УКРАЇНСЬКИЙ
БОТАНІЧНИЙ
ЖУРНАЛ

Фізіологія, біохімія та клітинна біологія рослин

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COMPARISON OF GROWTH RATES OF THE FILAMENTOUS FUNGUS *ASPERGILLUS NIGER* L. FROM CONTRASTING ENVIRONMENTS

Key words: growth rate, Aspergillus *niger, stress, water activity*

Abstract

Eighteen strains of filamentous fungus *Aspergillus niger* were isolated from contrasting environments in Israel: the hypersaline Dead Sea and the temperate «Evolution Canyon» I. A comparison of growth rates under different water activity stress was provided. Clear differences in growth rates were observed under 5—20 % and 40 % volumes of the Dead Sea water (DSW). No difference was observed when we used 25—35 % volumes of DSW. Strains from «Evolution Canyon» I grown at lower concentrations of the DSW revealed higher growth rates, while strains from the DSW demonstrated higher activity under higher stress (40 % volumes of DSW). Therefore, the following conclusion can be made: strains of *A. niger* isolated from the DSW are more adapted to stress associated with low-water activity.

Introduction

Aspergillus niger van Tieghem is one of the most common filamentous fungus reported from a great variety of natural habitats. It was found in different soils, food, and indoor and outdoor environments. *A. niger* is the third commonest species associated with invasive pulmonary aspergillosis and aspergilloma [9, 13]. It is also cultured for the industrial production of some chemical compounds. Various strains of *A. niger* are used in the industrial preparation of citric acid (E330), gluconic acid (E574), and the enzymes glucoamylase and α-galactosidase (Beano®, Bean-zyme™). In Israel, *Aspergillus niger* was documented from all soils that were subjected to mycological investigations [4—7, 22]. Thus, *A. niger* can be easily isolated from a wide range of habitats.

The present study is focused on *Aspergillus niger* isolated from the hypersaline and temperate environments (the Dead Sea water and «Evolution Canyon» (EC) I). We comparatively investigated natural populations of *A. niger* under different stress, associated with low-water activity (a_w) on local (opposite slopes of «Evolution Canyon» I) and regional («EC» I vs DSW) scales.

Evolutionary biology focuses a lot on how genetic diversity is maintained in nature. Stressful conditions can bring higher rates of mutation and recombination, which are very important in the evolution process [11, 19]. Stress-induced genetic variation may serve as an important source of material for natural selection to produce novel adaptations, and differential selection regimes in natural populations may cause complex spatial variation in population structure, and in the level and spectrum of genetic variation [8, 11]. Micro- and macrosite ecological contrasts are excellent critical tests for evaluating biodiversity patterns and dynamics and for assessing the relative importance of different forces in evolution [16].

Materials and methods

Study sites

(I) «Evolution Canyon» I is located at lower Nahal Oren, Mt Carmel National Park, Israel (32° 43'N, 34° 58'E). «EC» I has two slopes: the south-facing (tropical, xeric, «African», AS) and north-facing (temperate, mesic, «European», ES) slopes; the distance between slopes is 100 m at the bottom and 400 m at the top. The opposite slopes of lower Nahal Oren, designated «Evolution Canyon», display dramatic biotic contrasts. Higher solar radiation on the south-facing slope (up to 600 %) makes it warmer, drier, and spatiotemporally more heterogeneous than the north-facing slope. Consequently, local biodiversity differentiation across several hundred meters displays global patterns of divergence [14, 15].

(II) The Dead Sea is located in the Syrian-African rift valley between Israel and Jordan. The climate is very arid, with a mean annual rainfall near 60 mm and mean temperatures of the hottest and coldest months from 32—35 °C and 14—16 °C, respectively [1]. The DSW contains 340 g/l total dissolved salts. It has very a_w of < 0.669 with a pH that is relatively low (5.5—6.0) [12]. The increased drying of the DS can be observed nowadays. Divalent cations (Mg^{+2} and Ca^{+2}) now

dominate over monovalent cations (Na^+ and K^+). Cl^- makes up 99 % of the anion sum. Mycological studies of the water samples from the DS revealed soil mycobiota comprising 77 species from 26 genera [17].

Sampling

(I) «Evolution Canyon» I

Fourteen soil samples from «EC» I were taken from each of the 6 stations (3 stations on each slope from the AS and ES). Samples were taken from shady niches under trees (ES) and in sunny open niches (AS), from the upper soil layer (on 1—5 cm depth). Altogether, 84 samples were collected in spring (April) 2005.

