PARTICULARITIES OF LICHENS DISPERSAL AND MULTIPLICATION

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We presented in this review paper the study of lichens reproduction complexity and structures involved in asexual and sexual lichens reproductions besides particularities of dispersal and substrate colonization, lichens distribution in a wide variety of habitats related to substrate specificity and selectivity. The knowledge of these aspects is necessary to implement biotechnologies of lichens *in vitro* culture in order to improve secondary metabolites contents useful for pharmaceutical industries.

Keywords: lichens habitat, lichenology, lichens micropropagation, reproductive propagules, lichens distribution.

INTRODUCTION

The definition of lichens was revised through the time, the more recent one (Lücking *et al*., 2021) considering the scientific, Latinized name in association with primary mycobiont and the vernacular name with the entire lichen. Besides microbial syntrophies, lichens with a new phenotype resulted not only as a sum of their partners, but as models that inspired evolutionary events hypothesis from unicellularity to pluricellularity (Libby and Ratcliff, 2021) and adaptative coevolution between symbionts (Ametrano *et al*., 2022). Lichens are adaptated to a wide range of habitats, from polar (Folgar-Cameán *et al*., 2019) to arid areas. Beside plants, lichens are important sources of bioactive compounds (Kerboua *et al*., 2021, Voicu *et al*., 2019). Also, lichens have a rich content in carotenoids (Czeczuga *et al*., 1992), predominant being lutein epoxide and astaxanthin. Lichens are of special interest for antifungal and antibacterial potential (Popovici *et al*., 2022). Also lichens have decorative value (Devkota *et al*. (2017). Besides mycobiont, other lichen-associated fungi exhibite antioxidant activities, contributing to the symbiosis sustaining (Galinato *et al*., 2021). Lichens are seen nowadays as self-sustaining micro-ecosystems (Jung *et al*., 2021) new lichens species being continously identified (Orange *et al*., 2001, Van den Boom, 2020, Biju *et al*., 2021). Among lichen particularities, lichens reproduction expresses a special complexity (Tuovinen, 2017).

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MATERIAL AND METHODS

We reviewed the most recent scientific data from about 75 of articles regarding lichens dispersal and multiplication in natural conditions and in *in vitro* culture. We analyzed the abundance of lichens growing on tree bark substrate in Drumul Taberei Park.

RESULTS AND ISCUSSION

LICHENS DISPERSAL

Lichen distribution depends on a series of factors as type of habitat and substrate, climate and trace metal pollution of the environment.

The analysis of the distribution of lichens on different tree species (Figs. 2–5) revealed the correlation between the lichen abundance in a area with substrate specificity. Varieties of substrata colonized by lichens are elements that promote the increased number of species over Terra (Krishnamurthy and Upreti, 2001). *Xanthoparmelia* lichen genus is representative for high species diversity and evolution by changing habitat according to substrate preferences (Autumn *et al*., 2020), the typical habitat eing arid to semi-arid climate, important for evolutionary diversification.

Distance of dispersal is decisive for the successful colonisation of trees by epiphytic lichens (Ronnas *et al*., 2017); therefore, clonal propagules of the epiphytic lichens are dispersed on a distance of tens of meters while ascospores disperse over several thousand metres. Dispersal of the vegetative propagules can be achieved on long distance dispersal (Bohuslavová *et al*., 2018) to *Usnea* ssp. and *Leptogium puberulum* lichen species. Volcanic islands with a high degree of endemic biota as lichens are relevant for the significance of lichens isolation by distance and useful for providing information about the evolutionary history of lichens taxa related to geographic genetic patterns (Werth *et al*., 2021). Dispersal distance of lichens is evaluated by different methods consisting in mechanized propagule traps associated with DNA diagnostics; species-specific reproductive propagules of lichens were identified by using for the first time mechanized propagule traps and novel species-specific primers and optimized a standard DNA extraction and PCR protocol Eaton *et al*. (2017). Lichens differ in level attachment to substrate (Noton, 2014). The abundance of lichens from an geographical area depends also on the nutritional preferences of lichens and their host trees; therefore, lichens epiphytic species richness are tightly correlated with host species (Su, 2019, Vicol, 2016). A study on mapping species belonging of the genus *Parmelia* in Romania revealed that *P. sulcata* and *P. saxatilis* are the most widespread (Vicol, 2016). Physical landscape, landscape genetics, meaning population genetic and spatial variation sustain the understanding of lichen dispersal mechanism (Doering *et al*., 2020). Lichens transplantation process influence lichen community (Ellis *et al*., 2021); lichens influence ecosystems by the large number of species.

