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Short Communication

Stimulation of mycelial growth of pathogenic and seabed isolates of *Fusarium oxysporum* in presence of salts

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The mycelial growth of eleven *Fusarium oxysporum* strains, eight isolated from seabed soil of the South- eastern Mediterranean coast of Spain and three formae speciales from diseased plants were tested on potato-dextrose-agar adjusted to different osmotic pressures with either KCl or NaCl (-1.50 to -144.54 bar) at 10°C intervals ranging from 15 to 35°C. The extent of growth of *F. oxysporum* shows the same pattern for isolates from the seabeds that for isolates of *Formae speciales*. Maximal growth was observed at -13.79 bar of osmotic pressure at 25°C with an acute decrease at -41.79 bar and lower. At 35°C maximal growth was observed at -41.79 bar of osmotic pressure not only for seabeds isolates but also for pathogenic *F. speciales* isolates. These findings could indicate that *F. oxysporum* is well adapted to exist in moderate saline habitats. It does not seem that the pathogenicity of the isolates is a factor that determines the response to the salinity, but that behavior seems to be common to all the isolates studied and it could be speculated to be a specific behavior.

Key words: Salinity, soilborne pathogens, conductivity, osmotic pressure.

INTRODUCTION

The *Fusarium* genus is one of the most ubiquitous fungus in the terrestrial ecosystems and a relevant pathogen for the cultivated plants. It seems to have a special physiological mechanism that would allow it to survive in arid environment (Tresner and Hayes, 1971; Mandeel, 2006). Caracuel et al. (2003) demonstrated that the transcription factor PacC plays an essential role in salt tolerante of *Fusarium oxysporum* correlating with rapid and increased expresión of sodium efflux system in the studied strain. Authors worked with a single strain of *F. oxysporum* f. sp. *lycopersici* strain 4287 (race 2). Previous studies stated the existence of a Na+-(Li+)-ATPase as the main system involved in the efflux of these cations. Although it is unknown if such regulation also exists in non pathogenic isolates. The effect of this regulation, if any, on micelial growth remains to be elucidated.

Fusarium has been collected from such a salty environment as the marine bottom of Mediterranean Sea (Palmero et al., 2009b). This paper is closely connected to Palmero et al. (2008, 2009a) dealing with *Fusarium solani* and *Fusarium culmorum* strains.

In general, the *Fusarium* species have optimal growth between -5 and -25 bar and do not grow at all at -100 to -120 bar (Cook, 1981), with the exception of *Fusarium nivale* that displays its optimal growth between -1 to -2 bar and prevent growing at -50 bar.

Manandhar and Bruhel (1973) showed that *Verticillium dahliae* and *F. oxysporum* have an optimal growh rate *in vitro* between -10 and -30 bar (4 g/l of salt) and the conidial germination diminishes when the concentration of salt (NaCl) increases, in the same way, the mycelial growth stops growing when salt concentration reaches 80 g/l (Tran van Canh and Meyer, 1971).

