

Growth and development assessment of native strains of *Trichoderma* spp. in different culture media

Summary

Trichoderma spp. strains from the Catamarca province were evaluated in five synthetic culture media (Potato Glucose Agar, Sabouraud Agar, Amylolytic Agar, Czapek Agar, and a selective medium for fungi and yeasts) and one natural agro-industrial substrate (red grape pomace). The *Trichoderma* strains used were previously cultured and selected on Nutrient Agar. Qualitative characteristics such as mycelial density, conidia production, and pigment production, as well as quantitative characteristics such as growth dynamics at 24 and 48 h, were evaluated by measuring the growth area (mm²) using ImageJ software. Data were analyzed using ANOVA and Fisher's LSD test ($p < 0.05$). Significant differences in growth area were observed between strains and culture media at 24 and 48 h. Most strains showed significantly greater growth on the natural substrate VT. Only strain TAC2 showed significantly greater growth on Sabouraud medium. Qualitative observations coincided with quantitative results. These results highlight red grape pomace as a low-cost substrate and high potential for the growth of *Trichoderma* spp., with biotechnological applications.

Keywords: *Trichoderma*, Catamarca, media cultivation

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Introduction

Trichoderma is a fungus of life free, aerobic optional, symbionts of floors, avirulent. Account with possibility of dwell different guys of floor (agricultural, forestry, acids), with varied content of subject organic and ranges of temperatures. Their characteristics it They are located in the group of hypogeous, lignocollous fungi by Harman. Some strains of *Trichoderma* they arrive to colonize the surface of the root (epidermis) or the two layers below it.

The choice of strains of *Trichoderma* HE base in the qualities desirable that present, modes of action and ecological aptitudes by Martínez. Given the great skills that has the genus *Trichoderma* can be It has been applied to seeds as an inoculant, with its use extending to post-harvest and from the soil to the foliage.¹ Its aptitudes are also known. as biocontrol agent of agent phytopathogens, being used so much in floor as in the aerial part since it has the capacity to: compete for nutrients, space, mycoparasitize and produce antibiosis.^{1,2}

Within the framework of sustainable agriculture, the use of agro-industrial waste that is easily acquired and allows the production of viable conidia of this fungus is an alternative for its reproduction.³ *Trichoderma* has nutritional requirements such as: starch, pectin, and cellulose, among others. can be degraded, nitrogenous compounds and trace elements required in low concentrations for their growth.^{1,4} He aim of this job is assess growth and development of strains of *Trichoderma* native species of the province in different culture media.

Materials and methods

Starting with 11 native strains of *Trichoderma* spp. from the province of Catamarca that thrived in an assay with Nutrient Agar (NA), growth and development evaluation tests were carried out in different culture media. Five artificial culture media and one natural one (agro-industrial substrate, red grape pomace) were tested (Table 1).

Table 1 Media of crop and substratum agro-industrial used for assess he growth and development of *Trichoderma* spp.

Media	Synthetics	
	crop	I. Dad glucose agar (APG)
II. Sabouraud (SAT)		
III. Amylolytic (Amil)		
IV. Czapek (CZP)		
V. Selective of Fungus and Yeast (HyL)		
	Naturals	
	VI.	Orujo of vine ink (VT)

He substratum VT previous to his use was hydrous 24 h and sterilized in jars of glass covered with aluminum foil.⁶

HE they sowed by triplicate 4 strains of *Trichoderma* by box of Petri, the combination of The strains inoculated in the same Petri dish were randomly selected. Two perpendicular lines were drawn in each Petri dish, delimiting four quadrants of equal area. In the center of each of the four quadrants, 5 mm diameter potato glucose agar (PGA) discs were inoculated, each containing actively growing strains from the *Trichoderma* mother cultures (Figure 1). For VT pomace, only one disc was placed in the center of the dish. Subsequently HE incubated in conditions controlled to 25 ± 1 °C, with photoperiod of 16 Under 48 hours of light, the growth dynamics (mm²/time) of each strain were evaluated in the different culture media, and the edges of the growth area were marked. each a of the colonies. Subsequently the boxes of Petri were photographed of individually to then calculate the Growth Area (GA) of each colony using Image J software and Observing their qualitative growth and development characteristics allows us to understand their physiological state, viability, and dispersal capacity, which are evaluated based on the presence of mycelium, conidia, or pigments. The results obtained were analyzed statistically through analysis of variance (ANOVA) and The means were compared using Fisher's LSD (Least Significant Difference) test at a significance level of 0.05 using the statistical program InfoStat.⁶