Growth pattern of the circular lichens are tightly correlated with the carbon dioxide diffusion around them that can fix at the edges of the thallus for larger lichens or across the lichen thallus surface for the smaller lichens (Seminara *et al*., 2018). Lichen influence the climate of a region, functioning as a carpet of vegetation on soil (Aartsma *et al*., 2021). Estimation of climate change influence on lichen species reveal that lichens with green algae, saxicolous and epiphyte species respond better to climate change (Rubio-Salcedo *et al*., 2017). Lichens are recognized as excellent environmental monitoring (Vicol, 2022). The interactions of lichens with in the *ex vitro* and *in vitro* environment highlight that lichens are well adapted microecosystems (Voicu, 2021).

Analysis of functional traits as water holding capacity of lichens, specific thallus area, nitrogen and phosphorous content and tissue pH revealed phenotyipic plasticity of lichen community composition acros elevations gradient (Roos *et al*., 2019); the capacity to resist to drying state contribute to the use of some lichen thallus species as *Cetraria islandica* as ornaments (Fig. 1).

Lichens have genes involved in drought resistance; antioxidant capacity of lichens is enhanced by dry habitats (Gautam *et al*., 2021). With rock-inhabiting fungi resistant to abiotic stress, lichens form biocoenoses (Ametrano *et al*., 2019).

LICHENS MULTIPLICATION

In lichens there are two types of symbionts transmission: vertical transmission by grain-like structures developed on thallus, composed of fungal and algal partners and horizontal transmission of symbionts by sexual fungal spores in disc-shaped fruiting bodies (Grube and Spribile, 2012, Dal Grande *et al*., 2012).

LICHENS VEGETATIVE MULTIPLICATION

In vitro culture is a particular system of vegetative multiplication, especially for lichen thallus and even for their aposymbiotically culture (Voicu, 2021) and an alternative methodology to transplantation of lichens from *ex vitro* environment to *in vitro* environment with more possibility to monitor both evolution of growth process as the parameters involved. In order to develop new methodologies of lichens *in vitro* multiplication by vegetative propagules, modulating synthetic culture media composition is a necessary objective (Voicu *et al*., 2017) mainly for medicinal or threatened lichens (Voicu and Gavriloaie, 2017). Lichens presents a great spread and also the presence in almost all terrestrial ecosystems. Asexual reproduction in lichens is based on dissemination of specialized diaspores (Bowler and Rundel, 1975). Asexual reproductive way of a lichenized fungus changes with adverse environment (Merinero *et al*., 2017) is a way of well-adapted genotypes to cope with extreme and stable environments, sustaining the evolutionary events (Walser *et al*., 2004). Vegetative lichens reproduction by fragmentation, soredia and isidia depends on a series of factors (Krishnamurthy and Upreti, 2001); in this context, the isidia with large weight restrain its dispersal by wind. Asexual propagules deposition depends on dominant tree species substrata (Pasiche-Lisboa *et al*., 2019).

