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A novel dodine-free selective medium based on the use of cetyl trimethyl ammonium bromide (CTAB) to isolate *Beauveria bassiana*, *Metarhizium anisopliae* sensu lato and *Paecilomyces lilacinus* from soil

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Abstract: This study evaluated the quaternary ammonium compound cetyl trimethyl ammonium bromide (CTAB) as an alternative to the chemically related dodecylguanidine (dodine) for the selective isolation of entomopathogenic fungi. Oatmeal agar (OA) with chloramphenicol was used as basal medium, and three concentrations of CTAB (0.5, 0.6, 0.7 g/L) were evaluated and compared against OA + 0.46 g/L dodine. Selective isolation and growth studies were performed with the entomopathogens *Beauveria bassiana, Metarhizium anisopliae* s.l. and *Paecilomyces lilacinus* and five common non-entomopathogenic non-target species. The three entomopathogenic fungi sporulated earlier on OA + 0.6 g/L CTAB than on OA + 0.46 g/L dodine, while none of the non-target fungi sporulated on OA + 0.6 g/L CTAB. All entomopathogenic fungal isolates grew on OA + 0.6 g/L CTAB, despite some intra-species variation, whereas non-target fungi showed no growth or sporulation. OA + 0.6 g/L CTAB resulted in an efficient medium to isolate *B. bassiana*, *M. anisopliae* s. l. and *P. lilacinus* from soil samples. Results of our study suggest that OA + 0.6 g/L CTAB is a suitable, simple and inexpensive to prepare medium to replace OA + 0.46 g/L dodine for the selective isolation of these fungi.

Key words: *Beauveria bassiana*, CTAB, entomopathogenic fungi, *Metarhizium anisopliae*, *Paecilomyces lilacinus*, selective medium

Introduction

Entomopathogenic fungi, particularly *Beauveria bassiana* (Bals-Criv.)Vuillemin, *Metarhizium anisopliae* (Metschn.) Sorokin, *Paecilomyces lilacinus* (Thom) Samson, *Isaria fumosorosea* Wize and *Lecanicillium lecanii* (Zimm.) Zare and Gams, have been studied extensively as commercial biocontrol agents (Ramle et al. 2004, Faria and Wraight 2007, Jaronski 2007, Posadas and Lecuona 2009, Fernandes et al. 2010). In nature, entomopathogenic fungi tend to occur as diverse assemblages of species and strains (Fernandes et al. 2010). Explorations of this rich diversity by isolating new fungal isolates is expected to yield strains with attributes superior to those currently available for biological pest control (Fernandes et al. 2010).

A variety of fungicides and antibiotics have been used in selective media to isolate entomopathogenic fungi from environments such as soil (Wraight et al. 2007, Liu et al. 1993). Initially the media used for the general isolation of soil fungi, such as Veen's medium, were semi-selective (Veen and Ferron 1966). Veen's medium was useful but presented some limitations: *B. bassiana* grows slowly with poor development of aerial mycelium, making it difficult to identify morphologically in culture (Doberski and Tribe 1980).

The discovery by Doberski and Tribe (1980) that dodecylguanidine monoacetate (dodine) selectively inhibits non-entomopathogenic fungi in culture simplified isolation methods. Dodine is a soluble amphiphile (surfactant) consisting of a hydrophobic non-polar group and a positively charged hydrophilic polar head group (Vieira and Carmona-Ribeiro 2006). In vitro dodine severely affects the metabolism of fungal cells. Low concentrations of dodine inhibit growth, respiration of glucose and acetate and active transport of phosphorus and carbon (Cabral 1991). Media adaptations by Beilharz et al. (1982) and Chase et al. (1986) demonstrated that dodine-based media can be useful in quantifying propagules of entomopathogenic fungi in soils. Chase et al. (1986) showed that oatmeal agar medium with 0.6 g/L dodine facilitated isolation of *B. bassiana* but was inhibitory to *M. anisopliae* s.l. In addition, by reducing dodine concentration to 0.46 g/L and adding Benomyl (Benlate) 0.4 g/L, both species can be effectively isolated from soils. Liu et al. (1993) observed that low concentrations of dodine in combination with cycloheximide increased the recovery of *Metarhizium* spp. from soil.

