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ORIGINAL PAPER

The interactive effects of temperature and osmotic potential on the growth of marine isolates of *Fusarium solani*

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Abstract The mycelial growth of 18 *Fusarium solani* strains isolated from sea beds of the south-eastern coast of Spain was tested on potato-dextrose-agar adjusted to different osmotic potentials with either KCl or NaCl (−1.50 to −144.54 bars) in 10 °C intervals ranging from 15 to 35 °C. Fungal growth was determined by measuring colony diameter after 4 days of incubation. Mycelial growth was maximal at 25 °C. The quantity and frequency pattern of mycelial growth of *F. solani* differ significantly at 15 and 25 °C, with maximal growth occurring at the highest water potential tested (−1.50 bars); and at 35°C, with a maximal mycelial growth at −13.79 bars. The effect of water potential was independent of salt composition. The general growth pattern of *F. solani* showed declining growth at potentials below −41.79 bars. Fungal growth at 35 °C was always higher than that grow at 15 °C, of all the water potentials tested. Significant differences observed in the response of mycelia to water potential and temperature as main and interactive effects. The viability of cultures was increasingly inhibited as the water potential dropped, but some growth was still observed at −99.56 bars. These findings could indicate that marine strains of *F. solani* have a physiological mechanism that permits survival in environments with low water potential. The observed differences in viability and the magnitude of growth could indicate that the biological factors governing potential and actual growth are affected by osmotic potential in different ways.

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Introduction

Fusarium solani is one of the most ubiquitous fungi in terrestrial ecosystems [1] and an important pathogen in cultivated plants. There is a dearth of research dedicated to *Fusarium* spp. in marine habitats, because there is a particular focus on their occurrence in plants, soils and agricultural or other terrestrial environments. In literature, specifically dedicated to the study of Hyphomycetes, Pyrenomycetes and imperfect fungi (Deuteromycetes), it is difficult to find any mention of *Fusarium* occurring in marine environments. But the presence of ten *Fusarium* species has been reported in marshy waters [13]. Booth [2] observed *F. merismoides* in stagnant water and mud.

Stoner [21] isolated *F. oxysporum* and *F. solani* in mangroves and salt marshes occurring along the shoreline of various Pacific islands. Rebell [20] reported a high rate of recovery for *F. solani* isolated from calcareous beach sands mixed with decomposed marina and other vegetation in Florida and the Caribbean Islands. *Fusarium solani* commonly occurs in lesions sustained by marine animals [4, 12, 16, 17]. In addition, *F. solani* causes various infections in crustaceans [11, 15].

Tello et al. [23, 24] studied *Fusarium* species isolated from beach sands in Spain. The authors reported the presence of *F. solani* and other fusaria. Recently, Núñez et al. [18] corroborated the results obtained by Tello et al. [24] on the occurrence of *Fusarium* species in beach sand on the Mediterranean shore, including the existence of *F. solani* at 10 m depth in the bay of Almeria. It seems to have a special physiological mechanism of survival in aquatic salty environments.

On other hand, root and stem rots and other seedling diseases caused by two species (*F. solani* [Mart.] Appel & Wr. emend Snyder & Hansen, and *F. Roseum* Lk. emend Snyder & Hansen) are associated with dry soils. Studies showed that growth is optimal between −10 and −30 bars of water potential, reduced by half between −40 and −60 bars, and terminated only when water-potential falls below −100 bars [8, 10].

Vascular pathogens such as *F. oxysporum* [19], *Verticillium albo atrum* and possibly, *Cephalosporium gramineum* [3] appear to have the ability to grow under −100 bars of water potential.

Cook [6] refers to this ability to grow and cause disease in the context of low water potential and proposes that organisms unable to develop themselves in substrates possessing a water-potential of less than −40 bars would be restricted to areas (or years) with high rainfall or irrigated crops; those able to grow at less than −60 bars would be the only pathogens able to cause disease in dry climates.

There is no evidence indicating that fungal metabolism is directly impaired by high concentrations of common salt ions. Any harmful effects of a salt have to be interpreted in terms of the water-potential generated.

Pathogens such as *F. solani* are exposed to environments with low water potential. However, we were unable to locate any study on the effect of salt concentration on the growth of this marine fungus.

Observations of the influence of osmotic potential on the hyphal growth of *F. solani* are presented in this article.