(II) The DS water — from its surface and depth (50 m and 250 m)

Samples were collected in autumn (October) 2005, four times at seven different sites along the western shore of the Dead Sea. They were withdrawn from near-shore aquatic localities and from deep and surface water from the center of the sea, about 8 km northeast of En Gedi («En Gedi 320»). Samples were collected by means of Go-Flo sampling bottles. Afterwards, water for the experiments was collected the same way four times per year.

Fungal isolation

Aspergillus niger strains were isolated using the soil dilution plate method [3]. The dilution for the samples was 1:10 by weight. Ten grams of each soil sample taken were suspended in sterile water. For isolation of fungi from the DSW, 2 ml of the DSW (from the depth and surface) were poured into Petri dishes and mixed by rotation with molten agar media (MEA).

Eighteen strains were taken for this experiment. Six are from the middle station of the AS (from sunny, opened niches); six from the middle station of the ES (from shady niches, under trees and bushes), and six from the DSW: four from a depth (50 m and 250 m) and 2 froma surface distant from the shore.

Experimental design

The linear growth rate on solid substrate, e.g., agar medium, is a good approximation of biomass increase in liquid culture. In the present experiment, strains were grown on Petri dishes with 10—12 ml of glucose yeast extract agar (GY) (10 g/L glucose, 1 g/L yeast extract, 20 g/L agar; pH 6.0—6.5). Agar disks (4—5 mm in diam) were cut out from 4-day-old cultures on agar plates and inoculated at the center of Petri dishes with GY (35 g/L agar), prepared with different concentrations of the (DSW); each strain in two repetitions. Volumes of the DSW differed from 0 % till 80 %, with the step of 5 %. Water activity was modified with calculated amounts of the DSW and distilled water. Water activity of the DSW is 0.669 and a_w of the distilled water is 0.99. Calculations were made according to the following formula:

$$a_w = ((100 - X) \cdot 0.99 + 0.669\ X) / 100$$

a_w — final water activity; X — used volume of the Dead Sea water

In the case of the medium prepared without DSW, MEA (Malt Extract Agar) (30 g/L Malt extract, 3 g/L peptone, 15 g/L agar; pH 6.0—6.5) was used. For the media prepared with 10 % volumes of the DSW, both GY and MEA were used.

Measurements of the mycelium growth were recorded in two orthogonal directions every 24 hours until the Petri plates were completely colonized or if the log-phase was observed.

Data analysis

Under the same salinity treatment the divergence between species specific cumulative distributions was checked by the non-parametric Kolmogorov-Smirnov test. The *p*value of the test could be detected with the following statistic formula:

$$\sqrt{N} \cdot \max_i (S(x_i) - F(x_i))$$

that has Kolmogorov distribution. N — number of thresholds in the series; $x_i - i_{th}$ threshold of growth rates; $S(x_i)$ — fraction of rates of the first distribution that are less than threshold x_i; $F(x_i)$ — fraction of rates of the second distribution that are less than threshold x_i.

Results

The present experiment is the first of its kind in which ecological comparisons have been made between strains of fungi isolated from the Dead Sea and «Evolution Canyon» I.

Data analysis showed that the minimum water-activity limited growth was 0.779. After two months of incubation at 30 °C, none of the strains performed growth in the range of 0.779—0.728 a_w (65 %—80 % of volumes of DSW). At the range a_w 0.845—0.796 (45 %—60 % of volumes of DSW) colonies grew very slowly and no measurements could be obtained.

As the control we took a_w — 0.99 and fungus isolated from the DSW demonstrated higher growth rate compared with the «EC» I strains. Due to some methodological problems, *A. niger* strains at the control were grown on the MEA instead of GY. In order to be sure that the difference in the strains' behavior was not influenced by the type of media used in the experiment, the experiment with 10 % volumes of the DSW (a_w 0.958) was repeated with MEA. Results were the same from those with GY.

Optimal growth for all strains was observed at 0.974 water activity (a_w) — 5 % of volume of DSW (Fig. 1). Strains from different environments (AS, ES, and DSW) demonstrated a significant difference between their growth rates on the medium with that of water activity. The same difference was observed at 0.958, 0.942, and 0.926 a_w — 10 %, 15 % and 20 % of DSW volume correspondingly. On the media with these salinities, fungus from «Evolution Canyon» I obviously grew faster than strains from the Dead Sea.

