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Krystyna PRZYBULEWSKA[1], Anna STOLARSKA[2]
and Daria GŁĄBOWSKA[1]

CHANGE OF PROLINE CONTENT IN SELECTED SOIL FUNGI AS AFFECTED BY OSMOTIC STRESS

ZMIANA ZAWARTOŚCI PROLINY U WYBRANYCH GRZYBÓW GLEBOWYCH POD WPŁYWEM STRESU OSMOTYCZNEGO

Abstract: The study aimed at demonstrating intracellular proline synthesis under osmotic stress conditions in selected soil fungi on the example of *Trichoderma* sp. and *Trichotecium roseum*. Effect of the increase of sodium chloride (NaCl) salinity, in concentrations of 1 to 1000 mmol · dm^{-3} PDA medium, on proline content in the fresh matter of mycelium cultured on medium was examined.

The increase of medium salinity affects production of osmoregulatory substances in the form of proline in selected soil fungi. Its content in mycelium depends on salt (NaCl) concentration in medium as well as on species. Intracellular proline synthesis increased starting with the least salinity. As osmotic stress increased, proline content in mycelium almost quadrupled in *Trichoderma* sp. and quintupled in *Trichotecium roseum*.

Keywords: fungi, salinity, proline

Fungi play an important role in decomposition of organic matter and humification processes in soil. Their number and activity depend largely on different factors, both natural and anthropogenic ones, such as temperature, pH or osmotic pressure. Salinity induces increase of osmotic pressure, which limits water uptake or even makes it impossible and can be a reason of growth arrest or even of cell destruction [1–6].

Microorganisms show certain adaptative mechanisms and adaptabilities which allow them to outlast unfavourable conditions in environment [7–9]. Microorganisms are capable of intracellular accumulation of osmoregulatory substances which allow preserving a turgor indispensable for proper cell functioning [1, 10–12]. These substances include different organic compounds such as amino acids, peptides,

[1] Department of Microbiology and Biotechnology of Environment, West Pomeranian University of Technology, ul. J. Słowackiego 17, 71–434 Szczecin, Poland, phone: +48 91 449 64 24, email: krystyna.przybulewska@zut.edu.pl

[2] Department of Plant of Physiology, West Pomeranian University of Technology, ul. J. Słowackiego 17, 71–434 Szczecin, Poland.

saccharides, alcohols and others [13–14]. One of these compounds is proline which can be absorbed from environment by microorganisms or synthesised intracellularly by them to tolerate easily growth inhibition [1, 15–19].

The study aimed at demonstrating intracellular proline synthesis on the example of selected soil fungi under osmotic stress conditions.

Material and methods

Two soil fungi, ie *Trichderma* sp. and *Trichotecium roseum* (Photo 1 and 2) were used in the study. Species affinity was determined with microscopic methods and was done by gas chromatography fatty acid methyl ester (FAME) analysis performed by Microbial ID (Newark, DE, USA). Effect of the osmotic stress resulting from sodium chloride (NaCl) salinity in concentrations of 1, 10, 100 and 1000 mmol · dm^{-3} PDA medium was examined. Mycelium cultured on salt-free medium was a control. Experiment was set up in three replications. Culture incubation was carried out on solid media at 24 ^{o}C for 2 weeks. Mycelium was homogenised in 10 % aqueous solution of sulfosalicylic acid in order to determine proline content.

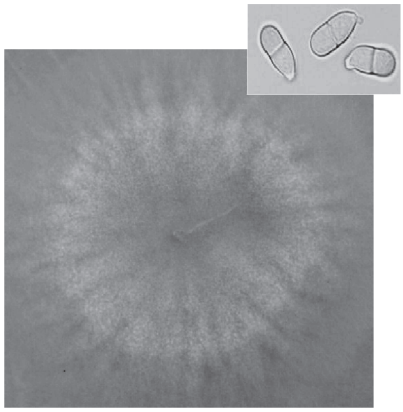

Photo 1. *Trichotecium roseum*

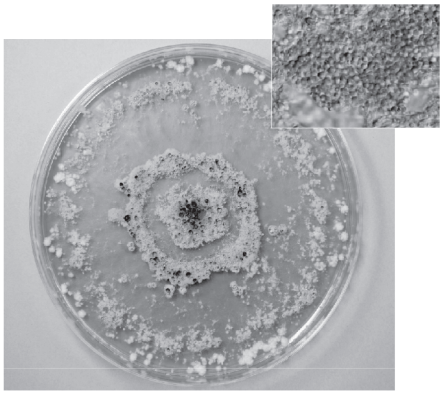

Photo 2. *Trichderma* sp.