Dodine has experienced a sharp reduction in its primary market as a fungicide and thus is increasingly difficult to obtain (Luz et al. 2007). Hence, there is a need to search for suitable alternatives. The quater-

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nary ammonium compound cetyl trimethyl ammonium bromide (CTAB) has a chemical structure similar to that of dodine, suggesting a similar biological activity toward fungi. The objective of this study was to evaluate CTAB as a possible alternative to dodine for the selective isolation of entomopathogenic anamorphic fungi from soil.

MATERIALS AND METHODS

Fungal isolates.—The fungal isolates used and their origin are listed (TABLE I). For preservation, *B. bassiana* was cultured on complete medium agar (CMA) composed of (g/L): K_2HPO_4, 0.4; NaH_2PO_4, 1.4; $MgSO_4$, 0.6; KCl, 1; NH_4NO_3, 0.7; glucose, 10; yeast extract, 5; agar, 15, chloramphenicol, 0.5. *M. anisopliae* s. l., *Aspergillus niger, A. ochraceus, P. lilacinus, Penicillium minioluteum* and *Trichoderma harzianum* were cultured on potato dextrose agar (PDA; Oxoid) amended with chloramphenicol (0.5 g/L). *Fusarium solani* was maintained on synthetischer nährstoffarmer agar (SNA), composed of (g/L): KH_2PO_4, 1; KNO_3, 1; $MgSO_4$, 0.5; KCl, 0.5; glucose, 0.2; sucrose, 0.2; agar, 20. All isolates were kept on agar slants at 8 C in a refrigerator then deposited in the collection of the Entomopathogenic Fungi Laboratory of IMYZA, INTA, Castelar, Argentina.

Development of the isolation medium.—Oatmeal agar (OA), consisting of 20 g/L rolled oatmeal and 20 g/L agar, amended with chloramphenicol 0.5 g/L to retard bacterial growth, was used as basal medium. Three concentrations of CTAB (Alfa-Aesar), 0.5 g/L, 0.6 g/L and 0.7 g/L (OA-CTAB 5, OA-CTAB 6 and OA-CTAB 7 respectively, were added to the basal medium to develop the testing medium. For comparison, OA with technical-grade dodine (95%) 0.46 g/L (OA-D) was prepared according to Chase et al. (1986).

Conidial suspensions.—Conidia suspensions were prepared for all fungi sporulated on standard media from week-old cultures (2 wk in the case of *F. solani*). Conidia were suspended in 5 mL 0.05% Tween 80 and conidia were adjusted to 10^5 conidia/mL with an improved Neubauer chamber.

Recovery studies.—Conidial suspensions were diluted 1/10, $1/10^2$ and $1/10^3$. Aliquots of 100 µL of each diluted suspension were aseptically spread with sterile glass spatulas uniformly over the surfaces of OA-CTAB 5, OA-CTAB 6, OA-CTAB 7 and OA-D plates. The control was performed on OA. Four replicates were performed for each treatment. All plates were incubated at 26 C for 72 h. Plates with 15–150 colonies, the maximum number that can be distinguished with accuracy, were selected and the number of colonies were counted and expressed as colony forming units (cfu) (Pitt and Hocking 2007). For each treatment the recovery percentage (%R) was calculated as:

$$[(\text{number of cfu on testing medium} / \text{number of cfu on OA})] \times 100.$$

ANOVA ($\alpha < 0.05$) was performed to evaluate the influence of the different media tested on the fungal species. Non-target fungi were analyzed with a Kruskal Wallis test because data were not normally distributed (Martínez González et al. 2001).

Radial growth assays.—Growth of the fungal strains was evaluated on OA-CTAB 6. Aliquots of 10 µL of each conidial suspension (10^5 conidia/mL) were inoculated with a calibrated loop on the surface of the culture medium (one drop on the center of each plate). Inoculations were performed in four independent replicates. Cultures were incubated at 26 C for 25 d. Growth diameter measurements (in millimeters) were taken in two orthogonal axes for each culture and summarized as the mean value of four replicates. Colony diameters were taken daily during the first 2 wk and subsequently twice a week. Attention was given to the time of the beginning of sporulation. Radial growth data were subjected to regression analysis. Growth rates (mm/d) were calculated from the regression slope of colony diameter versus time during the linear growth phase (Fargues et al. 1992). This parameter was taken into account to evaluate the influence of dodine and CTAB on fungal growth. To estimate the lag phase the linear growth phase was extrapolated to the initial inoculum diameter size ($y = 5$). The intercept on the time axis was defined as the lag. Parameters were compared by means of ANOVA.

