

How Flagella Expression May be Regulated by the Carbon and Energy Source?

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Abstract

Bradyrhizobium diazoefficiens has two flagellar systems: a subpolar, constitutive system and a lateral, inducible system. Contrary to other bacterial species, the lateral system is induced in liquid medium in response to the carbon and energy source. Since both flagella are moved by the proton-motive force, a relationship between the energy status of the cell and the signal that triggers lateral flagella expression might exist. Here I discuss how this relationship may control the induction of the lateral flagellar system, and its implications for improvement of *Bradyrhizobium*-based inoculants for soybean plants.

Keywords: Flagella; *Bradyrhizobium diazoefficiens*; soybean; symbiosis

Introduction

Bradyrhizobium is a soil bacterial genus that includes several species of importance due to their use as biofertilizers for soybean crops worldwide. Among these species, *B. diazoefficiens* and *B. japonicum* stand out as being the most widely employed.

These bacteria fix atmospheric N_2 in symbiosis with soybean plants, by reducing N_2 to NH_4^+ in a reaction catalyzed by bradyrhizobial nitrogenase. The NH_4^+ thus produced is supplied as N-source to the plant, in such a rate that all its N-needs may be satisfied. To this end, the *Bradyrhizobium* bacteria are inoculated to soybean seeds before sowing with the aim that these bacteria infect the roots and develop the N_2 -fixing symbiosis inside them. Since the symbiosis only occurs in specialized organs in the roots, seed-inoculated bacteria need moving from the site of inoculation to the sites of infection, and this movement has to occur in the soil, a porous and tortuous medium, which not always contain water enough for bacterial swimming. Therefore, the study of bradyrhizobial motility is of prime importance to improve this ecologically sustainable technology for soybean fertilization.

B. diazoefficiens USDA 110 is the type strain of this species [1], and its genome was completely sequenced in 2002 [2]. In addition, this strain is the most studied biochemically, genetically, and physiologically, as well as in the relevant aspects of its symbiosis with soybean plants. The motility of this strain was characterized in two kinds of bacterial movement: swimming [3-

5] and swarming [6]. Both movements are propelled by flagella, while other movements such as e.g. twitching or gliding were not reported. Although there exists evidence of the presence of pili, which constitute the device required for twitching, these appendages were studied only for their role in cell adhesion [7].

Remarkably, *B. diazoefficiens* USDA 110 possesses two entirely different flagellar systems: a subpolar system and a lateral system [4-6]. These systems are encoded in different gene clusters, and it seems that each one possesses its own regulatory system for the control of its expression. Indeed, the expression of the subpolar system seems constitutive in planktonic cells, while the lateral system is inducible [5,6]. Induction of the lateral system was observed as obeying to the carbon and energy source of the growth medium: when the sole carbon and energy source is arabinose, the lateral system is expressed, but it is inhibited when the only carbon and energy source is mannitol [6]. Although several other bacterial species are known to possess inducible lateral flagellar systems, in general the inducer is the medium viscosity or the proximity of a surface, which are perceived by the polar/subpolar flagellar system that under these circumstances behaves as a mechanosensor [8]. However, *B. diazoefficiens* is the only example known where the lateral flagellar system is induced by the carbon and energy source, and therefore the identity of the signal transducer is a complete enigma.

Structure and Rotation of Flagella

The flagellum consists in three main structures: the flagellar filament, the hook, and the basal body, which contains the motor [9]. Although the flagellar motor was not studied in *B. diazoefficiens*, there exists a great deal of knowledge in other species, in particular *Escherichia coli* [10]. The flagellar motor is embedded in the inner cell membrane and has two main rings: a stator formed mainly by the proteins MotA and MotB, and a rotor to which the rest of the flagellum is attached. The rotor is composed mainly by the proteins FliF, FliG, FliM and FliN, which play a central role in flagellar rotation. The Protonmotive Force (PMF) that is generated during cell respiration is the energy source for flagellar motor rotation. Protons pass from the periplasmic space towards the cytoplasm through a channel formed between FliG and MotA/MotB. Recent studies indicate that the rotor contains a ring of 26 FliG subunits faced against an external ring of MotA/

MotB subunits in such a way that an array of negatively charged amino acids in MotA, MotB and FliG interact with the protons that traverse the channel, producing changes in the conformations of the ring proteins, allowing movement of the rotor. These amino acids are disposed in such a way that the passage of protons from the periplasm to the cytoplasm moves the rotor against the stator thus producing a torque sufficient to impulse the cell body into the liquid environment [10-12].

In general, bacterial species that possess two flagellar systems use the PMF to move one of the flagella, while the other is moved by a Na⁺ gradient that is also formed between the periplasm and the cytoplasm. However, in *B. diazoefficiens* both flagellar systems seem to be moved by the PMF [4], thus sharing this energy source with the synthesis of ATP. Ultimately, the PMF comes from the oxidation of organic carbon and energy sources, and therefore the availability of energy in the cell might be in connection with the regulation of the lateral flagellum expression by the carbon and energy source.

Catabolism of Arabinose and Mannitol

L-arabinose is catabolized through a pathway that resembles the Entner-Doudoroff (ED) pathway for catabolism of hexoses [13,14] (Figure 1). The first step is oxidation of L-arabinose to

L-arabonate with formation of one mole of NADH per mole of L-arabinose. Then, L-2-keto 3-deoxy arabonate is formed, which splits in pyruvate and glycolaldehyde in a reaction catalyzed by an aldolase. The pyruvate continues through TCA cycle, while glycolaldehyde is oxidized in two sequential steps of NADH-producing reactions to glyoxylate, which is finally converted to formate and oxidized to CO₂ with production of another NADH [13-15]. Hence, a total of eight NADH moles plus one FADH₂ mole are produced per mole of arabinose completely oxidized to CO₂.