Materials and methods

To determine the interactive effects of temperature and osmotic potential on the growth of *F. solani* we used an 18 × 2 × 3 × 6 × 5 factorial experimental design, wherein strains (1–18) was the first factor, salt type was the second factor (NaCl and KCl),temperature (15–25–35 °C) was the third factor, osmotic potential (−1.50, −13.79, −41.79, −70.37, −99.56 and −144.54 bars) was the forth factor and each combination of isolate, temperature and water potential was replicated five times.

Isolates used in salinity and temperature tests

The origin and code of the isolates of *F. solani* tested can be seen on Table 1. All strains used in this study are stored in the University of Almería (Plant Prod. Department) and in the Polytechnic University of Madrid (E.U.I.T. Agrícola) culture collections.

Growth media

The culture medium used was PDA (potato dextrose agar). To achieve targeted osmotic pressures, different batches of the medium were amended with various amounts of NaCl and KCl according to the schedule in Table 2.

Study of mycelial growth

Each of the isolates was sub-cultured from Komada selective medium to PDA. To examine mycelial growth at various temperatures and osmotic pressures, 1-cm diameter agar circles were excised from the margins of 2-week-old PDA cultures and aseptically transferred to new PDA media.

These cultures were incubated in complete darkness at 15, 25, or 35 °C. Cultures were examined after 4 days under a dissecting microscope and colony margins were marked with permanent ink on the reverse side of the petri

Table 2 Relations between the osmotic potential of the medium (ψ) and the concentrations of KCl and NaCl

ψ (bars)	Product amount (g/l of PDA)	
	NaCl[a]	KCl[a]
−1.50[b]	0.0	0.0
−13.79	17.6	22.2
−41.79	52.0	68.8
−70.37	84.8	112.0
−99.56	115.2	152.8
−144.54	156.6	212.5

[a] Jakobsen et al. [14]

[b] Cook [5]

Table 1 Origin of *Fusarium solani* isolates used in tests (*D*: depth)

Sample code	Location		
	Sample depth	Coordinates (*X*, *Y*)	
34, 35, 36	Shoreline 0.10 m in depth	550,856	4,074,317
57, 59, 60 and 61	Sea bed at 6 m in depth	550,729	4,073,888
14, 15, 16 and 17	Mouth of Andarax River bed *D* = 0.1 m	551,169	4,074,231
4, 11, 12 and 13	Mouth of Andarax River bed *D* = 1.5 m	551,238	4,074,229
19	Mouth of Albuñol River bed *D* = 4.5 m	485,881	4,066,493
31 and 32	Mouth of Albuñol River bed *D* = 1.5 m	485,772	4,066,598

dishes. For each colony, the mean radial mycelial growth was calculated by measuring two different colony radii in each of five plates per combination of isolate, osmotic pressure and temperature. The growth was corrected by subtracting the 1 cm diameter of the original plug of inoculum.

Statistical analysis of data

Statistical analyses were performed using SPSS software, version 11.5.1, of Leadtools Company. For the magnitude of growth (measure of growth after 4 days), parametric and non-parametric analysis of variance were carried out. χ^2 tests were made to check whether the differences were significant.

To assess the temperature and osmotic pressure influence on growth, several comparative analyses of averages were performed; these analyses were parametric (one-way ANOVA) if Levene's test indicated no significant heterogeneity of variance, or non-parametric (Kruskal–Wallis test, Mann–Whitney post hoc test) where the heterogeneity of variance was significant.

Results and discussion

Growth versus salt composition

There were no significant differences in cultural viability versus salt composition ($\chi^2 = 0.160$, $P = 0.689$). Of all isolates tested, 58.6% grew in media amended with NaCl and 60.2% grew when KCl was used as a solute. Regarding the magnitude of growth versus salt composition, there was no significant difference [F (1,646) = 0.124, $P = 0.725$] with a mean of 0.718 cm (Table 3). These results are consistent with those obtained by Sung and Cook [22].

The fact that isolate viability and‘ growth was not significantly affected by salt composition indicates that ions formed by the salts do not have different chemical effects on the physiology of the fungi. Any differences in other parameters should therefore be due to physical effects (osmotic pressure), which only depend on the quantity of ions present.