Assay of intra-species variability in sensitivity/resistance to CTAB.—Conidial suspensions were prepared as described above, and 100 µL aliquots were spread on OA-CTAB 6 and OA plates. Four replicates were performed for each treatment. Plates were incubated at 26 C for 72 h. The number of colonies developed were counted and expressed as colony forming units (cfu). For each treatment the recovery percentage (%R) was calculated as in the recovery assay. Intra-species variability in sensitivity/resistance toward CTAB was evaluated with one-way ANOVA, in which the isolates were considered as a random factor. Tukey's tests were performed to post hoc comparisons. The fraction of the total variability represented by the intra-species variability was calculated.

Isolation of native strains from soil samples.—To test the performance of OA-CTAB 6 (i.e. its ability to isolate native strains from soil samples) four non-sterile soil samples were analyzed. Soil samples Nos. 1 and 2 were collected from Castelar (Buenos Aires province, Argentina), whereas soil samples Nos. 3 and 4 were collected from Ceres and Florencia respectively (Santa Fé province, Argentina). These samples were diluted in Tween 80 (0.05%), and aliquots of 100 µL soil suspension were spread over the surface of the OA-CTAB 6 and OA-D plates with a sterile glass spatula. Cultures were incubated 1 wk at 26 C. Colonies belonging to *B. bassiana*, *M. anisopliae* s. l. and *P. lilacinus* were counted on each medium and results compared. Three replicates were performed for each sample. Data were analyzed with a *t*-test.

RESULTS

Recovery studies.—The cfu values of the fungal isolates for the culture media tested are provided (TABLE II). The values recovered for *B. bassiana* showed no differences between the treatments and

TABLE I. Origin of fungal isolates used in tests to evaluate the effectiveness of CTAB

Isolate (identification code)	Isolation source	Locality
Beauveria bassiana		
Bb 98*	*Cyclocephala signaticollis* (Col.: Scarabaeidae)	Balcarce/Buenos Aires/Argentina
Bb 108**	*Anthonomus grandis* (Col.: Curculionidae)	Paraguay
LPSC 184**	*Diatraea saccharalis* (Lep.: Pyralidae)	Salto/Buenos Aires/Argentina
Bb 32**	*Diatraea saccharalis* (Lep.: Pyralidae)	Ipojuca/Pernambuco/Brazil
Bb 38**	*Nezara viridula* (Hem.: Pentatomidae)	Chapecó/Santa Catarina/Brazil
Bb 51**	Hemiptera: Pentatomidae	France
Bb 83**	Coleoptera: Elateridae	Novo Oriente/Ceará/Brazil
LPSC 902**	*Oliarus dimidiatus* (Hem.: Cixiidae)	La Plata/Buenos Aires/Argentina
Bb 94**	*Adelphocoris* sp. (Hem.: Miridae)	Acquapendente/Italy
LPSC 1060**	*Rondoresia bergi* (Ort.: Acrididae)	Alta Italia/La Pampa/Argentina
Metarhizium anisopliae		
Ma 8*	*Acromyrmex lundii* (Hym.: Formicidae)	Castelar/Buenos Aires/Argentina
Ma 32**	*Aphodius tasmania* (Col.: Scarabaeidae)	Adelande/Australia
Ma 40**	*Mahanarva posticata* (Hom.: Cercopidae)	Flexeira/Alagoas/Brazil
Ma 41**	Coleoptera: Scarabeidae	Brasilia/Goiás/Brazil
LPSC 503**	Soil	La Plata/Buenos Aires/Argentina
LPSC 84**	Soil	Coronel Suarez/Buenos Aires/ Argentina
Paecilomyces lilacinus		
Pl 1*	Soil	Castelar/Buenos Aires/Argentina
LPSC 183**	Soil	Coronel Suarez/Buenos Aires/Argentina
Pl 25**	Soil	Gdor. Virasoro/Corrientes/Argentina
LPSC 983**	Soil	Magdalena/Buenos Aires/Argentina
Pl 2**	Soil	Las Toscas/Santa Fe/Argentina
Pl 4**	Soil	Roque Saenz Peña/Chaco/Argentina
Non-target fungal isolates		
*Aspergillus niger**	Soil	Castelar/Buenos Aires/Argentina
*A. ochraceus**	Soil	Castelar/Buenos Aires/Argentina
*Fusarium solanii**	Soil	Castelar/Buenos Aires/Argentina
*Penicillium minioluteum**	Soil	Castelar/Buenos Aires/Argentina
*Trichoderma harzianum**	Soil	Castelar/Buenos Aires/Argentina

