

Extreme-environmental Microorganisms in Agriculture

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Abstract:

Agriculture, one of the most important human activities relevant to economy, society and environment, always faces many challenges, among which biotic and abiotic factors must be concerned for their limiting productivity and quality of crops. Sustainable agriculture is the leading direction of agricultural development. However, people only paid more attention to agricultural productivity but ignored the negative impacts of agricultural activities on the environment and ecology; and to obtain satisfied yield, more chemical fertilizers and pesticides have been applied in soils, which constitutes an uncontrollable vicious circle. Microorganism-derived fertilizers and pesticides are alternative to chemically synthesized products. Extreme microorganisms, isolated from hyper stressful environments have robust vitality in compare with ordinary organisms. Within the last few decades, a series of extreme microorganisms have been isolated. The application of the complete microbiomes or typical core microbiomes is going to be the key strategy for sustainable agriculture. Here, we selectively introduced certain useful microorganisms living in the saline obstacle environments and highlighted their function and application in sustainable agriculture.

Key words: Extreme microorganism; Halophilic fungi; soil-remediation

Introduction

To microorganisms, their roles in agriculture seem to be uncertain and over neglected. In the long history, people have been enjoying the material cycle and ecological balance promoted by microbial metabolism. The emergence of chemical fertilizers and pesticides, like the Hiroshima Atomic Bomb, has broken the silence of ecological balance. Chemical fertilizers and pesticides are double-edged swords for agriculture. And indeed, according to statistical data from UN food and agriculture Organization (FAO) and Ministry of Agriculture and Rural Affairs of the People's Republic of China (MOA), Fertilizer contributes near to 50% to the increase of world crop production, and pesticide use saves about 40% of the world's total crop production (MOA 2015, FAO 2015). However, fertilizers and pesticides lead to unwanted consequences, such as degraded soil fertility, excessive pesticide residues, and agricultural non-point source pollution. Particularly, Excessive use of pesticides and fertilizers influences the safety of the ecological environment and agricultural production, and further threatens human health and sustainable agricultural development. People are crazy to pursue the pleasure brought by ultra-high output of crops but have to stand the cost of unhealthy food. With finite resources, the pressure of the growing global population and human physical and mental health, we need a plan to stimulate action in areas of critical importance for agriculture.

In September 2015, the United Nations launched the Sustainable Development Goals. For developing countries with large populations such as China and India, this initiative has far-reaching significance. In order to deeply understand the connotation of the high-quality development of green agriculture and implement the ecological concept of "Nature is the true treasure"; and in order to promote the pace of ecological civilization in the developing countries and accelerate the rapid development of global modern agriculture, the International Symposium on Soil Fertility Improvement & Ecological Restoration in the Great Bend of Yellow River-Onsite Meeting for the Green Circulating Agriculture Based on Organic Fertilizer from Decomposed Straw was held in Tuoketuo County, Inner Mongolia, on July 31, 2019. The main theme of the conference is "Green, Cyclic, Health and Sustainability ". Fortunately, we were invited to attend and make keynote speeches at the conference. The Yellow River has bred the Chinese national culture, and the Great Bend of Yellow River (Hetao in Chinese) has laid the material foundation of China. "Harmful sometime the yellow river flooding, but makes wealth in the great bend". However, in today's Hetao, fertile fields disappeared, instead of which salt thorns are clustered. This is caused by man-made and unscientific farming system, especially the over-utility of chemical fertilizers, pesticides, and secondary disaster by flood irrigation. As fungal biotechnologists, our research has direct applications that contribute towards solving these problems. At the conference, we discussed a lot about how to emphasize the role of beneficial microorganisms in agriculture. We sincerely hope agronomists and micrologists who

work together with this fascinating group of organisms to improve the welfare of our planet and mankind.

Modern agriculture should be sufficient, organic and healthy agriculture. World agriculture is rapidly stepping into scale, intensiveness and modernization. Certainly not limited to China and Indian, there are many technical problems facing in the development of modern agriculture, among which the problems of soil conservation tillage and fertility upgrading, and harmless treatment of crop straw and efficient utilization are the most urgent ones. Human beings have always benefited from beneficial microorganisms, but we don't turn a blind eye to them until today. Therefore, A safe alternative to fertilizers and pesticides is becoming increasingly urgent.