Homogenate was filtered through Whatman No. 2 filter paper and filled up to a volume of 10 cm^3. Proline content was determined with the method of Bates et al [20].

Data were converted and given in μg of proline · g^{-1} of mycelium fresh matter. The obtained results were analysed statistically, using analysis of variance and testing the factors with the Duncan's test. Also a Pearson's correlation coefficient was calculated between medium salinity increase and mycelium proline content.

Results and discussion

The number and activity of fungi depends largely on various factors, both natural and anthropogenic ones [7–9]. Osmotic stress, resulting from the increase of medium salinity, has a significant effect on their development and enzymatic activity [4–6].

Based on the analysis of results, it is possible to state that the increase of medium salinity with sodium chloride (NaCl) significantly affected intracellular proline content in the mycelium of examined species. Increase in its content as affected by osmotic stress differed depending on the fungi examined (Fig. 1).

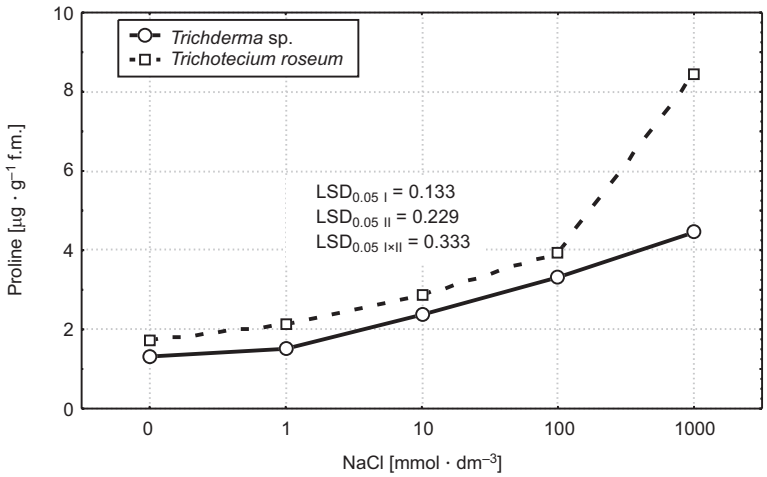

Fig. 1. Proline content in mycelium under sodium chloride (NaCl) medium salinity conditions

Proline content in the mycelium cultured on control medium without NaCl addition amounted to 1.32 µg · g^{-1} f.m. in *Trichoderma* sp., whereas its content was by 39 % larger in *Trichotecium roseum*. Salt addition to medium in the form of NaCl induced a significant increase of the content of examined amino acid irrespective of the species of examined fungus. The least amount of salt introduced into medium, ie 1 mmol · dm^{-3}, increased proline content by 15 % in *Trichoderma* sp. and by 24 % in *Trichotecium roseum* in relation to control (Photo 2). A tenfold and hundredfold increase of medium salinity increased proportionally its content in the mycelium of both examined species. Proline content in mycelium fresh matter was by 70–80 % larger at medium salinity with 10 mmol NaCl · dm^{-3}, while it exceeded its amount two- and a half-fold in relation to its content in the mycelium cultured on control medium. The largest proline content, ie 8.45 µg · g^{-1} f.m., was found in *Trichotecium roseum* cultured on the most salinated medium (1000 mmol NaCl · dm^{-3}). Significantly smaller proline content (33 %) under largest osmotic stress conditions was found in case of the second examined fungus, ie *Trichoderma* sp. Magnitude of the synthesis of osmoregulatory substances of that sort depends on the type of microorganisms and in case of this study depends even on fungus species. On average, its content was almost 20 % larger in *Trichotecium roseum* than in *Trichoderma* sp. (Fig. 2).