LPSC: Fungal Cultures from La Plata Spegazzini Culture. La Plata. Buenos Aires.
Bb, Ma, Pl: Fungal Collection of Entomopathogenic Fungi Laboratory, IMYZA, INTA, Castelar.
Fungal strains followed by * were used in the recovery and radial growth assays.
Fungal strains followed by ** were used only for the intra-species variability assays.

the control group ($F_{4;15} = 1.59$, $P < 0.23$); % R was always 100. The value for *M. anisopliae* s.l. on OA-D was significant with regard to the other treatments ($F_{4;15} = 21.5$, $P < 0.0001$). The mean % R across all OA-CTAB concentrations was 77 ± 11, and the highest recovery value was 90% on OA-CTAB 6. *M. anisopliae* s.l. was not recovered (% $R = 0$) on OA-D. Recovery of *P. lilacinus* displayed significant differences between the control and the treatments ($F_{4;15} = 9.98$, $P < 0.0003$). Although recovery of *P. lilacinus* was greater on OA-D, all variants of OA-CTAB media enabled successful recovery of this species as well. Among non-target fungi, *A. niger*, *A. ochraceus* and *P. minioluteum* did not grow on OA-D or any OA-CTAB media (% $R = 0$) whereas *T. harzianum* and *F. solani* were recovered on OA-CTAB 5 (% $R = 26$ and 94 respectively). The recovery studies led us to select 0.6 g/L CTAB as the most effective concentration because it allowed a good recovery of all three species of entomopathogenic fungi tested and the growth of non-target fungi was successfully inhibited.

Radial growth assays.—The results of growth rate, lag phase and time required for sporulation of the strains tested on OA, OA-CTAB 6 and OA-D are provided (TABLE III). The *B. bassiana* growth rate was strongly inhibited on OA-CTAB 6 and OA-D; nevertheless, the lag phase showed non-significant differences and sporulation was observed. The *M. anisopliae* s.l. growth rate was lowest on OA-CTAB 6 and the lag

TABLE II. Values of cfu obtained in the recovery assays on the culture media tested

Fungal isolate	OA[a]	OA-D[b]	OA-CTAB 5[c]	OA-CTAB 6[d]	OA-CTAB 7[e]
B. bassiana	8.1×10^4a	9.1×10^4a	9.8×10^4a	9.2×10^4a	8.2×10^4a
M. anisopliae	5.2×10^4a	0b	3.7×10^4a	4.7×10^4a	3.6×10^4a
P. lilacinus	6.0×10^4a	3.7×10^4b	3.0×10^4b	2.8×10^4b	3.3×10^4b
A. ochraceus	1.0×10^5a	0b	0b	0b	0b
A. niger	6.7×10^4a	0b	0b	0b	0b
P. minioluteum	1×10^5a	0b	0b	0b	0b
T. harzianum	9.5×10^4a	0c	2.7×10^4b	0c	0c
F. solani	7.9×10^4a	0b	7.4×10^4a	0b	0b

[a] OA: oatmeal agar.
[b] OA-D: oatmeal dodine.
[c] OA-CTAB 5: oatmeal CTAB 0.5 g/l.
[d] OA-CTAB 6: oatmeal CTAB 0.6 g/l.
[e] OA-CTAB 7: oatmeal CTAB 0.7 g/l.
Data followed by letters within each line are significantly different ($P < 0.005$).

phase increased in the presence of both CTAB and dodine. Sporulation was observed only on OA-CTAB 6 and OA (7 and 4 d respectively).

The lag phase of *P. lilacinus* was significantly greater for both dodine and CTAB than for the OA control. On all culture media tested, the time required to sporulation for *P. lilacinus* on OA-CTAB 6 was lower than that on OA-D (6 and 9 d respectively). For the entomopathogenic species, colonies grown on OA-CTAB 6 were generally small, compact and sporulated faster than on OA-D.

