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Influence of Salinity Stress on the Growth, Biochemical Changes and Response to Gamma Irradiation of *Penicillium chrysogenum*

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Abstract: The soil Fungus *Penicillium chrysogenum* was choosen, as test organism, from 10 soil samples and pathogenic fungi were tested for their response to salinity stress induced by different concentrations of NaCl. Its growth was increased in NaCl content up to 18%, where as on 19% NaCl its spores germinated only. The maximum vegetative growth was recorded on medium containing 10% NaCl. Intracellular total free amino acid pool was severely reduced to about 40-50% with increased salinity. The glycerol pool may balance the osmotic pressure in culture media of 5,10,15 and 18% NaCl. Exposure of *P.chrysogenum* to salt stress resulted in fungal resistance to gamma-irradiation. The accumulated 4 amino acids, either singly or in association with other amino acids, may contribute to induce gamma-irradiation tolerance.

Key words: *Penicillium chrysogenum*, salinity stress, NaCl, amino acids, glycerol, gamma-irradiation, osmotic pressure.

Introduction

All living cells respond to unfavourable environmental conditions, i.e. chemical and physical agents, by synthesizing a variety of low-molecular-weight compounds as protective responses (Wankhade *et al.*, 1996; Andreishcheva and Zviagilskaia, 1999). The production of low-molecular-mass hydrophilic compounds, such as amino acids, sugar alcohols, soluble sugars and proteins, as a response of the exposure to salt stress, reduce the osmotic potential of the intracellular solution to achieve osmotic equilibrium with the surrounding medium (Teixidó *et al.*, 1998 and Fulda *et al.*, 1999).

The response of micro-organisms to UV-light and gamma-irradiation under salinity stress condition has been studied by several investigators. Most studies dealt with the radiosensitizing action of NaCl, the damages caused by irradiation in the presence of NaCl in macromolecular components of living cells and how such effect was abolished by mutations or by the addition of certain solutes at the time of irradiation (Shin Chen, 1986 and Dion *et al.*, 1994).

This investigation elucidate the influence of salinity stress, induced by different concentrations of NaCl, on the growth of soil fungus *P. chrysogenum* and the physiological changes involved in its possible salinity tolerance and the response of the fungus to gamma-irradiation in presence and absence of salinity stress.

Materials and Methods

Organism: The soil fungus *P. chrysogenum* was choosen, from 10 soil and pathogenic fungi tested, as the test organism.

Growth conditions

For studying salinity stress: Aliquots of 50 ml Czapek's sucrose liquid medium having pH 5.5, not amended (control) or amended with different percentages of NaCl (2.5-20%), were added in 250ml flasks then autoclaved for 15 min. at 15 Lb/sq. inch 121°C and inoculated with 7-day old fungal discs (0.5 cm diameter). The flasks were incubated for 10 days at 25°C. After the incubation period the cultures were filtered, thoroughly washed and then oven dried at 80°C till constant weight.

For irradiation experiment: Discs (0.5 cm diameter) of 7-day old culture of *P. chrysogenum* were raised on sterilized Petri dishes containing Czapek's sucrose agar medium, not amended and amended with increased NaCl concentration (5, 10, 15 and 18%). After incubation for 10 days at 25°C, cultures were subjected to doses of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 kilo Gray (kGy) gamma-irradiation. Discs (0.5 cm diameter) of irradiated cultures were inoculated in flasks (250ml capacity), each containing 50ml Czapek's sucrose liquid medium. Flasks were incubated at 25°C for 10 days, then filtered, washed thoroughly, oven dried at 80°C tell constant weight. Mycelial dry weight was recorded, where the obtained values are expressed as average of 4 replicates.

Irradiation was carried out in Co^{60} gamma cell located at National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo. The gamma source gave a dose rate of 0.0258 kGy/min at the time of experiment.

Biochemical analysis: Dry mycelia of *P.chrysogenum*, subjected to various concentrations of NaCl, were utilized for the following biochemical analyses.

Amino acids analysis: Separation and estimation of free amino acid pool in the dry mycelium was determined using LC 3000 Amino acid Analyzer. The method adopted was that of Razak, (1980).

Protein analysis: The mycelial protein content was determined using Kjeldahl-method described in A.O.A.C. (1975).

Glycerol analysis: Free glycerol was extracted from the dried powdered mycelia with boiling water and estimated in the clear water extract as performed by Younis *et al.*, (1987).

