Symbiosis, 11 (1991) 255–262
Balaban, Philadelphia/Rehovot

Water Status and Urease Secretion from Two Ecotypes of *Xanthoria parietina*

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Received June 8, 1990; Accepted October 14, 1990

Abstract

Both epiphytic and saxicolous ecotypes of *Xanthoria parietina* are able to produce and secrete urease when they are floated on liquid media containing urea. Secretion appears to be related to the saturated thallus water content of each ecotype. The results are suggested to support the idea of enzyme movement within the lichen thallus.

Keywords: *Xanthoria parietina*, *Populus nigra*, *Robinia pseudoacacia*, basalt, urease, urease secretion, water content

Abbreviations: PEG, polyethyleneglycol; Tris-HCl, tris (hydroxymethyl) aminomethane hydrochloride

1. Introduction

Several lichen enzymes are secreted to the medium from thalli when they are appropriately incubated under laboratory conditions. Cellulase (Vicente, 1990), urease (Blanco et al., 1984) or arginase (Planelles and Legaz, 1987) are reported to be actively secreted to media containing, respectively, cellobiose, urea or arginine. In these cases, the protein must be glycosylated before secretion, since it is known that the attached carbohydrate determines the extracellular fate of the protein molecule (Davis and Davis, 1980). Arginase secreted

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by *Evernia prunastri* contains 280 residues of glucose, 27 of fructose, and 85 of mannose *per* molecule of protein (Planelles and Legaz, 1987), whereas the carbohydrate attached to urease, secreted from the same lichen species, is a homopolymer composed of 845 residues of galactose *per* molecule of protein (Perez-Urria and Vicente, 1989).

The physiological role of this secretion is, as yet, unknown. Urease (Legaz et al., 1986) and uricase (Masse, 1969) are mainly secreted by coprophylous species. Epiphytic lichens also secrete several enzymes whereas saxicolous or terricolous species secrete only small amounts of urease, or do not secrete at all (Blanco et al., 1984; Legaz et al., 1986). This has been explained by considering urease secretion as a response to the chemical nature of the medium. However, *Cladonia stellaris*, a terricolous species growing on a mineralized soil, is an exception in that it secretes more urease than that retained in its thallus (Perez-Urria et al., 1989). This has been explained as a function of the higher value of the saturated water content of this lichen thallus, whereas *C. rangiferina*, collected from the same soil, does not secrete urease and has a lower value of saturated water content.

In this paper we analyze urease secretion and water content of two ecotypes of the same lichen species, *Xanthoria parietina*, in order to further investigate the possible relationship between thallus water content and enzyme secretion.

2. Materials and Methods

Plant material and incubation conditions

Samples of *Xanthoria parietina* (L.) Th.Fr. were collected from *Populus nigra* L. and *Robinia pseudoacacia* L. in Montejo de la Sierra (Madrid) or from basalt (Toledo). Air-dried thalli were stored in polythene bags, in the dark at 7°C for no more than two weeks until required. Samples of 1.0 g of air-dried thalli, or after rehydration for 15 min with distilled water at room temperature, were incubated for 8 hr in the dark, at 26°C, on 40 mM urea in 0.1 M Tris-HCl buffer, pH 7.0. Ten per cent PEG 4000 was included in the medium, where indicated.

Preparation of cell-free extracts and assay of urease activity

At different times, both media and thalli were recovered. Incubation media were filtered through Millipore GS filters (0.22 μm pore diameter) and then dialyzed overnight against 5.0 L of Tris-HCl buffer at 4°C. These dialyzed media were used for enzyme assay. Thallus samples were washed with distilled water

and then macerated with 10 ml acetone for 5 min at room temperature to remove lichen phenols (Vicente et al., 1983). Thalline powder was dried *in vacuo* and macerated again with 10 ml 0.1 M Tris-HCl buffer, pH 7.0. Homogenates were centrifuged at 21,000 g for 20 min at 2°C and the supernatants were filtered through Millipore GS filters to use for enzyme assays.

Urease activity was measured by the Conway (1962) microdiffusion method as ammonia produced in a reaction mixture which contained, in a final volume of 2.0 ml, 1.0 mg protein, 37 mmol Tris-HCl buffer, pH 7.0, and 20 mM urea. After 20 min at 37°C, the reaction was stopped by adding 0.5 ml of saturated potassium carbonate solution. The ammonia produced during reaction was recovered on 1.0 ml 0.02 N sulfuric acid. A unit of specific activity was defined as 1.0 μmol of ammonia produced *per* mg protein *per* min. The amount of protein was estimated by the method of Potty (1969) using bovine serum albumin as a standard.

3. Results

Figure 1 shows the time-course of urease production and secretion by *X. parietina* thalli collected from *R. pseudoacacia*. When thalli were floated on urea without previous rehydration, a low amount of active urease was secreted, rising to a maximum after 4 hr incubation, whereas no urease activity was retained by the thalli. However, after 4 hr, activity of urease recovered in the media, diminished and was levelled off at 8 hr, whereas that retained by the thalli rapidly increased. The pattern of urease production and secretion by previously rehydrated thalli was similar to that described, but secretion showed two well-defined maxima, (2 hr and 6 hr), and the activity of secreted urease was about 4.5 times higher than that obtained from dry thalli.