LICHENS SEXUATE MULTIPLICATION

Lichens are characterized by a diversity in reproductive modes (Tripp and Lendemer, 2017) involving meiotic and mitotic recombination wich maintain their genetic diversity. Sexuate reproduction, similar to asexual, mitosporal, is specific to mycobiont. Between lichens fertility and population size is a direct proportional relation (Rolstad *et al*., 2013). As in almost all lichens, mycobiont is an ascomycete, its sexual reproduction is similar to that of some unlichenised taxa of the phylum *Ascomycota*. Lichens ascospores are criteria to determine lichens and to understand aerodynamic and dispersal of lichens (Pentecost, 1981, Honegger, 1985). In natural conditions they are released in clusters of eight from the asci; contacts were reported between free algal living cells and germinated ascospores to the epilithic lichens *Lecanora dispersa* and *Caloplaca aurantia* in the same niches (Garty and Delarea, 1988). In dispersal, spatial limitation is involved in evaluating environmental changes. Lichen meiospores are amenable for short-distance dispersal pathway and local colonization; climatic factors mainly rainfall events are also correlated with meriospore dispersal (Favero-Longo *et al*., 2014). An interesting feature of non lichenized and lichen-forming ascomycetes is the capacity to differentiate gametangia (ascogonia) or gametes (microconidia, meaning spermatia) (Honegger and Scherrer, 2008). Spores scattered by wind, water or less often by insects, reaching optimal conditions, germinate and give rise to a mycelium. Its subsequent evolution is determined by the presence/ absence of the corresponding alga; if it meets alga, after a certain period, it reconstitutes the lichen thallus. Arctic alpin lichens as *Thamnolia vermicularis* presents a reproductive strategy on long distance dispersal containing pycnidial conidiomata containing conidia (Lord *et al*., 2013). Fruiting body types as open apothecia or closed perithecia (cleistothecia), involved in meiospore dispersal are criteria for ascomycete classification (Schmitt *et al*., 2009). Structures involved in sexual and asexual lichens reproduction, as *Xanthoria parietina* apothecia or soredia of *Vulpicida pinastri* and *Hypogymnia physodes* have a rich content in phenols comparative to somatic structures (Hyvarinen *et al*., 2000). Lichens meiospores are species characteristics, having different colonization patterns (Morando *et al*., 2017). Lichen photobiont, namely green alga *Symbiochloris reticulata*, is mainly vertically transmitted via soredia propagule in *Lobaria pulmonaria* lichen (Werth, 2012). Disturbance on lichens mating systems by abiotic limiting factor trigger lichens clonality (Singh *et al.*, 2015). *In vitro* culture methods of lichens multiplication mainly for threatened species come to sustain measures of lichens conservations by thallus transplants in nature (Singh *et al*., 2012). Lichens can be cultivated *in vitro* starting from reproductive vegetative structures as soredia and isidia as well as apothecia (Lobakova and Smirnov, 2012). Soredia is used also in *in vitro* culture as a modality to obtain lichens symbionts separately and to micropropagate them further (Černajová and Škaloud, 2020). The lichen *Cladonia grayi* podetia has both sexual and asexual propagules; the upper margins are covered with brown fungal apothecia that are the site of meiotic spore production and releasing into the air; the podetial surface is covered with vegetative reproductive structures, namely green vegetative propagules called soredia. Coculture of the mycobiont *Cladonia gray* and green algal photobiont *Asterochloris glomerata* revealed that fungus is involved in regulation of the membrane transport proteins, signal transduction components, extracellular hydrolases, the ribitol transporter and ammonium transporter (Armaleo *et al*., 2019). Regarding the symbionts interactions in *in vitro* conditios, Du *et al*. (2019) observed the clumping together process of algae around the fungus (Bonfante, 2019). Interaction between symbiotic patterns are conditioned of specificity (Pardo-De la Hoz *et al*., 2018). Mycobiont *in vitro* culture can be developed from different type of inoculi to different lichen species. *In vitro* culture media suitable for mycobiont culture are: Lilly and Barnett medium containing ribitol or sucrose, amenable to *Ramalina conduplicans* lichen mycobiont (Yoshino *et al*., 2019); MY medium with 10% sucrose (Shanmugam *et al*., 2022); Malt-Yeast liquid culture medium (Cristian and Brezeanu, 2013); Czapek-Dox medium, Yeast nitrogen base medium without aminoacids, Lilly and Barnett medium with ribitol or sucrose as a carbon source (Yoshino *et al*., 2019); D-mannitol (a sugar alcohol that occur in lichens and is transported between symbionts) in concentrations of 1 and 2% added to the culture medium (Pichler *et al*., 2021). Cryopreservation methods were used for some lichen species as *Pseudoevernia furfuracea* L. (Banciu and Cristian, 2015) Conservation strategies are focused on lichens populations characteristics correlated with the substrate (Scheideger and Werth, 2009). Reproductive strategies of lichens (Brunialti *et al*., 2021) revealed that sexually reproducing species have specific abundance in non old-growth forest and vegetative ones are predominantly corelated with old-growth forests forming nested assemblages. Mat-forming lichens are the most representative vegetation in altitudinal habitat, this capacity of insulating the soil, beside bryophytes, having an positive effect on microclimate and a suitable substrate for seedlings establishment (Van Zuijlen *et al*., 2020). Certain lichen species as *Calopadia puiggarii* have a great colonization potential because of the sexual and asexual reproduction by abundant dispersal of ascospores, conidia and photobionts (Sanders, 2014). Most of lichen thalli contain *Trebouxia* (Dal Grande *et al*., 2018). Lichen diversity offer a vast field of approaches as the study of *in vitro* culture, the complete life cycle of lichens (Sanders, 2014) and also air quality biomonitoring (Khastini *et al*., 2019, Käffer *et al*., 2021).