The carried out study confirms information that also soil fungi, as exampled by *Trichoderma* sp. and *Trichotecium roseum*, are capable of intracellular accumulation of specific osmoregulatory substances which are stress proteins, in this case proline, under

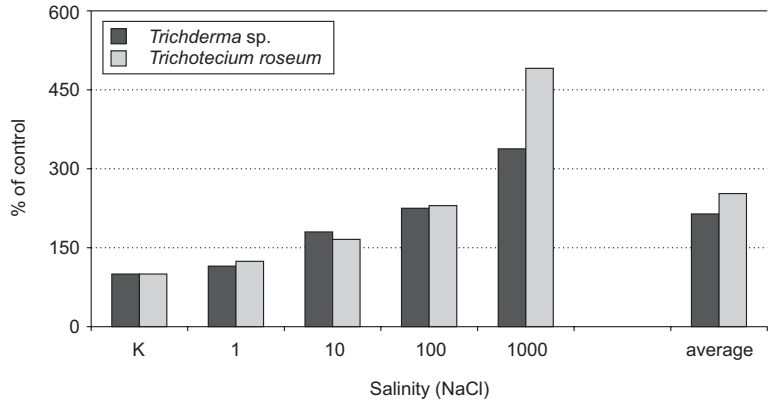

Fig. 2. Proline content in mycelium under sodium chloride (mmol NaCl · dm^{-3}) medium salinity conditions expressed as percent of control

medium salinity increase conditions. These results are corroborated by other authors in studies on bacteria or other eukaryotic organisms [1, 15–18].

In addition, the obtained regression equation, ie y = 0.0043x + 2.252, confirmed by significant correlation coefficient r = 0.84, points to the presence of dependence between medium salinity increase and mycelium proline synthesis.

Conclusions

1. Increase of medium salinity affects production of osmoregulatory substances in the form of proline in selected soil fungi. Its content in mycelium depends on salt (NaCl) concentration in medium as well as on fungus species.

2. Intracellular proline synthesis increased starting with the least medium salinity (1 mmol NaCl · dm^{-3}) and amounted on average to 20 % when compared with control. As salinity increased, its content increase in mycelium almost quadrupled in *Trichoderma* sp. and quintupled in *Trichotecium roseum* under the strongest osmotic stress conditions.

References

[1] Csonka L.N.: *Physiological and genetic responses of bacteria to osmotic stress*. Microbiol Rev. 1989, **53,** 121–147.

[2] Lopez C.S. Heras H., Garda H., Ruzal S. Sanchez-Rivas C. and Rivas E.: *Biochemical and biophysical studies of Bacillus subtilis envelopes under hyperosmotic stress*. Int. J. Food Microbiol. 2000, **55(**1–3), 137–142.

[3] Koch S. Oberson G. Eugster-Meier E., Meile L. and Lacroix Ch.: *Osmotic stress induced by salt increases cell yield, autolytic, and survival of lyophilization of Lactobacillus delbrueckii supsp. lactis*. Int. J. Food Microbiol. 2007, **117**(1), 36–42.

[4] Przybulewska K., Stolarska A. and Błaszak M.: *Wpływ zasolenia podłoża chlorkiem sodu (NaCl), azotanem sodu ($NaNO_3$) i ich mieszaniną na aktywność enzymatyczną wybranych grzybów glebowych. Część II. Hydroliza tłuszczu*. Wyd. Uczelniane UTP, Bydgoszcz 2008, 150–151.