Among the five non-target fungal species all displayed decreased growth rate and increased lag phase in the presence of CTAB or dodine, except for *A. niger*. Moreover, these fungi were not able to sporulate on both culture media with dodine or

TABLE III. Growth rate, lag phase and time to sporulation in radial growth assays on selected media

Fungal isolates		OA	OA-D	OA-CTAB 6
B. bassiana	Growth rate	4.88a	2.11b	0.8c
	Lag phase	0.73a	1.76a	1.41a
	Sporulation (days)	4	7	6
M. anisopliae	Growth rate	3.92a	2.1b	0.58c
	Lag phase	1.07a	3.8b	4.11b
	Sporulation (days)	4	NS	7
P. lilacinus	Growth rate	2.44a	2.03b	0.6c
	Lag phase	0.02a	5.31b	2.6c
	Sporulation (days)	4	9	6
A. niger	Growth rate	8.88a	2.52b	0.81c
	Lag phase	0.42a	2.96b	0.09a
	Sporulation (days)	2	NS	NS
P. mineoluteum	Growth rate	6.37a	1.93b	2.12b
	Lag phase	0.39a	5.22b	7.55c
	Sporulation (days)	2	NS	NS
A. ochraceus	Growth rate	4.63a	1.52b	0.47b
	Lag phase	0.27a	4.05b	7.81c
	Sporulation (days)	3	NS	NS
F. solani	Growth rate	9.58a	2.95b	2.53b
	Lag phase	0.43a	5.56b	2.95c
	Sporulation (days)	2	NS	NS
T. harzianum	Growth rate	30.5a	3.22b	0.95b
	Lag phase	0.6a	5.83b	7.27b
	Sporulation (days)	3	NS	NS

NS: non-sporulated colonies.
Data followed by the same letter within the same line do not differ statistically ($P < 0.05$; ANOVA).

CTAB. Growth of *T. harzianum* was strongly inhibited on OA-CTAB 6; *A. niger* and *F. solani* exhibited a 30-fold decrease in their growth rate compared to OA.

Assay of intra-species variability in sensitivity/resistance toward CTAB.—All isolates of *B. bassiana* were able to grow on OA-CTAB 6. Significant differences between the isolates in their sensitivity/resistance to CTAB were observed ($F [8, 27] = 9.05$; $P < 0.01$). The intra-species variability represented a high percentage of the total variability (67%). In general the recovery was satisfactory (FIG. 1A).

Similarly all isolates of *M. anisopliae* s.l. were successfully recovered on OA-CTAB 6 (FIG. 1B). Significant variability in sensitivity/resistance toward CTAB among the isolates was observed only in Ma 40 ($F [4, 15] = 18.19$; $P < 0.001$). A high proportion of the total variability (81%) was explained by the intra-species variability. *P. lilacinus* presented the lowest variability in the response to CTAB (FIG. 1C). All isolates were recovered in a percentage greater than 80%, and the variability between the isolates was not significant ($F [4, 15] = 2.43$; $P = 0.09$). In this case, a low portion of the total variability (31%) was explained by the intra-species variability.

Isolation of native strains from soil samples.—The addition of CTAB to the OA medium enabled the isolation of native entomopathogenic fungi from soil. Statistically the same number of colonies of *B. bassiana* and *M. anisopliae* s.l. were isolated on OA-CTAB 6 and OA-D from the four samples analyzed. The number of colonies of *P. lilacinus* was higher on OA-CTAB 6 than on OA-D in three of the samples evaluated (TABLE IV).

Non-target fungi were isolated in comparable quantities on both media, with the exception of sample No. 2, where the number of colonies of contaminant fungi was significantly higher on OA-D than on OA-CTAB 6 (19 and eight colonies respectively; TABLE IV). The four soil samples tested showed differences in the number and diversity of colonies. Particularly, in sample No. 3, the plates of OA-D presented a low number of colonies, most of which corresponded to large colonies of *Trichoderma* spp.