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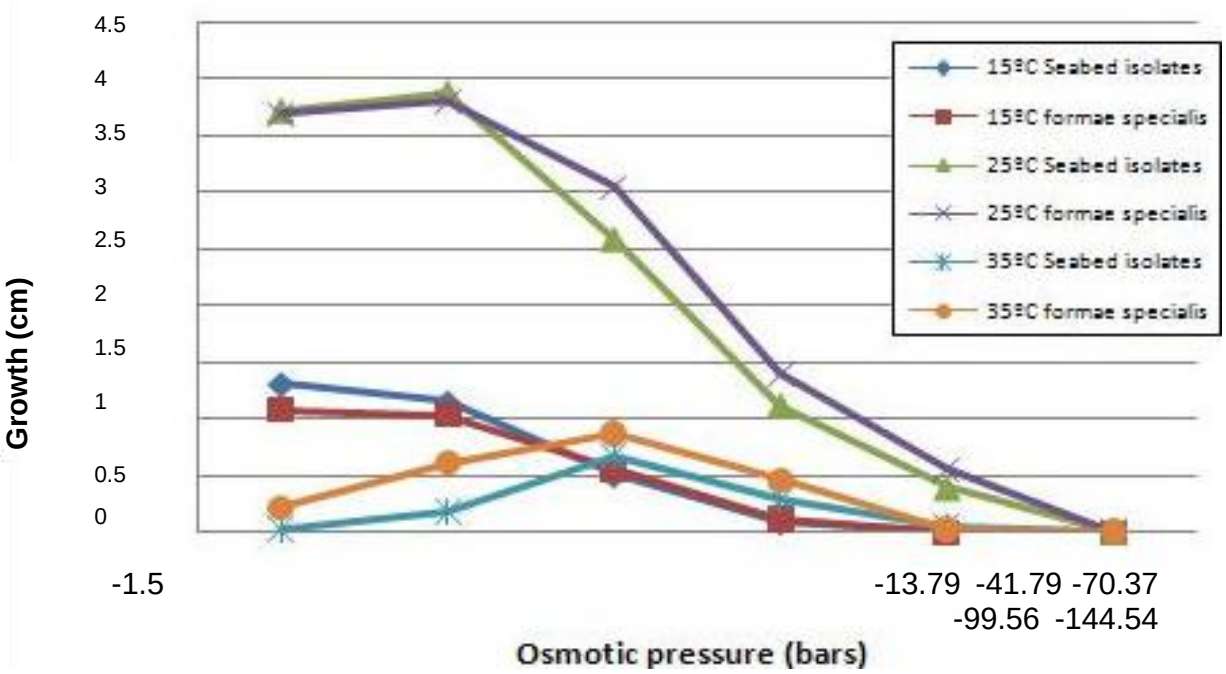

Figure 1. Growth of pathogenic *Formae speciales* and seabeds isolates of *F. oxysporum* versus temperature and osmotic pressure (variance does not exceed 5% of the means).

The aim of this research is to study the interactive effects of temperature and osmotic pressure on the growth of the isolates of *F. oxysporum*. The different sources of *Fusarium* isolates used in the study (diseased plants and marine bottoms of the Mediterranean coast) will provide information on whether or not the physiological effect of the salinity is linked to the pathogenicity of the isolate or not.

To determine the interactive effects of temperature and osmotic pressure on the growth of *F. oxysporum*, a 11 x 2 x 3 x 6 x 5 factorial experimental design was used, wherein strains 1 to 11 were the first factor, salt type was the second factor (NaCl and KCl), temperature (15, 25 and 35°C) was the third factor, osmotic pressure (-1.50, -13.79, -41.79, -70.3, -99.56 and - 144.54 bar) was the fourth factor; each combination of isolate, temperature, and osmotic pressure was replicated 5 times.

A total of 11 strains were used in salinity and temperature tests. Three *Formae speciales* were used (*F. oxysporum* f. sp. *melonis* race 1, *F. oxysporum* f. sp. *lycopersici* race 0 and *F. oxysporum* f. sp. *radicis cucumerinum*) and eight isolates collected from the mouth of the Andarax Riverbed (4 m in depth) and from the mouth of the Albuñol Riverbed (1.50 m in depth) in previous works (Palmero et al., 2009a).

There were no significant differences in the number of isolates that exhibited growth at different salt composition for the *F. oxysporum* isolates from seabeds (2 = 0.062; P = 0.804) and neither for the pathogenic formae specialis (2 = 1.548; P = 0.21). Of all the isolates, 56.7% of the seabed isolates tested and 54.6% of the isolates of the *F. speciales* grew in media amended with NaCl. While 65.3% of the seabed isolates and 63.0% of the isolates of the *F. speciales* grew when KCl was used as a solute. No significant difference in the amount of growth was observed with the different salt compositions for the seabeds isolates [F (1.286) = 0.000; p = 0.983] and for the pathogenic *F. speciales* tested [F (1.214) = 0.109; p = 0.742] with a mean of 0.972 and 0.885 cm for the seabed and *F. speciales* isolates, respectively.