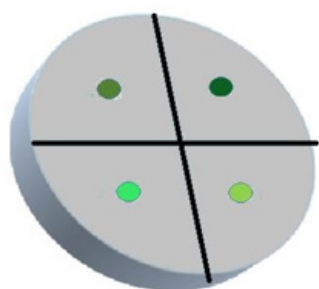


Figure 1 Scheme of sowing of tacos of different strains of *Trichoderma* spp. for evaluate growth and development in different culture media (each color represents a strain).

Results

The strains of *Trichoderma* presented different characteristics qualitative in the different culture media both artificial as a natural medium, which are presented in Table 2 and Figure 2 observe and B and D: growth on the reverse, strains: TPaM3, TN-walnut, TAC2, TSN1.

Table 2 Qualitative description of the growth of *Trichoderma* strains in different culture media. In the Phytopathology Lab. UNCa. Catamarca

Strains	Characteristics growth	Media of Crop					
		SAT	AMIL	APG	CZP	H and L	VT
TPaM3	Mycelium	XXX	X	XX	XX	XXX	XXX
	Conidia	-	-	-	VO	X	-
	AND surpass with limit	IF	-	IF	-	-	IF
	Pigment	-	-	-	-	-	-
TN-walnut	Mycelium	30	30	30	20	30	30
	Conidia	-	-	-	-	-	X
	B.C overpass su limit	-	SI	-	SI	SI	SI
	Pigment	-	-	-	-	YOU	-
TAC2	Mycelium	XXX	XXX	XXX	XX	XXX X	XXX X
	Conidia	-	-	YOU	GRANDMOTHER	GRANDMOTHER	GRANDMOTHER
	B.C overpass su limit	SI	SI	-	SI	SI	-
	Pigment	YOU	-	YOU	-	-	GRANDMOTHER
TSN1	Mycelium	XXX	X	XXX X	X	XXX X	XX
	Conidia	-	-	GRANDMOTHER	GRANDMOTHER	-	-
	AC sobrepasa su limite	-	-	-	-	-	-
	Pigmento	VC	-	-	-	-	-
TM5A	Micelio	XXX	X	XXX	XXX	XXX	XXX
	Conidios	VC	VO	X VO	VO	VC	X VO
	AC sobrepasa su limite	-	-	SI	SI	-	SI
	Pigmento	VC	-	-	-	-	-
TLT2	Micelio	XXX	X	XXX X	X	XXX X	XXX
	Conidios	-	-	-	-	-	-
	AC sobrepasa su limite	SI	-	-	SI	-	-
	Pigmento	VC	-	-	-	-	-
TAC3	Micelio	XXX	X	XXX	X	XXX X	XXX X
	Conidios	-	-	-	-	-	-
	AC sobrepasa su limite	SI	SI	SI	-	-	-
	Pigmento	VC	-	-	-	-	-
TA203	Micelio	XXX	X	XXX	XX	XXX	XXX
	Conidios	VC	-	X YOU	GRANDMOTHER	-	X GRANDMOTHER
	B.C overpass su limit	-	SI	-	-	SI	SI

Table 2 Continued...

	Pigment	YOU	-	YOU	-	-	-
TVLI	Mycelium	XXX	X	XXX X	XXXX	XXX	XXX X
	Conidia	-	-	VO	-	VC	VO
	AC surpasses his limit	YEAH	YEAH	YEAH	-	YEAH	-
	Pigment	-	-	-	-	VC	-
TYac on	Mycelium	XX	X	XX	XXXX	XX	XXX
	Conidia	-	-	VC	-	VO	VC
	AC sobrepasa su limite	SI	SI	SI	SI	-	SI
	Pigmento	VO	-	-	-	VO	-
TT	Micelio	XXX	XX	XXX	XX	XXX	XXX X
	Conidios	-	-	VO	-	VC	VO
	AC surpasses his limit	YEAH	YEAH	YEAH	YEAH	YEAH	YEAH
	Pigment	-	-	-	-	VC	-

References: Sabouraud (**Sab**); Amylolytic (**Amil**); Potato Glucose Agar (**APG**); Czapek (**Czp**); Fungus and Yeast (**HyL**) and Pomace of vine ink (**VT**). **X** : incipient; **XX** : scarce; **XXX** : dense; **XXXX** : abundant. Conidium/pigments: (**VC**): light green; (**VOs**): dark green; **AC** : Growth Area.