Most halophilic organisms from different extreme-environments have been isolated mainly from oceans or related places with a high concentration of salt, such as the famous Dead Sea (Moubasher et al. 1990; Arakaki et al. 2013). In cold desert, Antarctic, or other specific extreme environments, many extreme organisms exist with specific resistance against to the single extreme environmental factor, but also confer strong resistant ability to other extreme conditions. Till now, many studies on biodiversity and physiology have focused on the characterization of halophilic fungi present in saline and hypersaline ecosystems. Many species in ascomycetes and some in basidiomycetes have been described with the ability to grow in these environments.

Halophilic microorganisms

Ancient Earth was covered in a global ocean (Burnham and Berry 2017). Studies on the microbial communities of deep subsurface sediments, saline lakes, or desert soils with variable salinity levels have indicated the presence of Bacteria and Archaea, containing a number of ubiquitous phyla including Actinobacteria, Bacteroidetes and Proteobacteria (Fierer et al., 2009). A series of strains isolated from the vent of submarine volcano show not only halotolerance but also thermotolerance. In addition, *Pyrococcus abyssi*, *Sulfolobus acidocaldarius*, *Thermococcus kodakarensis*, and *Thermotoga neapolitana* make them best laboratory models for understanding the mechanisms that they have evolved to live under hostile environmental conditions. Another halotolerant *Exiguobacterium* strain was isolated from Salar de Huasco, is ideal for the study on resistant mechanism and the evolution of adaptation (Remonsellez et al., 2018).

Early researches focused on prokaryotes grow under salt stress and populate saline ecosystems such as Eubacteria, Archaea and Algae (Oren 2002). Microbial eukaryotes also appeared in deep-sea subsurface sediments; and fungi are the most consistently detected eukaryotes in the marine sedimentary subsurface (Edgcomb et al. 2011). Most marine-derived fungi belong to halotolerant fungi which live in saline environments but do not necessarily require certain concentrations of salt; the rest of marine-derived fungi are classed as halophilic fungi because these fungi require salt concentrations of at least 0.3 M (sodium salt, e.g. NaCl) to grow optimally, and even they thrive in high-salt environments (Arakaki et al. 2014). Over the last two decades, marine fungi have been discovered accordingly in the saline environments such as in the Dead Sea, Atlantic Ocean, China Sea (Grishkan et al. 2003; Nazareth et al. 2012), and the solar salterns near to seacoast (Cantrell et al. 2006; Nayak et al. 2012). A large number of studies on biodiversity and physiology have focused on the characterization of halophilic fungi present in the sea related saline and hypersaline ecosystems, among which Ascomycetes and Basidiomycetes have been described (Gunde-Cimerman et al. 2000; Gunde-Cimerman and Zalar 2014). In general, fungal communities in hypersaline environments are dominated by *Aspergillus*, *Penicillium* and some of their teleomorphic genera. Other genera such as *Alternaria*, *Cladosporium*, *Fusarium*, *Chaetomium*, *Wallemia* and *Hortaea* were also reported (Moubasher et al. 2018). Some new species were also described from hypersaline environments including three *Gymnoascus* species.

Some special extreme-environments are favor to isolate the halotolerant or halophilic microorganisms. A variety of filamentous fungi have been isolated from the Dead Sea, including *Gymnascella marismortui* isolated from the surface water down to a depth of 300 m (Buchalo et al. 1998). *G. marismortui* is adapted to high-salt conditions and requires high salt concentrations (Buchalo et al. 1998; 2000). In addition, 476 fungal isolates were isolated consistently from the Dead Sea and probably form the stable core of the fungal community, including *Aspergillus terreus*, *A. sydowii*, *A.*

versicolor, *Eurotium herbariorum*, *Penicillium westlingii*, *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*. However, most fungal isolates from the Dead Sea belong to the genera *Eurotium* and *Aspergillus* (Yan et al. 2005).

Lake Magadi is a hypersaline location in the East African Rift valley, Kenya. 52 fungal isolates in Lake Magadi were characterized with different pH, temperature and salinity ranges, respectively (Orwa et al. 2020). These isolates were affiliated to 18 different genera with *Aspergillus*, *Penicillium*, *Cladosporium*, *Phoma* and *Acremonium* being dominant. Interestingly, the different isolates could produce diverse extracellular enzymes, such as proteases, chitinases, cellulases, amylases, pectinases and lipases. In addition, antimicrobial metabolites were noted for isolate 11M affiliated to *Penicillium chrysogenum* (99%). Cell free extracts and crude extracts from isolate 11M had inhibitory effects on both animal and plant pathogens, indicating the promising application potential in biological protection.