Dry thalli of *X. parietina* collected from *P. nigra* secreted little amounts of urease to the medium continuously. High levels of urease activity were retained by the thalli with a maximum at 2 hr incubation (Fig. 2). When the thalli were rehydrated before incubation in urea, practically no activity was retained by the cells whereas a maximum secretion was after 2 hr incubation.

The saxicolous ecotype of *X. parietina* retained very low urease activity inside the thallus. Maximal secretion was after 6 hr incubation, which was 4 times higher with rehydrated thallus samples (Fig. 3).

The addition of 10% PEG to the incubation media, slightly decreased the activity retained by the thalli of all the specimens. However, no active urease was recovered from the media in which samples of the three ecotypes had been floated (Fig. 4). It was interesting to note that a linear correlation could

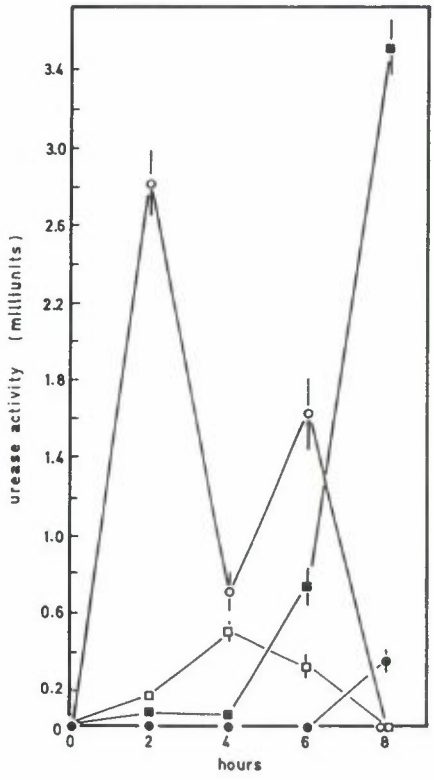

Figure 1. Urease produced (■) or secreted (□) by dry thalli and produced (•) or secreted (○) by rewetted thalli of *X. parietina* collected from *R. pseudoacacia* and floated on 40 mM urea for 8 hr. Values are the mean of three replicates. Vertical bars give standard error where larger than the symbols.

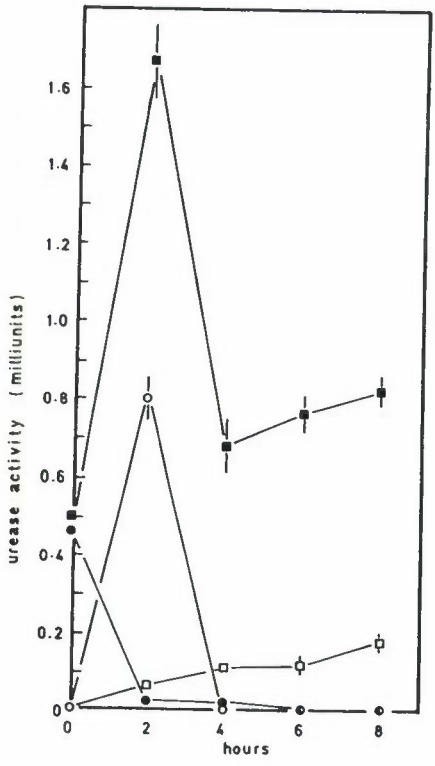

Figure 2. Urease produced or secreted by dry or rewetted thalli of *X. parietina* collected from *P. nigra* and floated on 40 mM urea for 8 hr. Symbols as in Fig. 1. Values are the mean of three replicates. Vertical bars give standard error where larger than the symbols.

be established between the relative water content of the thalli after 15 min of rehydration prior to incubation in urea-containing media and secretion of urease (after 2 hr) for the different ecotypes of *X. parietina*. The lichen growing on *P. nigra* needed the lowest amount of water to achieve thallus saturation and secreted a low amount of urease. In contrast, the saxicolous ecotype had the highest saturated water content and secreted much more active urease (Table 1).

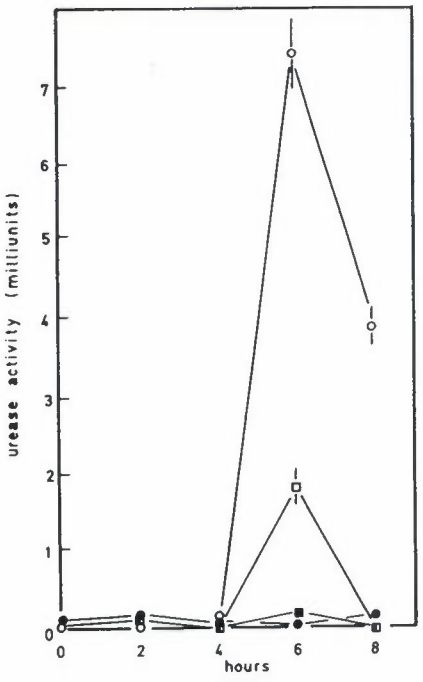

Figure 3. Urease produced or secreted by dry or rewetted thalli of *X. parietina* collected from basalt and floated on 40 mM urea of 8 hr. Symbols as in Fig. 1. Values are the mean of three replicates. Vertical bars give standard error where larger than the symbols.