Fig. 1. *Xanthoria parietina* on *Tilia cordata*.

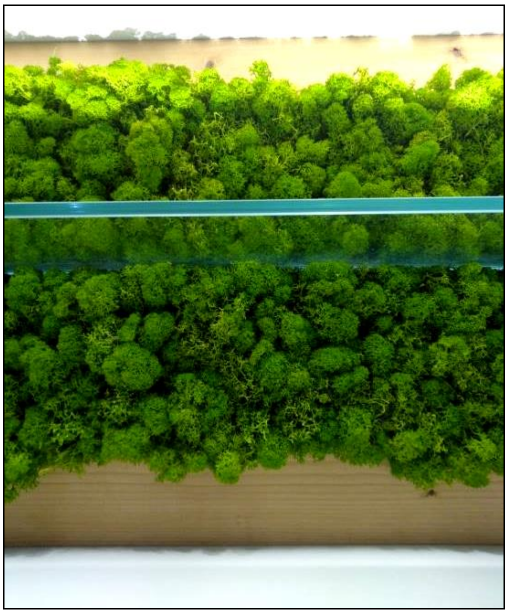

Fig. 2. *Cetraria islandica* thallus as an ornament in a shop.

Fig. 3. *Xanthoria parietina* on *Acer* species.

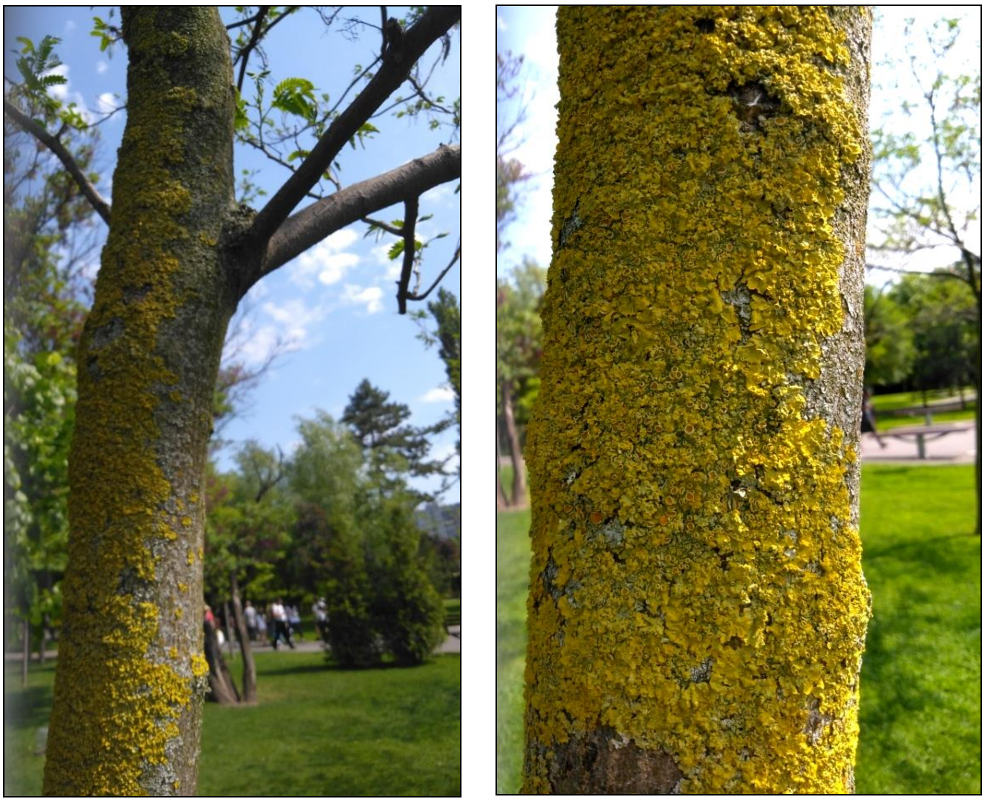

Fig. 4. *Xanthoria parietina* on *Robinia* species bark in Drumul Taberei Park (original photos, D. Voicu).

CONCLUSIONS

The present study contributes as a documentary on knowledge of mechanism of lichens distribution in nature and factors interacting with adaptation to the habitat. The propagule dispersal of lichens is related to microenvironment particularities. Lichen species diversity and adaptability determine the success of establishment on different substrates. Studying the capacity of lichens to colonise a wide range of niches we can draw research directions for conservation status of these amazing symbioses.

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