China has remarkable biodiversity and many typical hypersaline environments. Research aimed at isolating and characterizing halotolerant or halophilic fungi from seas, has progressed rapidly. A series of promising halophilic fungi, including *Aspergillus glaucus* CCHA, have been reported (Liu et al., 2011). Three marine-derived isolates were collected in Wenchang, Hainan Province, China, and identified as extremely halotolerant fungi: *Wallemia sebi* PXP-89, *P. chrysogenum* PXP-55, and *Cladosporium cladosporioides* PXP-49 (Xu et al. 2011). In addition, 188 marine-derived fungi were collected from the sediment in Zhoushan Sea area, the mangrove at Yunxiao Country and Jiulongjiang estuary in Fujian Province, China (Xiao et al. 2005), of which the ethyl acetate extract of strain 164 exhibited strong lethal effect on nematode *Rhabditis* sp. In another research, 31 nematode-trapping fungi recorded from mangrove habitat of Hong Kong were identified *Arthrobotrys*, *Monacrosporium*, and *Dactylella* (Swe et al. 2009). The South China Sea covers a vast area. The diversity of fungal communities in nine different deep-sea sediment samples of the South China Sea were isolated by culture-dependent methods followed by analysis of fungal internal transcribed spacer sequences (Zhang et al. 2013), in which 13 of 27 identified species were firstly reported. Moreover, 3 isolates might be novel phylotypes of genera *Ajellomyces*, *Podosordaria*, *Torula*, and *Xylaria*.

Many terrestrial halotolerant fungi have been characterized. Chamekh et al. (2019) identified 136 isolates from the soil of the Great Sebkhia of Oran located in northwestern of Algeria. *Wallemia* sp. H15, *Gymnoascus halophilus* H19 and H20 are obligatorily halophilic, but most isolates are halotolerant, which can still grow on PDA medium without NaCl, indicating the dominant flora of halotolerant fungi. 74% of the strains could grow at 12.5% NaCl and 5 strains (*A. subramanianii* strain A1, *Aspergillus* sp. strain A4, *P. vinaceum* and the two strains of *G. halophilus*) at 17.5%. The only strain that could grow at 20% was *Wallemia* sp. The optimum growth of most strains is 2.5% or 5% NaCl. The concentration of 10% is optimal for the growth of *G. halophilus*. The halophilic fungus *A. glaucus* CCHA from air-dried wild vegetation has been analyzed (Liu et al. 2011). This species shows extreme salt tolerance, with a salinity range of 5 to 32% (NaCl) required for growth. Interestingly, *A. glaucus* CCHA survives in solutions with a broad pH range of 2.0-11.5, indicating that it is a haloalkaliphilic fungus. Further investigation indicated that increasing the pH value (> 8.0) can induce *A. glaucus* CCHA to produce a variety of organic acids, including citric acid, oxalic acid and malic acid. In addition, *A. glaucus* CCHA shows resistance to aridity, heavy metal ions, and high temperature (Liu et al. 2011). The extremophilic nature of *A. glaucus* CCHA suggests that it has great promise in soil remediation applications (Fig 1).



Control Field

Test Field

Fig X.1 Mycoremediation of salt-affected soil using amendments supplemented with saline tolerant fungi (*Aspergillus glaucus* CCHA and *Aspergillus terreus* (ratio = 1:1)). The experiment was conducted in salt-affected soil in Dalian, Liaoning province, China. The photos were taken in 2020.

Prospects

Abiotic stresses that influence agriculture include soil salinity, drought and extreme high or low temperatures. Second salinization or such as contaminations caused by overusing chemical fertilizers actually belong to abiotic stress as well. Scientific utilization of beneficial extreme-microorganisms is an important means to reduce harms to agriculture. Microbial application for amelioration of saline soils is gaining popularity due to its better amelioration and reduction in economic and environmental costs. Microorganisms with different roles play function synergistically in a defined extreme environment. The identification and application of the complete microbiomes or typical core microbiomes is going to be the key strategy for sustainable agriculture. It is clear that despite the advances, more researches are required to realize the potential of sustainable fungal environmental biotechnology. Agronomists and micrologists must work together with this fascinating group of organisms to improve the welfare of our planet and mankind.

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