0.001). By it so much, HE explored dynamic of the growth of different strains of *Trichoderma* in different media of crop. The areas of growth (mm²) in the different culture media are presented in Table 3.

Table 3 Dynamic of the growth of different strains of *Trichoderma* in different culture media.

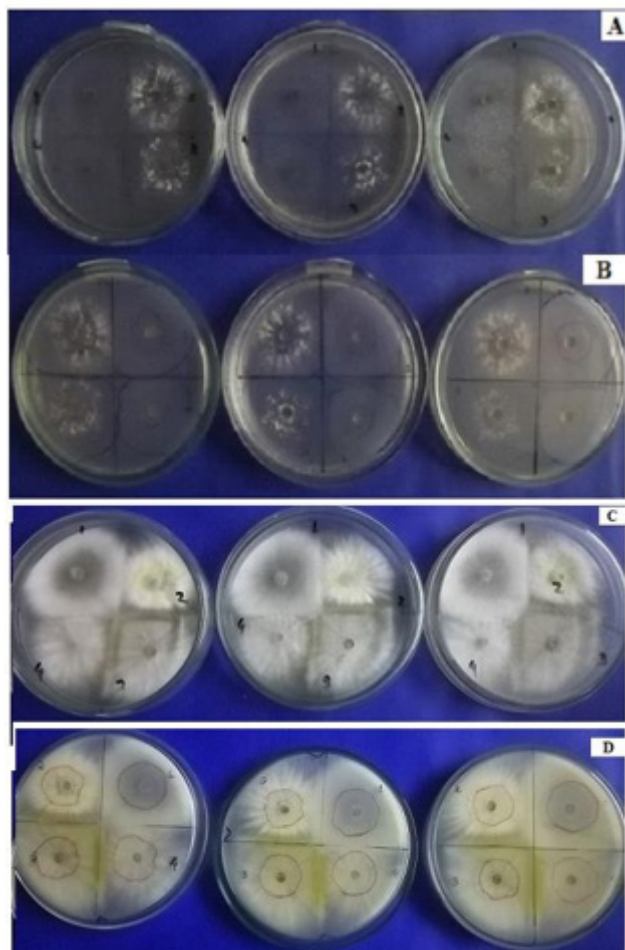


Figure 2 Growth of *Trichoderma* spp. strains in culture media: **A** and **B**: Amylolytic (Amil) and **C** and **D**: Sabouraud (Sab). **A** and **C**: growth on the obverse and **B** and **D**: growth on the reverse, strains: TPaM3, TN-walnut, TAC2, TSN1.

HE they found differences significant in the interaction strain and half of crop to the 24 h ($p < 0.001$) and at 48 h ($p < 0.001$) in the growth area (mm²). As well as in the main effects of strains (24 h: $p < 0.001$ and 48 h: $p < 0.03$) and of culture media (24 h: $p < 0.001$ and 48 h: $p <$

CEPA	HALF OF CULTIVATION	GROWTH AREA			
		(mm ²) 24 h	(mm ²) 48 h		
TA203	CZP	4988	TO	90858	A
	AMIL	9066	AB	123289	A
	SAB	15090	AB	130322	A
	APG	19662	B	92337	A
	HyL	19690	B	103427	A
	VT	50898	C	475576	B
TAC 2	CZP	18283	A	143703	A
	AMIL	20052	A	126637	A
	SAB	21192	A	285588	B
	APG	25395	A	133811	A
	HyL	30101	A	157591	A
	VT	30425	A	137696	A
TAC3	CZP	8897	A	62809	A
	AMIL	17430	AB	128575	A
	SAB	36082	CD	139334	A
	APG	26209	BC	105922	A
	HyL	26980	BC	126140	A
	VT	43282	D	355965	B
TLT2	CZP	14015	A	103617	A
	AMIL	16682	AB	104444	A
	SAB	27447	B	111324	A
	APG	22883	AB	124243	A
	HyL	17956	AB	104409	A
	VT	52486	C	414717	B
TM5A	CZP	4112	A	91293	A
	AMIL	12216	A	77193	A
	SAB	28403	B	111184	A
	APG	30444	B	123563	A
	HeL	37192	B	114110	A
	VT	53009	C	501725	B

Table 3 Continued...