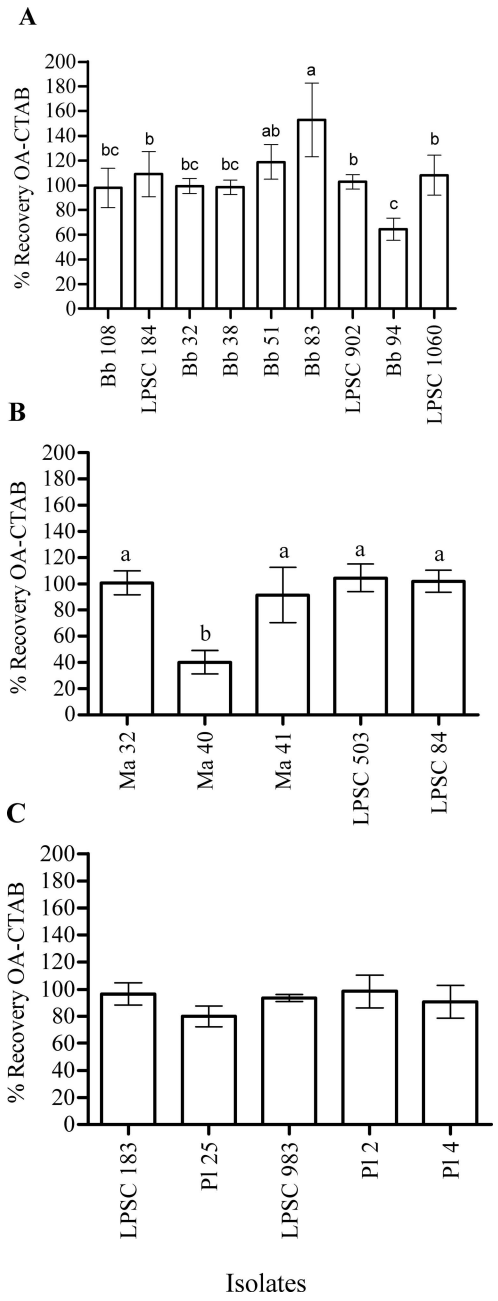

FIG. 1. Strains' resistance/sensitivity toward CTAB of (A) *B. bassiana*, (B) *M. anisopliae*, (C) *P. lilacinus*. Letters above bars indicate statistical significance (Tukey's test $\alpha = 0.05$). Absence of any letter indicates non-significant differences. (For origin of strains see TABLE I.)

DISCUSSION

Recovery studies.—The three species of entomopathogenic fungi evaluated were successfully recovered with CTAB. The recovery of *B. bassiana* was similar on all culture media used. Chase et al. (1986) observed that 0.46 g/L dodine did not affect the growth of *B. bassiana*, suggesting that this species is not inhibited by this fungicide. Similarly, in the present work *B. bassiana* displayed a high resistance to both dodine and CTAB. In conclusion, it is feasible to isolate *B. bassiana* using CTAB as an inhibitor in a culture medium. It is possible that a high tolerance to dodine and CTAB might be a general feature of *Beauveria* spp. and that the same tolerance to dodine and CTAB is shared by *B. brongniartii* (Luz et al. 2007).

Regarding *M. anisopliae* s.l., our inability to recover this fungus on OA-D is in agreement with the results

TABLE IV. Number of colonies of entomopathogenic and non-target fungi isolated from soil with OA-CTAB 6 and OA-D media

Sample number	Fungal strain	Number of colonies/ Petri plate	
		OA-CTAB 6	OA-D
1	*B. bassiana*	6	7
	M. anisopliae	2	1
	P. lilacinus	20*	7*
	Non-target fungi	8	8
2	*B.bassiana*	2	1
	M. anisopliae	2	1
	P. lilacinus	7**	3**
	Non-target fungi	8*	19*
3	*B. bassiana*	0	0
	M. anisopliae	0	0
	P. lilacinus	5*	2*
	Non-target fungi	1	3
4	*B. bassiana*	0	1
	M. anisopliae	2	2
	P. lilacinus	1	0
	Non-target fungi	3	3

Values in the same line followed by * are significantly different, with $P < 0.05$ (*t*-test).

Values in the same line followed by ** are significantly different, with $P < 0.01$ (*t*-test).

by Chase et al. (1986), who found that oatmeal medium amended with 0.55 g/L dodine allows *B. bassiana* growth but inhibits *M. anisopliae*. However, these authors found that lower concentrations of dodine and benomyl (0.46 g/L and 0.38 g/L) were satisfactory for isolation of *Metarhizium* spp. from soil. Likewise, Sneh (1991) successfully isolated *Metarhizium* spp. from soil by using 0.3 g/L dodine and 0.8 g/L benomyl. In contrast, our results showed that *M. anisopliae* s.l. was inhibited even with 0.46 g/L dodine. Of note in the present work, using all three concentrations of CTAB it was possible to recover this fungus at rates higher than 71%. CTAB was useful to isolate *M. anisopliae* s.l.