A different pattern was observed on the media with higher volumes of DSW. At water activities of 0.91—0.878 (25 %—35 % volumes of the DSW), no significant difference between the strains' behavior was observed. Still, when a_w reached 0.862

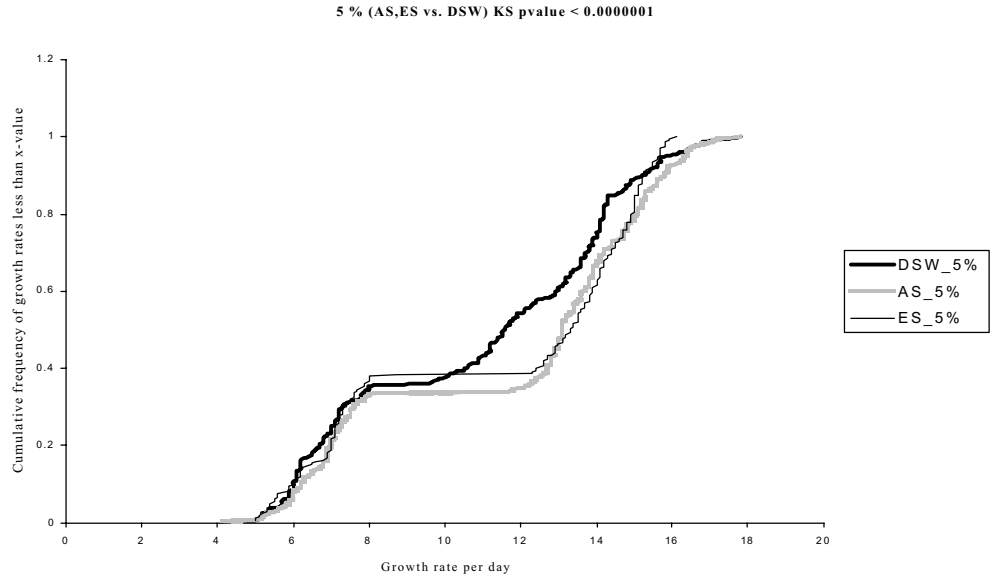

Figure 1. Growth rate of *A. niger* strains from African and European slopes of «Evolution Canyon» I and the Dead Sea on the GY medium with 5 % volume of DSW (a_w 0.974)

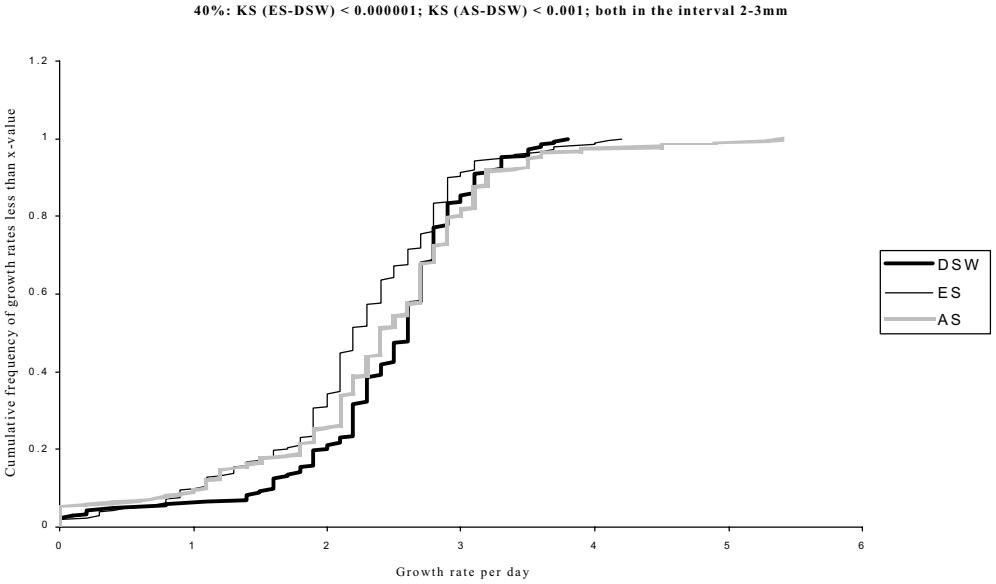

Figure 2. Growth rate of *A. niger* strains from African and European slopes of «Evolution Canyon» I and the Dead Sea on the GY medium with 40 % volume of DSW (a_w 0.862)

(40 % volume of the DSW), salt-tolerant strains from the Dead Sea started to show higher growing rates (Fig. 2). These factors are pretty good indicators for the appropriateness of media.

Discussion

Experimental studies on the combined influence of water activity and temperature on growth and spore production of *Aspergillus niger* strains [2, 18] demonstrated that the studied strains grew optimally at a_w 0.97—0.98.

Comparative investigation of the survival rate of conidia of the *A. niger* strains from the northern «EC» II (Upper Galilee) and the DS shore in different concentrations of the DS water showed 1.4—2.5-fold higher spore viability of the DS shore strains. Therefore, the potential for the introduction of foreign fungal species into the Dead Sea is very low. Examining and comparing species isolated from the Dead Sea to the same species from other environments can answer the question as to what extent have the species isolated from the Dead Sea adapted to its habitat, hypersaline brine [10].