The extent of growth of *F. oxysporum* at different osmotic pressures shows the same pattern for isolates from the seabeds that for isolates of *F. speciales* (Figure 1). Maximal growth was observed at -13.79 bar of osmotic pressure at 25°C with an acute decrease at -41.79 bar and lower. At 35°C, maximal growth was observed at -41.79 bars of osmotic pressure not only for seabeds isolates, but for pathogenic *F. speciales* isolates. The highest growth occurred at 25°C at all osmotic pressures. The differences in extent of growth at different osmotic pressures were statistically significant (Figure 1).

The 2 test showed that there were significant differences in the growth capacity versus osmotic pressure for isolates from the seabeds (2 = 149.908; P < 0.001) and for isolates of *F. speciales* (2 = 77.147; P < 0.001).

There was minimal or no effect on cultural viability at 15°C in the first two pressure tests, with an acute decrease in growth capacity (>50%) at -70.37 bar. Cultural

viability at 25°C was still present at -99.56 bar, where all of the isolate cultures were viable. At 35°C the viability decreased visibly, but the addition of salts to the environment produced an increase of the growth capacity, the increase was more accused in the case of isolates procedents from the marine bottoms where it reaches to 87.5% at -70.37 bar, although in lesser extent the higher increase of growth capacity in isolates of *F. speciales* was also observed at -70.37 bar.

There was a clear differences in the growth response of pathogenicity. *F. oxysporum* graphic at 15°C, where growth was maximal at the highest osmotic pressure and the response at 25 and 35°C, where growth was maximal at -13.79 and -41.79 bars, respectively.

It is worth noting the difference in the response of the number of isolates that exhibited growth and the extent of growth. This was particularly striking at 25°C, where growth capacity only diminished at very low osmotic pressures, while the average growth initially increased with decreasing osmotic pressure to -13.79 bar and declined gradually with increasing salinity. Different patterns occurred at 35°C, where the pressure of -41.79 bar produced the maximum number of isolates that exhibited growth and also the maximum mycelial growth as well for isolates from the seabeds as for isolates of *F. speciales*.

The study of the interaction between temperature and osmotic pressure showed statistically significant differences in the response to temperature for isolates from the seabeds [F (2.287) = 363.369; $P < 0.001$; $^2 = 0.729$] and for isolates of *F. speciales* [F (2.215) = 243.377; $P < 0.001$; $^2 = 0.711$]. Statistically significant differences were also observed in the response to osmotic pressure for isolates from the seabeds [F (5.287) = 120.684; $P < 0.001$; $^2 = 0.691$] and for isolates of *F. speciales* [F (5.215) = 82.853; $P < 0.001$; $^2 = 0.677$] and in the interaction between temperature and osmotic pressure for isolates from the seabeds [F (10.287) = 39.907; $P < 0.001$; $^2 = 0.596$] and for isolates of *F. speciales* [F (10.215) = 33.918; $P < 0.001$; $^2 = 0.631$].

A significant interaction between temperature and osmotic pressure causes the previously noted change in the growth response at lower osmotic pressures; it seems that this interaction is not different in function of the origin or pathogenic capacity of the isolates.

Our results suggest that the survival of *F. oxysporum* could be possible in moderately saline soils with high soil temperatures (near 35°C).

Hyphal growth may be stimulated at low osmotic pressures (-41.79 bar) over 25°C. This agrees with the results of Cook et al. (1972) for *F. culmorum* and are consistent with those obtained by Cook and Christen (1976) for *F. culmorum* and *Fusarium graminearum* and by Palmero et al. (2008, 2009a) for *F. solani* and *F*. *culmorum*.

Experimental results show that *F. oxysporum* has the capacity of growing at low osmotic pressures which could explain the prevalence of the *Fusarium* genus in soils in the seabeds of the Mediterranean Sea and within semi-arid environments. Pathogenicity of the isolates is not a factor that determines the response to the salinity, but seems to be common to all the isolates studied. It could be a specific behavior. Further work is necessary to understand the physiological mechanism of salt tolerance in *F. oxysporum* as well as its effects on their pathogenicity.

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