PROCEEDINGS OF THE FOURTH SYMPOSIUM ON THE NATURAL HISTORY OF THE BAHAMAS

Edited by
W. Hardy Eshbaugh

Conference Organizer
Donald T. Gerace

Bahamian Field Station, Ltd.
San Salvador, Bahamas
1992

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Printed in USA by Don Heuer

ISBN 0-935909-41-9

DEAMINATION AND GLUCOSE REPRESSION OF SEAGRASS RHIZOPLANE ISOLATES

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ABSTRACT

Seagrasses growing in oligotrophic, marine waters and carbonate sediments obtain much of their mineral nutrition from microbial transformations. Nitrogen transformations, by associated microflora, are particularly important in these nitrogen poor environments. Nitrogen fixation (conversion of diatomic, elemental nitrogen to the ammonium ion) accounts for a large part of the plants' requirements, but recycling of organic nitrogenous exudates and detritus is also believed to be a critical process in maintaining healthy seagrass meadows. The purpose of the work described in this paper was to compare glutamate deamination rates among root-rhizome segments and rhizoplane bacterial strains from three species of seagrasses growing near San Salvador, Bahamas. Results indicated that deamination rates among bacterial isolates varied considerably and may not reflect rates of the overall population. Overall deamination rates were higher for *Halodule*, followed by *Syringodium*, then *Thalassia* root segments. Glucose depressed deamination of all root segments except for *Halodule*. Recycling of nitrogen via deamination may be more important for the seagrass *Halodule* and *Syringodium* than for *Thalassia* in tropical, oligotrophic, carbonate environments.

INTRODUCTION

In the estuarine environment, seagrass ecosystems are among the most productive and dynamic. Large amounts of organic matter are deposited into the coastal sediments each year by the growth of extensive root-rhizome systems and incorporation of abscised, often epiphytized leaves (Thayer, Kenworthy & Fonseca, 1984). Many seagrass meadows maintain very dense stands of living biomass, suggesting a rather large requirement for mineral nutrients. Among these nutrients, available nitrogen has been of interest to a number of seagrass researchers for some time (Smith, 1987). Nitrogen fixation (the reduction of elemental diatomic nitrogen to the ammonium ion) has been shown to occur in both the phyllosphere and the rhizosphere of seagrasses (Capone, 1983; Smith & Hayasaka, 1982a), but rates do not appear to account for all of the nitrogen required to support many of the vast seagrass meadows (Iizumi, Hattori & McRoy, 1982).

Another mechanism of providing nitrogen to seagrasses is recycling of organic nitrogenous compounds. Both nitrogen fixation and recycling appear to be a function of the microflora associated with the plants (Boon, Moriarty & Saffigna, 1986). The major recycling process involves deamination of amino acid residues from either detrital material or root exudates (Smith, Hayasaka & Thayer, 1984).

The purpose of this study was to compare deamination rates of seagrass root segments to determine the relative importance of this process among seagrass species. In addition, deamination rates of isolated rhizoplane bacterial strains were measured to determine if certain strains may be more significant with respect to deamination than

others. The role of glucose as a modulator of deamination was also investigated.

MATERIALS AND METHODS

Core samples were collected from Graham's Harbor in San Salvador, Bahamas. Root-rhizome segments were extruded aseptically and either plated onto GASW (glycerol artificial seawater) medium (Smith & Hayasaka, 1982b) or placed into 60mL serum bottles. Root segments were assayed at the time of inoculation and at 24 h intervals for ammonium, for six days. The change in ammonium concentration per gram dry weight root tissue (where appropriate) was then calculated using a standard curve (Strickland & Parsons, 1972). A series of serum bottles for each root- rhizome or strain experiment contained the following: 30mL 3.2% Rila salts; 30mL Rila salts + 1% glucose; 30mL Rila salts + 1% glutamate; and 30mL Rila salts + 1% glucose + 1% glutamate. All assays were done in triplicate. Isolated strains were suspended in sterile Rila salts from GASW streak plates. The ammonium concentration was determined using the Solorzono method (Strickland & Parsons, 1972).

The change in ammonium concentration over time was then calculated and the results expressed as mM of ammonium produced per day per gram (dry weight) of root-rhizome tissue or mM ammonium produced per day (for pure cultures). Negative values indicate net ammonium consumption within the experimental vials. Patterns of ammonium evolution and uptake produced by the bacterial isolates were compared with their corresponding root-rhizome segments to determine the possible importance of strains *in situ* with respect to the overall deamination process.

Bacterial strains were also assayed for the ability to use glutamate and the deamination product, alpha-ketoglutarate, as sole carbon sources by inoculation onto Biolog GN (TM) plates.

RESULTS

Vials containing root-rhizome segments without added amendments (Rila) all showed a net uptake of ammonium (Table 1). Uptake rates were greatest for *Halodule* segments, followed by *Syringodium* then *Thalassia*. The addition of glucose reduced the rate of uptake for all the species. The addition of glutamate resulted in net production of ammonium for all seagrass root-rhizome segments, but the rate of production for *Halodule* segments was twice the rate of *Syringodium* and four times the rate for *Thalassia*. The addition of glucose to glutamate amended vials depressed the rate of ammonium production by approximately 2.5 times for *Thalassia* and *Syringodium*. *Halodule* segments, however, showed an increase in production by about 1.7 times when exposed to glucose plus glutamate.