Lipid analysis: Extraction of lipid following the procedure outlined by Viladomat *et al.* (1986). The extracted lipid was subjected to alkaline hydrolysis using Schmidt-thannhouser procedure outlined by Clark and Switzer (1977). The content of liberated glycerol was determined according to the procedure described by Younis *et al.* (1987). The obtained value was considered as the glycerol content of hydrolyzed lipid (GHL).

Results

Table 1 shows that the soil fungus *P. chrysogenum* exhibited outstanding growth behaviour and tolerated a wide range of NaCl concentrations, therefore it was choosen as the test organism for this investigation.

The data of Table 1 reveal also a progressive increase in mycelial dry weight of *P. chrysogenum* with the increase in NaCl concentration up to 10% NaCl concentration which

Table 1: The growth behaviour of ten soil and pathogenic fungi grown on Czapek's sucrose liquid medium not amended and amended with different concentrations of NaCl and incubated for 10 days at 25°C.

Fungi	Mycelial dry weight (mg) with NaCl concentrations (% w/v)									
	0	2.5	5	7.5	10	12.5	15	18	19	20
Alternaria alternata	375	381	-	-	-	-	-	-	-	-
Aspergillus flavus	282	240	229	202	186	166	-	-	-	-
A. wentii	299	283	245	231	216	190	178	-	-	-
Cunninghamella echinulata	249	216	199	-	-	-	-	-	-	-
Fusarium oxysporum	383	325	297	271	205	132	-	-	-	-
F. moniliforme	399	345	284	210	84	-	-	-	-	-
F. graminarum	487	410	269	192	111	-	-	-	-	-
P. chrysogenum	432	466	508	576	644	580	487	361	*	-
Rhizoctonia solani	245	171	-	-	-	-	-	-	-	-
Trichoderma viride	153	132	-	-	-	-	-	-	-	-

* Only germinated spores.

Table 2: Influence of medium NaCl concentrations on the intracellular free amino acid composition of P. chrysogenum.

Free Amino acids	Amino acid concentration (μg/g dry weight) With NaCl concentration (% w/v)				
	0	5	10	15	18
1- Phosphoserine	80.73	65.13	48.52	33.18	20.19
2- Phosphoethanolamine	150.59	-	-	-	-
3- Aspartic acid	132.36	105.62	61.28	48.11	26.62
4- Therionine	28.16	35.10	41.58	66.54	123.43
5- Serine	99.37	32.78	-	-	-
6- Glutamic acid	254.23	186.66	124.91	78.13	40.70
7- Proline	118.01	-	-	-	-
8- Glycine	113.99	129.56	209.07	234.98	366.61
9- Alanine	317.66	222.12	147.02	81.17	64.13
10- Valine	187.00	208.10	296.23	324.00	398.78
11- Methionine	24.31	-	-	-	-
12- Isoleucine	132.07	-	-	-	-
13- Leucine	253.96	63.20	-	-	-
14- Tyrosine	169.69	112.00	89.50	61.19	39.01
15- Phenylalanine	249.86	121.99	95.44	54.30	32.11
16- b-Amino-Iso-butyric acid	415.20	-	-	-	-
17- γ-Amino n-butyric acid	212.50	69.80	-	-	-
18- Ornithine	40.37	-	-	-	-
19- Lysine	135.27	43.10	21.28	-	-
20- Arginine	266.27	289.00	323.30	376.18	431.88
Total free amino acid concentration (μg/g dry weight)	3381.50	1684.41	1458.13	1357.78	1539.46

Table 3: The effect of different NaCl concentrations on protein, free glycerol and glycerol of hydrolyzed lipid (GHL) content of P.chrysogenum.

NaCl Conc. (% w/v)	Amounts in mg/g dry weight		
	Protein	Free glycerols	Glycerol of hydrolyzed lipids
0	16.62	12.87	6.49
5	21.70	17.56	20.19
10	24.18	34.99	38.34
15	25.16	58.16	22.51
18	22.83	44.22	9.53

produced the maximum dry biomass. The increase in NaCl concentration above 10% caused retreat in the amount of fungal dry weight below the maximum value. However, the lowest dry weight was abtained with 18% NaCl. The fungus failed to grow on 20% NaCl, while its spores germinated only on 19% NaCl.

