and W. T. Carll. 1966. Mycotoxicoses: toxic fungi in tobaccos. Science 152:1634-1635.
2. Fulton, N. D., K. Bollenbacher, and G. E. Templeton. 1965. A metabolite of *Alternaria tenuis* that inhibits chlorophyll production. Phytopathology 55:49-51.
3. Harvan, D. J., and R. W. Pero. 1976. The structure and toxicity of the *Alternaria* metabolites. PP. 344-355. *In* J. V. Rodricks (ed.). Mycotoxins and other fungal related food problems. ACS Monograph. No. 149. Washington, DC.
4. Kumagai, T., and Y. Oda. 1973. Blue and near ultraviolet reversible photoreaction with intracellular particulate fraction of the fungus, *Alternaria tomato.* Plant Cell Physiol. 14:1107-1112.
5. Leach, C. M. 1964. The relationship of visible and ultraviolet light to sporulation of *Alternaria chrysanthemi.* Trans. Brit. Mycol. Soc. 47:153-158.
6. Leach, C. M. 1967. Interaction of near-ultraviolet light and temperature on sporulation of the fungi *Alternaria, Cercosporella, Fusarium, Helminthosporium,* and *Stemphylium.* Can. J. Bot. 45:1999-2016.
7. Leach, C. M., and E. J. Trione. 1966. Action spectra for light-induced sporulation of the fungi *Pleospora herbarum* and *Alternaria dauci.* Phytochem. Photobiol. 5:620-630.
8. Lukens, R. J. 1963. Photoinhibition of sporulation in *Alternaria solani.* Amer. J. Bot. 50:720-724.
9. McCallan, S. E. A., and S. Y. Chan. 1944. Inducing sporulation of *Alternaria solani* in culture. Contrib. Boyce Thompson Inst. 13:323-335.
10. Pero, R. W., H. Posner, M. Blois, D. Harvan, and J. W. Spalding. 1973. Toxicity of metabolites produced by the "Alternaria." Environ. Health Prospect. June:87-94.
11. SAS Users's Guide: Basic 1982 Edition. SAS Institute Inc., Cary, North Carolina.
12. Snedecor, G. W., and W. G. Cochran. 1980. Statistical methods, 7th ed. Iowa State University Press, Ames.
13. Spalding, J. W., R. W. Pero, and R. G. Owens. 1970. Inhibition of the G2 phase of the mammalian cell cycle by the mycotoxin, alternariol. J. Cell Biol. 47:199a.
14. Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York.
15. Stinson, E. E., D. D. Bills, S. F. Osman, J. Siciliano, M. J. Ceponis, and E. G. Heisler. 1980. Mycotoxin production by *Alternaria* species grown on apples, tomatoes and blueberries. J. Agric. Food Chem. 28:960-963.
16. Templeton, G. E. 1972. *Alternaria* toxins related to pathogenesis in plants. pp. 169-192. *In* S. Kadis, A. Ciegler and S. J. Ajl (eds.), Microbial toxins, Vol. VIII. Academic Press, New York.
17. Vakalounakis, D. J., and C. Christias. 1981. Sporulation in *Alternaria cichorii* is controlled by a blue and near ultraviolet reversible photoreaction. Can. J. Bot. 59:626-628.