Table 3 The effects of salt composition on the growth of marine isolates of *Fusarium solani* (mean ± SD)

Type of salt	*n*	Growth (cm)	Maximum (cm)
NaCl	324	0.733 ± 1.1120	4.1
KCl	324	0.703 ± 1.0310	3.8
Total	648	0.718 ± 1.0716	4.1

Growth versus osmotic pressure and temperature

The χ^2 test showed that there were significant differences in the cultural viability versus osmotic pressure ($\chi^2 = 369.038$, $P < 0.001$) (Table 4).

Optimal pressures for growth of isolates of *F. solani* were −1.50 and −13.79 bars, with no significant difference between the two. Experimental results obtained with other species of the genus [7, 9, 10] corroborated these results, where low pressures were advantageous to fungal growth.

Cultural viability versus osmotic pressure and the three temperature studies are expressed in Table 5.

There was minimal or no effect on cultural viability in the first two pressures test, with an acute decrease in cultural viability (>50%) at −70.37 bars. Cultural viability was still present at −99.56 bars, where 28.7% of isolate cultures were viable. No growth occurred at −144.54 bars (Fig. 1).

There are clear differences in the growth response of *F. solani* graphic at 15 and 25 °C, where growth was maximal at the highest osmotic pressure, and the response at 35 °C, where growth was maximal at −13.79 bars. It should be noted that the growth at 35 °C exceeded the growth observed at 15 °C, at all pressures. In this case, all growth differences between different osmotic pressures were statistically significant. It is also worth noting that the difference in the response of cultural viability and growth.

Table 4 The effect of osmotic pressure on the cultural viability of marine isolates of *F. solani*

Osmotic pressure (bars)	Growing (%)	Not growing (%)	Total (%)
−1.50	98.1	1.9	100.0
−13.79	98.1	1.9	100.0
−41.79	84.3	15.7	100.0
−70.37	47.2	52.8	100.0
−99.56	28.7	71.3	100.0
−144.54	0.0	100.0	100.0

Table 5 The interactive effects of temperature and osmotic pressure on the cultural viability of marine isolates of *Fusarium solani*

Osmotic pressure (bars)	Temperature (°C)		
	15	25	35
−1.50	100.0	100.0	94.4
−13.79	100.0	100.0	94.0
−41.79	77.8	100.0	75.0
−70.37	0.0	100.0	41.7
−99.56	0.0	75.0	11.1
−144.54	0.0	0.0	0.0

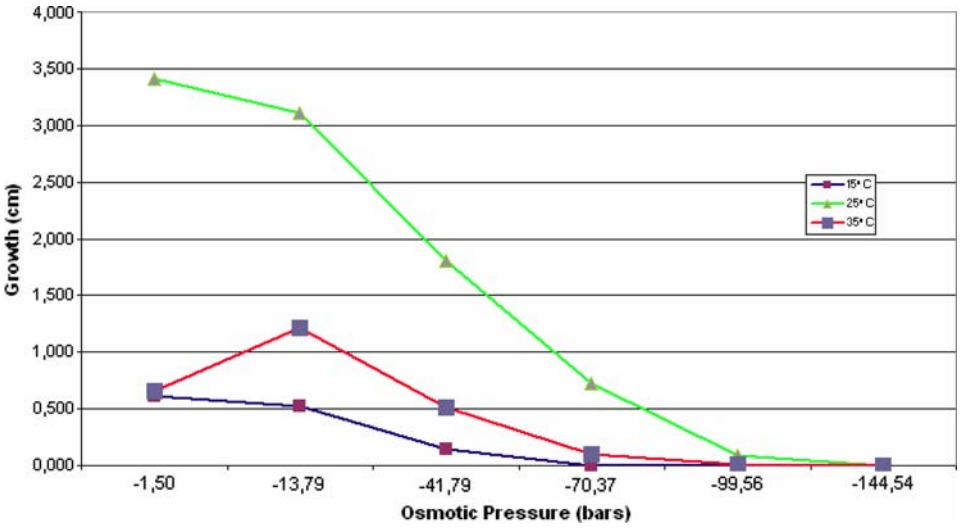

Fig. 1 Growth of *Fusarium solani* isolated from seabed versus temperature and osmotic pressure (variance does not exceed 5% of the means)

This was particularly striking at 25 °C, where cultural viability only diminished at very low osmotic pressures, while the average growth declined gradually with increasing salinity. It was also quite noticeable at 35 °C, where the average growth initially increased with decreasing osmotic pressure. These results indicate that the physiological determinants of viability and growth magnitude are affected quite differently by the presence of dissolved salts in the culture medium.