The result of Table 2 show a pronounced reduction in the amount of the total free amino acids ranged from 50-60% of the control value with the increase in NaCl concentration. Such reduction was accompanied with disappearance of 10 amino acids of total 20 of the control treatment. The amounts of six of the ten amino acids were decreased with the increase in concentration of NaCl and decrease was obvious above 5% NaCl.

The other four amino acids namely; therionine, glycine, valine and arginine showed distinct increase in concentration with the increase in NaCl concentration.

Table 3 manifests a remarkable increase in the protein content of *P.chrysogenum* cells and increase in the free intracellular glycerol up to 15% NaCl followed by a decrease in glycerol content as NaCl concentration was increased to 18%. Furthermore, Table 3 demonstrates rise in glycorol content, estemated after the hydrolysis of lipid (GHL), as NaCl concentration increase from zero to 10% followed by a rapid decrease above 10% NaCl.

Fig. 1 shows a gradual decline in fungal growth, when the fungus raised on a medium not amended and amended with different concentration of NaCl and irradiated with increased doses of gamma- irradiation (0-5 kGy) up to the dose 2.0 kGy. This was followed by a distinct sharp drop in fungal growth

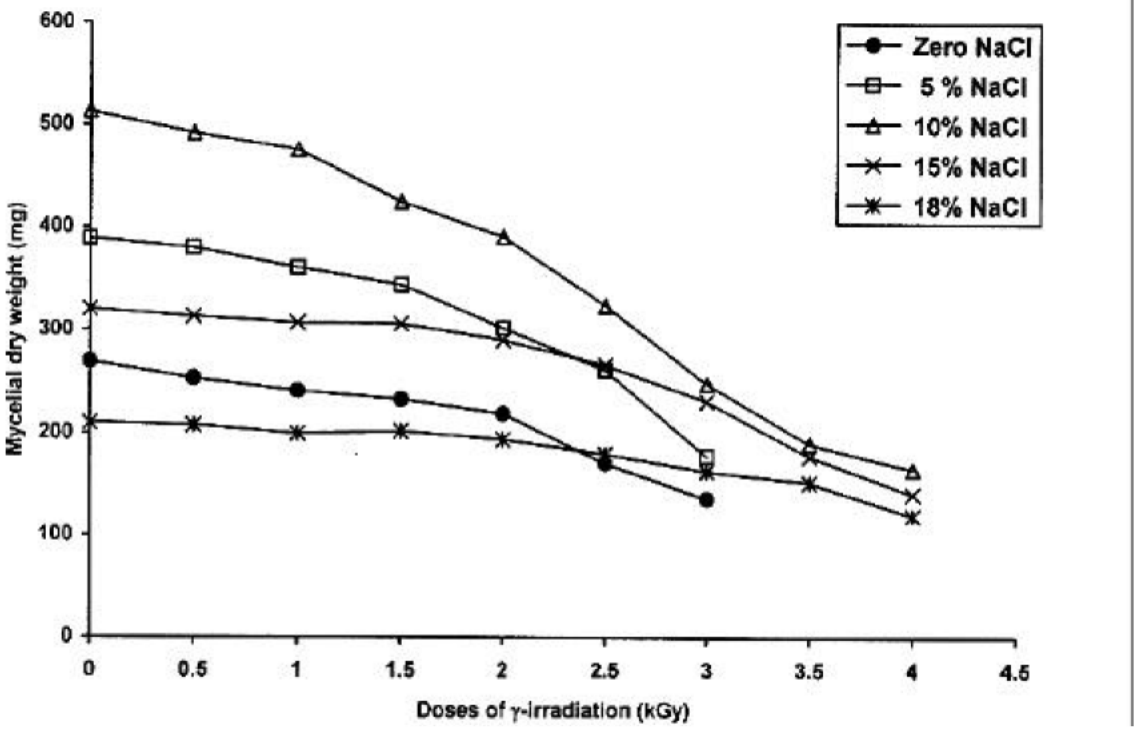

Fig. 1: The effect of medium NaCl concentrations on the response of *P.chrysogenum* to gamma-irradiation.

above 2.0 kGy. On the media not amended and amended 5% with NaCl the fungal growth could be measured up to the dose 3.0 kGy, whereas the doses above 3.0 kGy were lethal to *P.chrysogenum*.

A remarkable radioresistance was recorded when the fungus irradiated on a medium amended with NaCl concentration of 10,15 and 18% where the sub-lethal dose raised to 4 kGy.