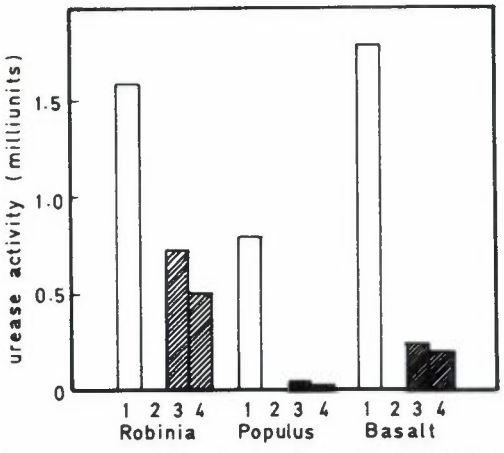

Figure 4. Urease secreted (empty bars) or retained (dashed bars) by *X. parietina* thalli incubated on 40 mM urea (1 and 3) or on 40 mM urea + 10% PEG (2 and 4) for the time period in which maximal secretion occurred.

Table 1. Relationship between thallus water content and urease secretion for different ecotypes of *Xanthoria parietina**

Lichen on	Dry weight (g)	Fresh weight (g)	FW-DW/DW (y)**	FW-DW/FW (z)***	Secreted urease activity (milli-units) (x)****
P. nigra	0.3956	1.1584	1.93	0.66	0.81
P. pseudo-acacia	0.2204	0.7647	2.45	0.71	2.81
Basalt	0.2909	1.5350	4.27	0.81	7.40

* Fresh weight was measured after 15 min rehydration with distilled water. Thalli from *P. nigra* and *R. pseudoacacia* were incubated for 2 hr on urea whereas those from basalt were incubated for 6 hr. Samples were used in triplicate.

** Relationship between FW-DW/DW and secreted activity is $y = 0.36x + 1.55; r^2 = 0.99$.

*** Relationship between FW-DW/FW and secreted activity is $z = 0.22x + 0.64; r^2 = 1.00$.

**** A milliunit was defined as 1.0 nmol of ammonia produced *per* mg protein *per* min

4. Discussion

Two different hypotheses have been used to explain enzyme secretion by lichen thalli. According to the first one, enzyme secretion depends on an exogenous source of organic nutrients which occurs on the natural substrate (Legaz et al., 1986), such as urea from animal excrements in the soil (Galinou, 1954; Masse, 1969), or translocated in the phloem from leaves of phytophores (Ludders and Bunemann, 1969). Those would be extracellularly hydrolyzed by urease secreted from coprophylous or epiphytic lichens, respectively, and the ammonia produced then taken up by the thalli. This hypothesis is supported by the fact that all the epiphytic or terricolous species growing on organic soils are able to secrete urease, such as *E. prunastri* (Blanco et al., 1984), *Pseudevernia furfuracea* (Vicente and Legaz, 1988), *Parmelia roystonea* (Xavier Filho and Vicente, 1978) or *Caloplaca regalis* (Legaz et al, 1986).

The second hypothesis suggests the possibility of protein translation between symbionts within the thallus (Vicente and Perez-Urria, 1989). In that case, enzyme produced by highly saturated thalli could be release to the media during translocation through the intercellular spaces open to the surrounding (Perea-Urria et al., 1989). The low level of urease secretion from *Cladonia verticillaris* growing on sandy soils (Vicente and Xavier Filho, 1979), and the

high level of urease secretion from *C. stellaris* growing on mineral soils (Perez-Urria et al., 1989) or *X. parietina* growing on granite (Vicente and Legaz, 1988) support this hypothesis. As it has been noted above, *C. rangiferina* has a low thallus saturation water content and, consequently, does not secrete urease to the medium.

In the present paper, it has been shown that both epiphytic and saxicolous ecotypes of *X. parietina* are able to secrete urease to urea-containing media. Rehydrated thallus samples secreted more urease to the media than that released from dry thalli which had not been previously rewetted (Figs. 1–3). In addition, secretion was completely abolished by adding 10% PEG to the incubation media, since PEG impedes the uptake of water by the cells. However, this treatment did not significantly affect urease retention by thalli.

A linear relationship between secreted urease activity and relative water content is established for the three specimens used here. This was found whether relative water content was expressed *per* gram dry weight (Green and Snelgar, 1982) or *per* gram fresh weight (Vicente and Velasco, 1986). This provides further support for the conclusion that urease secretion is a consequence of the movement of the enzyme inside the thallus (Vicente and Legaz, 1988).

Acknowledgements

This work was supported by a grant from the Direccion General de Investigacion Cientifica y Tecnologica (Spain) PB87 0081.

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