Tresner and Hayes [25] describe the occurrence of unusual physiological effects in experiments conducted with media containing NaCl. The effects included a profusion of normally innocuous bright purple and red pigments produced by isolates of *Aspergillus* and *Penicillium* grown in saline mediums. This increased pigmentation reflected the occurrence of some unknown metabolic abnormality taking place in organisms growing under very saline environments. This effect was also been observed in our study, but not in all of the isolates studied. Some isolates produced profuse pigmentation when cultured in saline environments, but they did not do so on culture media with no extra salt.

Study of the interaction between temperature and osmotic pressure

The two-way ANOVA (temperature × osmotic pressure) showed statistically significant differences in of the response to temperature [F (2,647) = 977.092, $P < 0.001$, $\eta^2 = 0.756$], in the response to osmotic pressure [F (5,647) = 528.447, $P < 0.001$; $\eta^2 = 0.807$], and in the interaction between temperature and osmotic pressure [F (10,647) = 150.537, $P < 0.001$; $\eta^2 = 0.705$]. Sorted according to η^2, these data are as follows (Table 6).

A significant interaction between temperature and osmotic pressure causes the previously noted change in the growth response at lower osmotic pressures.

Table 6 Analysis of variance for the main and interactive effects of temperature and osmotic pressure on the growth of marine isolates of *Fusarium solani*

Variable	F	p	Partial η^2
Osmotic pressure (O. P.)	528.447	0.001	0.807
Temperature	977.092	0.001	0.756
Temperature × O. P.	150.537	0.001	0.705

The growth response of isolates of *F. solani* at 15 and 25 °C was very similar, and was clearly affected by the osmotic pressure of the medium, gradually decreasing with increasing salt concentration in the culture medium. However, this does not match the pattern observed at 35 °C. A 10 °C increment in temperature has spectacular effects, allowing a favoring effect of the salts in the medium and producing a double hyphal growth versus controls.

This work confirms that there can be a stimulation of the hyphal growth at low osmotic potentials (−13.79 bars). This is consistent with the results obtained by Sung and Cook [22] for osmotic potentials but not for matrix potentials. The experiments also confirm that increasing temperatures in the range of 15–35 °C decrease the potential for growth. These results are consistent with those obtained by Cook and Christen [7] for *F. culmorum* and *F graminearum*. Taken together, these findings may explain the (ubiquitous occurrence?) of *F. solani* in the sea beds of the Mediterranean Sea.

Experimental results allow to asseverate that isolates studied are well adapted for existence in marine habitats. We ignore if the positive effect on growth observed with high temperatures and salinity content is due to the effect of temperature on pressure response or the effect of pressure on the temperature. This ability to grow in saline media with high temperatures confers an adaptive advantage over

other microorganisms, not only in marine media but also in warm salinity soils.

Marine isolates may be better adapted for saline habitats with a potential lower than −1.5 bars, but higher than −41 bars; however, this optimum is only expressed in optimal temperature regimes.

Further work is necessary to understand the physiological mechanisms of salt tolerance in this species, particularly with respect to differences in growth optimal conditions. Practical implications, as the possible proliferation of *F. solani* in farmland or glasshouse crops irrigated with saline water which endure high soil temperatures, should also be attempted.