Paecilomyces lilacinus was successfully recovered with both OA-D and all OA-CTAB media. This result is in agreement with those of Luz et al. (2007) and Mitchell et al. (1987) who found high recovery rates of *P. lilacinus* on dodine containing media.

Non-target fungi were completely inhibited by both dodine (0.46 g/L) and CTAB (0.6 and 0.7 g/L). Beilharz et al. (1982) observed that ubiquitous fungi, such as *T. viride*, *Gliocladium roseum*, *Fusarium* spp., *Penicillium* spp. and *Verticillium* spp., were somewhat inhibited by dodine but often developed despite its presence. In addition, Luz et al. (2007) observed that dodine selectively blocked the growth of *A. niger*, *A. flavus*, *F. roseum* and *C. cladosporioides*.

Radial growth assays.—The growth rates of all fungi decreased in the presence of dodine and CTAB; however, growth inhibition was significantly lower in entomopathogens than in non-target species. On the other hand, *T. harzianum* and *F. solani*, which are fast growing fungi, were strongly suppressed on OA-CTAB 6. Beilharz et al. (1982) found that these genera were suppressed with 0.65 g/L dodine and stated that *T. viride* was the only real weed species, sometimes appearing as small restricted colonies but occasionally overrunning the plates.

In all fungi tested, early colony growth showed a variable behavior with a general tendency to increase the lag phase in the presence of dodine and CTAB.

B. bassiana and *P. lilacinus* both sporulated on the three culture media evaluated, while *M. anisopliae* s.l. sporulated only on OA-CTAB 6 and OA but not on OA-D. The sporulation of *M. anisopliae* s.l. on OA-CTAB 6 is an important factor because early sporulation of target fungi allows their rapid identification (Flowers and Hendrix 1969).

Moreover, the suppression by CTAB in non-target species reduces their potential to spread on the isolation plates and facilitates the morphological identification of entomopathogenic fungi, which generally conidiate normally on selective media.

Assay of intra-species variability in sensitivity/resistance toward CTAB.—Regardless of the origin, all entomopathogenic fungi displayed tolerance to CTAB. Shapiro-Ilan et al. (2002) showed a natural variation in dodine resistance among *B. bassiana* isolates. On the other hand, Liu et al. (1993) observed intra-species variability in susceptibility to dodine among *Metarhizium* spp. In the present study, intra-species variability in *B. bassiana* and *M. anisopliae* s.l. also was observed on CTAB. Luz et al. (2007) found that one strain of *P. lilacinus* was resistant to several fungicides including dodine. We also found high resistance of this fungus to CTAB.

Isolation of native strains from soil samples.—In all soil samples evaluated we were able to isolate native strains of entomopathogenic fungi on media containing dodine or CTAB. OA-CTAB 6 was a useful medium to isolate *B. bassiana*, *M. anisopliae* s.l. and *P. lilacinus* from soil samples. *Trichoderma* sp., a common, fast-growing fungus, was isolated only on OA-D. The absence of this fungus on plates with OA-CTAB 6 let us corroborate that 0.6 g CTAB/L successfully inhibits fast-growing, non-target fungi that often overran the isolation plates. Fernandes et al. (2010) suggested using CTC medium (chloramphenicol, thiabendazole, cycloheximide) for the isolation of *M. acridum* and other *Metarhizium* spp. and *Beauveria* spp. from soil. Nevertheless, OA-CTAB

is simpler than CTC because it requires oatmeal and CTAB, which are less expensive than thiabendazole and cycloheximide.

CTAB is a widely available, inexpensive compound often stocked in molecular biology laboratories. Our study demonstrates the effectiveness of CTAB as an alternative to dodine in media formulation for the selective isolation of several common soilborne entomopathogenic fungi. CTAB-containing media yield compact colonies of entomopathogenic fungi that conidiate normally while inhibiting the growth and sporulation of representative non-entomopathogenic fungi. Due to the variability in the response to fungicides among species and isolates of the same species of entomopathogenic fungi, further studies are required to confirm the effectiveness of OA-CTAB for the selective isolation of other entomopathogenic fungi not investigated in the present study. Moreover, it will be important to test CTAB activity on the isolation of these fungi from different soil types and other sources. Nevertheless, OA-CTAB shows promise as a replacement for dodine as an effective medium for the selective isolation of environmental strains of *B. bassiana*, *M. anisopliae* s.l. and *P. lilacinus*.

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