J. Indian Inst. Sci., Sept.–Oct. 1990, **70**, 447–450.

Short Communication

Ammonia leaching by resting cells of a photosynthetic purple non-sulfur bacterium, *Rhodobacter sphaeroides* O.U. 001*

K. SASIKALA AND CH. V. RAMANA
Microbial Physiology Lab, Department of Botany, Osmania University, Hyderabad 500 007, A.P. India.

Received on August 10, 1989; Revised on January 29, 1990.

Abstract

Resting cells of *Rhodobacter sphaeroides* O.U 001 were tested for their ability to leach out ammonia in the absence of combined nitrogen sources in the assay medium, with or without malate under various gas phases. A maximum of 159 μM ammonia (mg dry wt)$^{-1}$ was produced in 72 hours under air as gas phase in the absence of malate.

Key words: *Rhodobacter sphaeroides* O.U. 001, resting cells, ammonia leaching, gas phases.

1. Introduction

Ammonia represents the primary source of nitrogen fertilizers presently used in agriculture. As it improves crop productivity its demand is increasing rapidly. Presently, ammonia is being produced commercially by Haber-Bosch process which is expensive and energy-intensive. Microbial ammonia production which occurs at ambient temperatures and pressures is receiving a great deal of attention in recent years and is expected to become an alternative process for commercial production soon.

A number of cyanobacteria which fix nitrogen are known to leach out ammonia when grown on nitrogen in the presence of MSX (L-methionine-D, L-sulfoximine), an analogue of glutamine[1]. Ammonia leaching was observed during growth on amino acids in the photosynthetic bacteria, *Rhodopseudomonas capsulata*[2] and *Rhodobacter sulfidophilus*[3]. Weare and Shanmugam[4] reported ammonia leaching by cell suspensions of nitrogen-fixing *Rhodospirillum rubrum* in the presence of MSX. To the best of our knowledge, there are no reports of ammonia leaching by resting cells of photosynthetic bacteria other than mutant strains[5,6] due to fixation of nitrogen in the absence of MSX. In the present investigation, resting cells of *Rhodobacter sphaeroides* O.U. 001 were tested for ammonia leaching in the absence of MSX under various gas phases.

*Dedicated to the loving memory of our research supervisors, Drs (Mrs) B. Renuka Rao and M. Vinayakumar.

2. Materials and methods

2.1. *Organism and growth conditions*

A purple non-sulfur photosynthetic bacterium *Rhodobacter sphaeroides* O.U. 001 (ATCC 49419; DSM 5864), isolated locally[7], was used in the present investigation. The organism was grown anaerobically in light (4,000 lux) at a temperature of $30 \pm 2°C$, on the medium described by Biebl and Pfennig[8], with malate (30 mM) and sodium glutamate (10 mM) as carbon and nitrogen sources, respectively (pH 7·0).

2.2. *Preparation of resting cell suspension*

Log-phase cells were harvested by centrifugation and washed repeatedly with saline (0·3% w/v) till no traces of ammonia were detectable in the supernatant. The pellet was suspended in the basal medium (pH 6.9, devoid of nitrogen source) without and with malate (1 g/l). The cell suspension was distributed (2 ml in each) into 15 ml capacity test tubes, sealed with suba seals, evacuated and replaced with the required gas phase (gases used in the experimentation were of ultrapure grade).

2.3. *Cell mass and ammonia determination*

The cell mass was determined by measuring the absorption of a bacterial suspension at 660 nm. The bacterial dry weight was calculated with the following empirical relation determined for this organism: Absorption at 660 nm of $0·1 = 0·37$ mg dry wt ml^{-1}. Ammonia was estimated in the culture supernatant by the method of Solorzano[9].

3. Results and discussion

Photoproduction of ammonia in the presence of MSX is well documented among the members of cyanobacteria and photosynthetic bacteria[10]. Our results (Table I) show that ammonia leaching can also take place in the absence of MSX.

Ammonia leaching is found both in the presence and in the absence of malate in the medium. However, in the absence of malate, it was observed under all gas phases tested in contrast to the presence of malate where ammonia leaching was observed only in the presence of dinitrogen in the gas phase.

Production of ammonia when incubated without malate even in the absence of dinitrogen must be from the intracellular reserves. The difference between ammonia produced in the presence and in the absence of dinitrogen actually gives ammonia leaching due to dinitrogen fixation. The reason for the non-production of ammonia in the absence of dinitrogen when malate was present in the medium might be the utilization of intra-cellular nitrogen reserves of the bacterium for growth which was observed only in the presence of malate (Table I). Similarly, in the presence of $Ar + 10\%$ N_2, the fixed nitrogen must have been utilized for growth which was more than that in the presence of argon alone.

Table I
Photoproduction of ammonia by resting cells of *R. sphaeroides* O.U. 001 under various gas phases

Gas phase during assay	μM ammonia formed (mg dry wt)$^{-1}$		Increase in biomass* (mg dry wt/ml)	
	with malate	without malate	with malate	without malate
Air	98.0	159.4	1.2	0.03
Ar	0.0	61.4	0.38	0.03
N_2	4.6	143.8	1.1	0.05
H_2	0.0	52.1	0 58	0 03
Ar + 10% H_2	0.0	16.2	0 4	0.04
Ar + 10% N_2	0.0	52.4	0 7	0.05
Ar + 10% N_2 + 10% H_2	9.6	42.7	0.67	0.07
N_2 + 10% H_2	49.1	108.2	1 0	0.03

Results expressed are average values of two independent experiments done in triplicate. Temperatures, pH and light intensity of the assay were 30 + 2 C, 6.9 and 4,000 lux, respectively. Ammonia was estimated after 72 hours of incubation.
*Initial biomass of assay was 1.6 mg dry wt/ml.

The interrelationship between nitrogen fixation and hydrogen metabolism in micro-organisms is well documented[11–13]. Mortenson[13] reported that dihydrogen inhibits nitrogen fixation and hence it was of interest to see the effect of dihydrogen on the ammonia leaching activity of *R. sphaeroides* O.U. 001 which could both consume and evolve hydrogen (unpublished results). The investigation revealed a decrease in ammonia leaching activity in the presence of hydrogen proving that in *R. sphaeroides* O.U. 001 also nitrogen fixation is inhibited by dihydrogen.

Ammonia leaching was maximum in air as the atmosphere (semiaerobic) in the absence of malate which is the most ideal condition for practical exploitation. The strain is also capable of growing in the absence of any combined nitrogen source aerobically by fixing atmospheric nitrogen (unpublished results). The use of dinitrogen-grown cells for ammonia production is more practical and is presently under study.

Acknowledgements

This work is supported by a research grant from the Department of Non-Conventional Energy Sources (DNES), New Delhi, to late Drs (Mrs) B. Renuka Rao and M. Vinayakumar. The authors are grateful to Dr M. Subrahmanyam, RRL, Hyderabad for valuable suggestions and to Prof. C. G. K. Ramanujam, Head, Department of Botany, and Prof. H. Polasa, Head, Department of Microbiology, for constant encouragement and support. KS thanks the UGC, New Delhi, for the award of a research fellowship.

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