References

1. Backhouse D, Burgess LW, Summerell BA (2001) Biogeography of Fusarium. In: Summerell BA, Leslie JF, Backhouse D, Bryoen WL, Burguess L (eds) Fusarium. Paul E. Nelson Memorial symposium. APS Press, Bethesda, pp 122–137
2. Booth C (1971) The genus *Fusarium*. Ed. Commonwealth Mycol. Inst., Kew, England. p. 237
3. Bruehl GW, Cunfer B (1971) Physiologic and environmental factors that affect the severity of snow mold of wheat. Phytopathology 61:792–799
4. Cabanes FJ, Alonso JM, Castella G, Alegre F, Domingo M, Pont S (1997) Cutaneous hyalohyphomycosis caused by *Fusarium solani* in a loggerhead sea turtle (*Caretta caretta* L.). J Clin Microbiol 35(12):3343–3345
5. Cook RJ (1973) Influence of low plant and soil water potentials on diseases caused by soilborne fungi. Phytopathology 63:451–458
6. Cook RJ (1981) Water relations in the Biology of *Fusarium*. In: Nelson PE, Toussoun TA, Cook RJ (eds) *Fusarium*. Diseases, biology, and taxonomy. The Pennsylvania State University Press, Pennsylvania, pp 237–242
7. Cook RJ, Christen AA (1976) Growth of cereal root rot fungi as affected by temperature and water potential interactions. Phytopathology 66:193–197
8. Cook RJ and Papendick RI (1970) Effect of soil water on microbial growth, antagonism, and nutrient availability in relation to soilborne fungal diseases of plants. In: Tousson TA, Bega RV and Nelson PE (Eds.). Root diseases and soil-borne pathogens. 2nd International Symposium on Factors Determining the Behaviour of Plant Pathogens in Soil. London, England. Imperial College, July 14–28, 1968. University of California. Berkeley, California, USA. pp 81–88
9. Cook RJ, Papendick RI (1972) Influence of water potential of soils and plants on root disease. Annu Rev Phytopathol 10:349–374
10. Cook RJ, Papendick RI, Griffin DM (1972) Growth of two root rot fungi as affected by osmotic and matrix water potentials. Soil Sci Soc Amer Proc 36:78–82
11. Corolini A (1989) Fusariosis in the shrimp *Penaeus semisulcatus* cultured in Israel. Mycopathologia 108(2):145–147
12. Frasca J, Duna JL, Back JD (1996) Mycotic dermatitis in an Atlantic white-sided dolphin, a pygmy sperm whale, and two harbour seals. J Am Vet Med Assoc 2008:727–729
13. Gordon WL (1960) The taxonomy and habitats of *Fusarium* species from tropical and temperate regions. Can J Botany 38:643–658
14. Jakobsen M, Filtenborg O, Bramsnaes F (1972) Germination and outgrowth of the bacterial spore in the presence of different isolates. Lebensm Wiss u Technol 5(5):159–162
15. Khoa LV, Hatai K, Aoki T (2004) *Fusarium incarnatum* isolated from black tiger shrimp, *Penaemus monodon* Fabricius, with black gill disease cultured in Vietnam. J Fish Dis 27(9):507
16. Leslie AD, Gulland FMD (2001) Marine mammal medicine, 2nd edn. CRC press, West Palm Beach, p 1120
17. Migaki G, Jones SR (1983) Mycotic diseases in marine mammals. In: Howard EB (ed) Pathobiology of marine mammal diseases, Vol. II. CRC Press, Boca Ratón, pp 1–25
18. Núñez FJ, Palmero D, Iglesias C, de Cara M, Sinobas J, Tello JC (2006) Biogeografía de especies de *Fusarium* en el litoral mediterráneo de España. Bol San Veg Plagas 32:137–149
19. Papendick RI, Cook RJ, Shipton PJ (1971) Plant water stress and the development of *Fusarium* foot rot in wheat. Phytopathology 61:905
20. Rebell G (1981) *Fusarium* Infections in human and veterinary medicine. In: Nelson PE, Toussoun TA (eds) *Fusarium* disease, biology and taxonomy. The Pennsylvania State University Press, Pennsylvania, pp 210–220
21. Stoner MF (1981) Ecology of *Fusarium* in non-cultivated soil. In: Nelson PE, Toussoun TA (eds) *Fusarium* disease, biology and taxonomy. The Pennsylvania State University Press, Pennsylvania, pp 276–286
22. Sung J, Cook RJ (1981) Effect of water potential on reproduction and spore germination of *Fusarium roseum* "*Graminearum*", "*Culmorum*" and "*Avenaceum*". Phytopathology 71(5):499–504
23. Tello JC, Lacasa A y Rodríguez MC (1990) Presence of some *Fusarium* species on Spanish beaches. Proceedings of 8th Congress of the Mediterranean Phytopathological Union. Agadir (Morocco), 137–138
24. Tello JC, Rodríguez MC, Lacasa A (1992) Importancia de *Fusarium* en las arenas de playas de España. ITEA 88:77–94
25. Tresner HD, Hayes JA (1971) Sodium chloride tolerance of terrestrial fungi. Applied Microbiology 22:210–213