[5] Przybulewska K., Stolarska A. and Błaszak M.: *Wpływ zasolenia podłoża chlorkiem sodu (NaCl), azotanem sodu ($NaNO_3$) i ich mieszaniną na aktywność enzymatyczną wybranych grzybów glebowych. Część III. Hydroliza skrobi*. Ecol. Chem. Eng. A 2009, **16**(4), 405–410.
[6] Przybulewska K. and Wieczorek A.: *Effect of medium salinity with sodium chloride (NaCl), sodium nitrate ($NaNO_3$) and their compound on the enzymatic activity of selected soil fungi. Part 1. Protein hydrolysis*. Ecol. Chem. Eng. A. 2007, **14**(11), 1198–1202.
[7] Boch J., Kempf B. and Bremer E.: *Osmoregulation in Bacillus subtilis*: *synthesis of osmoprotectant glycine betaine from exogenously provided choline*. J. Bacteriol. 1994, **176**, 5364–5371.
[8] Pawłowski S., Gulewicz P. and Grajek W.: *Wpływ ciśnienia osmotycznego na stan fizjologiczny komórek bakteryjnych*. Post. Biol. Komór. 2002, **29**(3), 435–448.
[9] Kogej T., Stein M., Volkmann M., Gorbushina A.A., Galinski E.A. and Gunde-Cimerman N.: *Osmotic adaptation of the halophilic fungus Hortaea werneckii: role of osmolytes and melanization*, Microbiology 2007, **153**, 4261–4273.
[10] Kempf B. and Brehmer E.: *Uptake and synthesis of solutes as microbial stress responses to high-osmolality environments*. Arch. Microbiol. 1998, **170**, 426–431.
[11] Poolman B. and Glaasker E.: *Regulation of compatible solute accumulation in bacteria.* Molecular Microbiol., 1998, **29**, 397–407.
[12] Mager W.H. and Varela J.C.S.: *Osmostress response of the yeast Saccharomyces*. Molec. Microbiol. 1993, **10**, 253–258.
[13] Galinski E.A. and Truper H.*: Microbial behaviour in salt-stressed ecosystems*. FEMS Microbial. Rev. 1994, **15**, 95–108.
[14] Galińska E.A.: *Osmoadaptation in bacteria*. Adv. Microbial. Physiol. 1995, **37**, 273–328.
[15] Whatmore A.M., Chudek J.A. and Reed R.H.: *The effects osmotic upshock on the intercellular solute pools of Bacillus subtilis.* J. Gen. Microbiol. 1990, **136**, 2527–2535.
[16] Chris A., Zeeshan M., Abraham G. and Prasad S.M.: *Proline accumulation in Cylindrospermum sp.* Environ. Exper. Bot. 2006, **57**, 154–159.
[17] Lucht J. and Bremer E.: *Adaptation of Escherichia coli to high osmolarity environments: osmoregulation of the high-affinity glycine betaine transport system proU*. FEMS Microbiol. Rev. 1994, **14**(1), 3–20.
[18] Kavahara Y., OhsumiI T., Yoshihara Y. and Ikeda S.: *Proline in the osmoregulation of Brevibacterium lactofermentum*. Agr. Biol. Chem. 1989, **53**, 2475–2479.
[19] Von Blohn C., Kempf B., Kappes R.M. and Bremer E.: *Osmostress response in Bacillus subtilis: Characterization of a proline Uptake system (OpuE) regulated by high osmolarity and alternative transcription factor sigma b.* Mol. Microbiol. 1997, **25**, 175–187.
[20] Bates L.S.: *Rapid determination of free proline for water stress studies*. Plant Soil 1973, **39**, 205–207.

ZMIANA ZAWARTOŚCI PROLINY U WYBRANYCH GRZYBÓW GLEBOWYCH POD WPŁYWEM STRESU OSMOTYCZNEGO

Katedra Mikrobiologii i Biotechnologii Środowiska
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

Abstrakt: Celem badań było wykazanie syntezy wewnątrzkomórkowej proliny w warunkach stresu osmotycznego u wybranych grzybów glebowych na przykładzie *Trichoderma* sp. i *Trichotecium roseum*. Badano wpływ stresu osmotycznego w wyniku zasolenia chlorkiem sodu (NaCl), w stężeniu od 1 do 1000 mmol · dm^{-3} pożywki PDA na zawartość proliny w świeżej masie grzybni wyrosłej na podłożu.

Wzrost zasolenia podłoża wpływa na wytwarzanie substancji osmoregulacyjnych w postaci proliny u wybranych grzybów glebowych. Jej zawartość w grzybni jest zależna od stężenia soli NaCl w podłożu oraz od gatunku. Synteza proliny wewnątrzkomórkowej zwiększała się począwszy od najmniejszego zasolenia. W miarę wzrostu stresu osmotycznego zawartość proliny w grzybni zwiększyła się prawie czterokrotnie u *Trichoderma* sp. i pięciokrotnie *Trichotecium roseum*.

Słowa kluczowe: grzyby, zasolenie, prolina