Deamination rates of bacterial isolates varied considerably (Table 2). A comparison of patterns of ammonium production/uptake rates among specific isolates and their respective seagrass species segments showed a similar pattern for some isolates but not for others. Among the *Halodule* strains, SSHC3 and SSHC10 were more similar than SSHC8. SSHC3 Rila vials, however, showed net ammonium production and deamination was glucose depressed. SSHC10 did not show significant deamination rates with glutamate alone. Among the four *Syringodium* isolates, only SSFB7 showed a pattern similar to root-rhizome segments and significant deamination rates. The only *Thalassia* isolate did not show a pattern similar to root segments.

Table 3 compares the ability of specific strains to use glutamate and alpha-ketoglutarate as sole carbon sources with glutamate deamination rates. In general, strains that could use glutamate as a sole carbon source had relatively low deamination rates. The highest deamination rates were observed with strains that could not use glutamate but could use alpha-ketoglutarate as a sole carbon source (strains 8210 and 1942). One strain (8231) could use neither glutamate nor alpha- ketoglutarate as a sole carbon source, but had a relatively high deamination rate (402 uM h-1).

DISCUSSION

Both ammonium uptake and deamination rates were higher for *Halodule*, followed by *Syringodium* over *Thalassia* root-rhizome segments (Table 1). This may indicate overall higher microbial activity associated with the rhizoplane of these respective seagrass species. This, in turn, may

Table 1. Ammonium evolution/uptake (mM NH4 produced /day/mg dry wt.) of seagrass root- rhizome segments.

Seagrass	Rila	Glucose	Glutamate	Glucose+Glutamate
Halodule	-3.28(o.57)	-0.69(0.53)	4.22(1.42)	7.17(1.43)
Syringodium	-1.26(1.16)	0.11(0.09)	2.68(1.62)	0.93(0.38)
Thalassia	-0.25(0.50)	-0.19(0.05)	0.84(0.45)	0.38(0.26)

(Standard Error)

Table 2. Ammonium evolution/uptake (mM NH4 produced/day) by seagrass rhizoplane bacterial isolates.

Isolate	Rila	Glucose	Glutamate	Glucose+ Glutamate
SSHC3	0.74(0.56)	0.79(0.12)	0.50(0.22)	0.69(0.10)
SSHC10	-2.47(0.40)	0.57(0.13)	0.33(0.26)	1.51(7.45)
SSHC8	-0.59(0.22)	-6.76(0.20)	0.17(3.40)	0.83(0.18)
SSSFB2	-0.44(0.86)	9.13(6.89)	-0.24(0.27)	-0.50(0.73)
SSSFB3	2.71(1.15)	-4.93(0.05)	1.29(1.39)	0.92(0.88)
SSSFB4	-1.07(1.22)	-0.36(0.48)	-0.62(0.47)	1.31(1.04)
SSSFB7	-1.59(0.14)	-0.30(0.16)	5.13(0.21)	1.65(0.68)
SSTFB9	0.02(0.29)	-0.46(9.60)	-0.50(0.36)	0.37(6.27)

(Standard Error)
SSH= *Halodule* isolate;SSS=*Syringodium* isolate; SST= *Thalassia* isolate

Table 3. Comparison of Deamination Rates of Glutamic Acid with the Ability to Use Glutamate and alpha-ketoglutarate as a Sole Carbon Source Among Selected Isolates.

Source	Strain	Use as a Sole Carbon Source: Glutamate	alpha-ketoglutarate	Deamination Rate(umoles/h)
Halodule	8111	+	-	239
	8210	-	+	542
	8221	+	-	295
	8231	-	-	402
	8411	+	-	284
Syringodium	19211	+	+	184
	19222	+	+	158
	19411	+	+	332
	1942	-	+	479
	19611	+	+	184
Thalassia	2041	+	-	211
	2042	+	+	167
	20621	+	+	390
	20631	+	-	320

reflect the fact that in San Salvador, *Halodule* is smaller than *Syringodium*, which is smaller than *Thalassia*. The smaller the root-rhizome system, the greater the surface area per gram of tissue and root surface available for microbial colonization. It is interesting that glucose increased deamination rates of *Halodule* segments but depressed rates of the other two seagrasses. It may be that the greater microbial population was carbon limited which may have counteracted any normal glucose repression of metabolic activity.

Some bacterial isolates reflected deamination patterns similar to their source of isolation and some did not (Table 1 and 2). This is not surprising for two reasons. First, only eight strains were tested and only one strain from *Thalassia*. There could be hundreds of different strains associated with each seagrass rhizoplane, only part of which may be culturable using the methods and medium described in this report. Secondly, while pure culture studies are indicative of environmental potential, they seldom represent actual microbial activity. Co-metabolism and nutritional cross-feeding are common occurrences in natural environments. Nevertheless, one isolate, SSFB7, did show a pattern similar to the seagrass species from which it was isolated. This isolate will be studied in more detail in future studies.

Deamination rates were generally higher for strains that could use alpha-ketoglutarate but not glutamate as a sole carbon source (Table 3). This indicates that deamination may be extracelluar among these strains and that a different energy source may be required to initiate the process. Strain 8231 could deaminate glutamate but could not use either compound as a sole carbon source. Those strains that could use glutamate as a sole carbon source probably used this compound as a nitrogen source also, accounting for low turnover rates.

Studies are continuing on additional isolates. These studies should indicate if the pure culture approach will point out specific isolates which play a major role in recycling nitrogen via deamination.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation through the University of South Carolina at Aiken's Ruth Patrick Science Education Center and the Bahamian Field Station. The authors thank Drs. Jeff Priest, Don and Kathy Gerace, James Yates and Bill Pirkle for helpful advice and support.This is Contribution No.866 of the Belle W. Baruch Institute of Marine Biology and Coastal Research.

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