TN- Quite	CZP	10629	A	110099	A
	AMIL	22469	AB	126819	A
	SAB	25145	B	132793	A
	APG	24465	B	117117	A
	HyL	30133	B	119540	A
	VT	65555	C	707642	B
TPaM3	CZP	4010	A	98696	A
	AMIL	19712	B	111501	A
	SAB	35983	CD	125989	A
	APG	47410	DE	124080	A
	HyL	33797	C	111454	A
	VT	53787	E	462505	B
TSN1	CZP	6399	A	58561	A
	AMIL	14545	AB	99413	A
	SAB	33667	C	143664	A
	APG	25163	BC	113672	A
	HyL	15925	AB	79075	A
	VT	36714	C	337810	B
TT	CZP	12777	AB	116671	A
	AMIL	21378	BC	147684	A
	SAB	23386	BC	126078	A
	APG	7149	A	82029	A
	HyL	33086	C	141040	A
	VT	54000	D	600115	B
TVL1	CZP	9252	A	95565	A
	AMIL	8623	A	99134	A
	SAB	16442	AB	122171	A
	APG	19797	AB	99472	A
	HyL	24834	B	104584	A
	VT	50743	C	492587	B
TYacón	CZP	12217	A	118679	A
	AMIL	14974	A	127966	A
	SAB	19796	A	140159	A
	APG	18469	A	123453	A
	HyL	14233	A	126555	A
	VT	24249	A	459012	B

Different letters in the same variable denote significant differences according to the LSD Test (Minimum significant difference) for $P < 0.05$.

Trichoderma strains showed greater growth on the VT substrate, with significant differences. Only strain TAC2 at 48 h showed the greatest growth with a significant difference on SAB. This coincides with the results obtained for the qualitative characteristics evaluated. Therefore, the strains that expressed the greatest growth and development, both quantitatively and qualitatively, were: TA203, TAC2, TM5A, TPaM3, TSN1, TT, TVL1, and TYacón.

Discussion

In this study, with different media of crop, the strains of *Trichoderma*, they can having been found affected due to the combination and influence exerted by each of them due to their particularities, in the consortium or groups of strains evaluated.

The plasticity of the strains of *Trichoderma* for adapt to the different media of cultivation and continue his growth and development HE

evidence in he bit growth that They were present and in the presence of conidia in CZP. This medium has organic sources of C (sucrose) and inorganic of N (nitrate of sodium), between others minerals as Na, Faith and K. This coincides with the results obtained by Michel Aceves et al.⁷ where *Trichoderma* produces viable mycelium and spores on organic substrates with a high percentage of moisture and low mineral content.

So also HE achievement only he growth mycelial of *Trichoderma* in half for Amylolitics that contain only starch as a source of C, in addition to N and other nutrients in low concentrations.

Meanwhile, the greatest growth and The development of *Trichoderma* strains was obtained with the other media cultivation employees, already that contain source of C, amino acids, vitamins and factors of growth. He pomace of VT this constituted by sources organic of C (as fructose), of nitrogen (N), phosphorus (P) and potassium (K) present and to his availability, It is also composed of lignin and cellulose, which are required for the nutrition of these microorganisms.^{1,3,8,9} Similar results to those of this study were obtained in other substrates of agricultural origin.^{3-5,9-11} The results obtained in this work demonstrate the potential of VT grape pomace for the large-scale multiplication of *Trichoderma*. This natural culture medium is an abundant resource in our area, since it is a residue agro-industrial. Therefore that he The use of this bio-input will enable the reproduction of the inoculum in large volumes and in an economical manner. it which will allow the management agroecological with the incorporation of antagonists at low cost.

Conclusion

The results of the variables qualitative as quantitative were similar. The best growth and development results of the eleven *Trichoderma* strains were shown in VT during all he rehearsal. While that to the 24 h of incubation HE noticed elderly growth and development regard to the 48 h of assessment, in this last the variables studied They had lower values.

Acknowledgments

None.

Conflicts of interest

Author declares there is no Conflicts of interest.

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