This is the first study where such a complicated mixture with large amounts of different chemical elements as DSW was used for adapting a_w. Previously, in all similar experiments, the water activity of the medium was modified with calculated amounts of the non-ionic solute glycerol and the ionic solute NaCl. The use of glycerol to modify media water availability produced a higher growth rate than with NaCl, probably because glycerol can be utilized as a carbon source and can act directly as a compatible solute. In contrast, high concentrations of NaCl can be toxic, and this may explain the differential growth patterns observed [18]. These studies showed that the wild-type strains grew optimally at 0.95 a_w on glycerol-modified media, but optimally at 0.99 a_w when NaCl was used [18]. All the strains grew in CYE (Czapek Yeast Extract) medium from 0.86 to 0.99 a_w (when glycerol was used). The germination of *A. niger* strains has been reported at 0.77 a_w [20] and 0.80 a_w. The slow growth of *A. niger* isolates was reported at 0.76 a_w on malt extract agar (MEA) [22], whereas in another study the minimum water-activity limit for growth was 0.82 using the same culture medium [18]. The water of the Dead Sea has a very complicate structure. Therefore, it was impossible to predict fungal growth rates on the medium prepared with different volumes of the DSW.

Nevertheless, our data correlate good with the abouve said. Optimal growth for this fungus was not 0.99 a_w, but lesser water activity — 0.974 a_w (5 % volumes of DSW). Limiting growth a_w was 0.779 (65 % volumes of DSW). Thus, in spite of extremely high concentration of NaCl in the DSW brine, fungal growth is influenced not only by this salt. Therefore, media adjusted with DSW allow *A. niger* to grow with the rate that is in between, NaCl and glycerol adjusted media.

Results of the comparison between different populations («<EC» I vs DSW) seemed confusing. Low concentrations of DSW (5—20 % volumes of DSW; 0.974—0.926 a_w) gave advanatge to «EC» isolates, growth in media with moderate stress (25—35 % volumes of DSW; 0.91—0.878 a_w) didn't show any differences in strains' behavior. Nevertheless, when a_w reached 0.862 (40 % volumes of DSW), population isolated from DSW began to show higher growth rate.

Two hypotheses could be used to explain such interesting behavior. The first is that low concentrations of the Dead Sea water do not activate salt-resistant pathways in the salt-tolerant strains, while high volumes do. *A. niger* is considered to be a xerotolerant species. Therefore, high-water activity is a bit stressful for *A. niger*. Due to the constant salt stress of the habitat, to which strains from the Dead Sea are subjected, *A. niger* are more resistant to the stress associated with low-water activity.

The second explanation could be in the different pathways that the DSW strains and «EC»s strains use to resist osmotic stress. DSW strains are more resistant to stress associated with water availability. Probably, the pathway that is used by DSW strains gives *A. niger* advantages in surviving under salt stress. Any of these hypotheses support the suggestion that *Aspergillus niger* from the Dead Sea water is a salt-tolerant species which has some genetic advantages over the *A. niger* from «Evolution Canyon» I.

In any case, future investigations will be provided to confirm or disprove each of these hypotheses.

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Submitted 05.09.2006

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ТЕМПИ РОСТУ ІЗОЛЯТІВ *ASPERGILLUS NIGER* З КОНТРАСТНИХ УМОВ ІСНУВАННЯ

Порівняння темпів росту ізолятів *Aspergillus niger* з контрастних умов існування: гіперсолоних — Мертвого моря і помірних — «Еволюційного каньйону» (Ізраїль) дало можливість дійти висновку про більшу адаптованість ізолятів *A. niger,* виділених з води Мертвого моря, до стресу, асоційованого з низькою активністю води.

Ключові слова: ріст, Aspergillus niger, *стрес, водна активність*

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ТЕМПЫ РОСТА ИЗОЛЯТОВ *ASPERGILLUS NIGER* ИЗ КОНТРАСТНЫХ УСЛОВИЙ СУЩЕСТВОВАНИЯ

Сравнение темпов роста изолятов *Aspergillus niger* из контрастных условий существования: гиперсоленых — Мертвого моря и умеренных — «Эволюционного каньона» (Израиль) дало возможность сделать вывод о большей адаптированности изолятов *A. niger,* выделенных из воды Мертвого моря, к стрессу, ассоциированному с низкой активностью воды.

Ключевые слова: рост, Aspergillus niger, *стресс, водная активность*