It is worthy to mention that the irradiated fungus started growth after 2 days of incubation on both not amended and amended media with NaCl.

Discussion

Fungi showed various responces to different environmental stresses such as salinity stress. The growth of *P.chrysogenum* is maximum at 10% NaCl and tolerated NaCl concentration up to 18%. The results are in full agreement with those obtained by Mert and Emekci (1987) on *Aspergillus flavus* and *P.chrysogenum*. Omori *et al*. (1995) reported that a mutant TK-2 of *Saccharomyces* was tolerant to 18% NaCl.

The participation of free amino acids in the osmo-regulation of *P. chrysogenum* was excluded. Our results hold fairly with those of Hernandez *et al*. (1994) on the marine yeast *Rhodotorula rubra*. On the other hand, EL-Abyad *et al*. (1994) reported that *Fusarium solani* and *Sclerotia rolfsii* appeared to be more tolerant to salinity stresses than other two formae speciales of *F.oxysporum* and both accumulate free amino acids under salinity stress.

The recorded increase in protein content following the increased salinity was coupled with a concomitant decrease in the total free amino acids and explained partially the increase in dry weight with the increased salinity. In agreement with our results. EL-Abyad *et al*. (1994) found that in *Fusarium solani* and *Sclerotia rolfsii* both synthesis of amino acids as well as their incorporation into proteins are stimulated under salinity stress. Furthermore, Wankhade *et al*. (1996) reported that osmotic component of salinity stress has been shown to induce ten protein in a salt tolerant / osmosensitive cowpea *Rhizobium* 4a (groundnut isolate).

An increase in the glycerol content estimated after hydrolysis of lipid (GHL) was observed with the increase in NaCl concentration. The data obtained by Hernandez *et al*. (1994) hold fairly with present work. They found that the total lipid content of the cells of the marine yeast *Rhodotorula rubra* increased with salinity.

The observed close correlation between intracellular free glycerol and medium salinity suggesting its participation in osmo-regulation. The glycerol pool concentration (17.56, 34.99 and 58.16 mg/g dry wt.) may balance the osmotic pressure in culture medium of 5, 10 and 15% NaCl.

The sharp decrease in dry weight at 18% NaCl was accompanied with a decrease in the accumulated free glycerol and may suggest that the fungus under such condition has lost more glycerol to the surroundings. Such results hold fairly with the findings of Adler *et al*. (1985). It was reported low and high molecular weight polyols accumulated (mainly glycerol) as osmotically active compounds and offset an increased external osmotic pressure but does not disrupt enzyme structure and function (Andreishcheva and Zviagil'skaia, 1999). These findings explained the increased growth of *P. chrysogenum* with the increase in NaCl concentration.

With regard to the effect of γ-irradiation on *P.chrysogenum* raised either on a medium not amended or amended with

NaCl, our data are consistent with the findings of Wang *et al.* (1998) on *Armillaria mellea* and those of Gherbawy, (1998) on *Aspergillus niger*. They reported that higher doses of γ-rays were inhibitory to mycelial dry weight. However results are in contrast with those of Gautam *et al.* (1998) who found that dose of 2.0 kGy has no effect on the growth of *Agaricus bisporus*.

Our data revealed that *P. chrysogenum* aquired resistance to γ-irradiation in the presence of high concentration of NaCl. The findings of Igarashi *et al.* (1978) are in full agreement with present results, where they found that Cl^- did not sensitiz bacterial spores or fungi to gamma-rays. On the other hand, Igarashi *et al.* (1978) reported that the radioresistant vegetative form of bacteria and yeasts was particularly sensitive to NaCl present during irradiation.

The radioresistance of *P.chrysogenum* on increased concentration of NaCl may attribute to four amino acids singly or in association with other amino acids, where they are thought to play a role in radioprotection.

Furthermore, Adam and Attaby (2000) reported that the variation in response to γ-irradiation of *F. moniliforme* and *T. viride* may be referred to the presence of amino acids as well as gibberellic acid. The appearance of the fungal growth, after gamma- irradiation of *P. chrysogenum* on all treatments, started after 2 days of incubation.

In conclusion, stressed mycelium of *P. chrysogenum* can generate a sufficiently negative osmotic potential to reverse the flow of water by synthesis of soluble organic solute(s) responsible for osmotic adjustment. In the same-time, it produces some other organic solutes for its protection from radiosensitization by sodium chloride.

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