D-mannitol is oxidized to fructose with production of one NADH mole per mole of mannitol in a reaction catalyzed by mannitol dehydrogenase [16]. Then, the fructose produced may be catabolized by the ED or the Emden-Meyerhof-Parnas (EMP) pathways [17], or the Pentose-Phosphate (PP) pathway [18] with production of 10 additional NADH moles plus two FADH₂ moles (ED, EMP pathways), or 11 additional NADH moles plus two FADH₂ moles (PP pathway) in the complete oxidation of one mole of fructose to CO₂. In Figure 1, the yields of NADH plus FADH₂ with arabinose are compared with those with mannitol catabolized by the ED pathway as an example.

Thus, assuming that 10 H⁺ moles are passed to the periplasm per mole of NADH oxidized and 6 H⁺ moles are passed per mole of

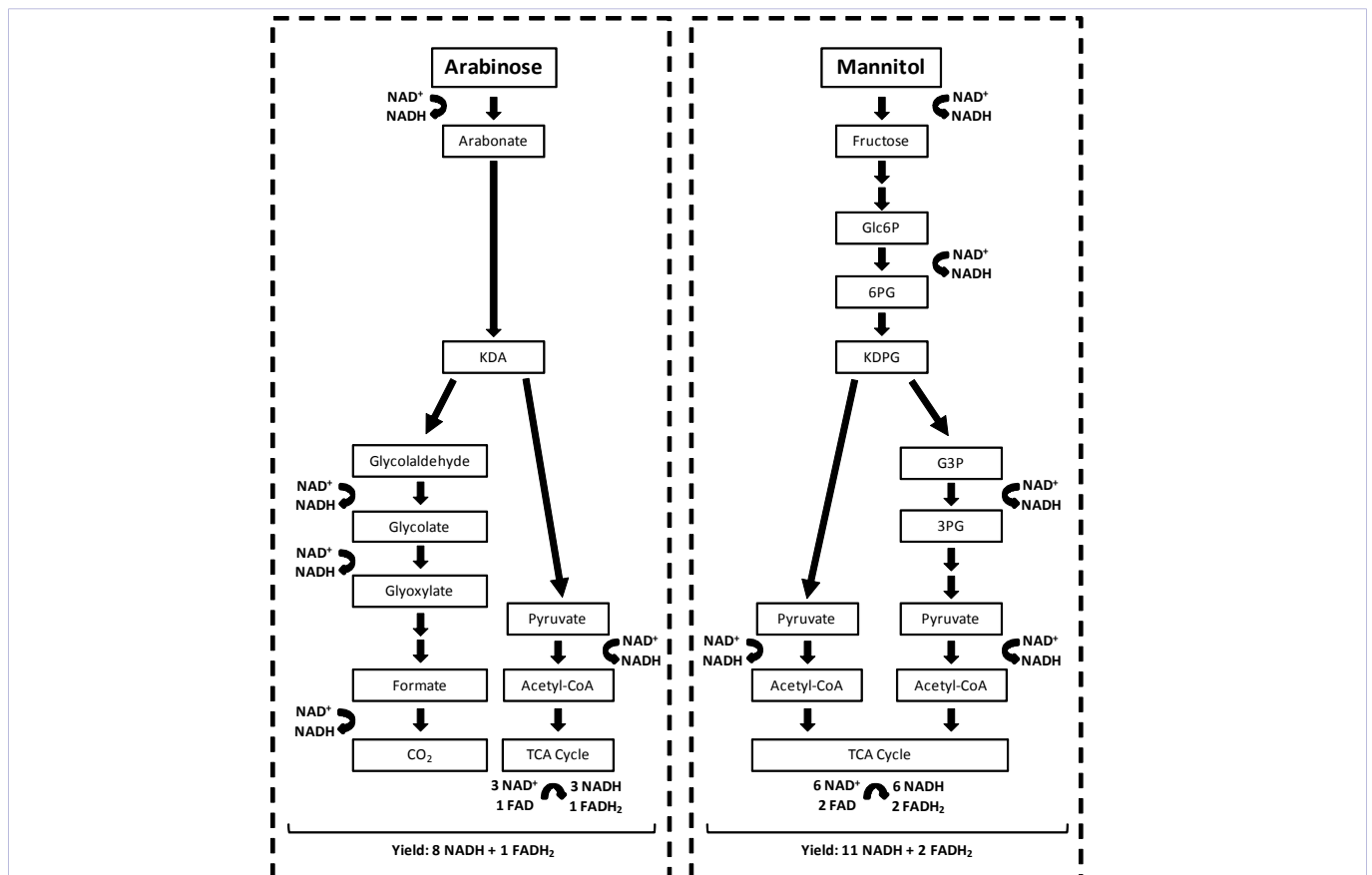


Figure 1: Comparison of the catabolism of arabinose (left) and mannitol (through the ED pathway, right) in *Bradyrhizobium diazoefficiens* with emphasis in the reactions where reducing power is generated. KDA: 2-keto-3-deoxyarabonate; Glc6P: glucose-6-phosphate; 6PG: 6- phosphogluconate; KDPG: 2-keto-3-deoxyphosphogluconate; G3P: glyceraldehyde-3-phosphate; 3PG: 3-phosphoglycerate.

FADH₂ oxidized, it results that 86 H⁺ moles are passed per mole of arabinose or 122-132 H⁺ moles are passed per mole of mannitol completely oxidized to CO₂. Despite this higher yield of mannitol with respect to arabinose, O₂ consumption rate was reported many years ago as roughly twice higher with arabinose than with mannitol [19], but these observations should be repeated with *B. diazoefficiens* USDA 110, and modern culture conditions and analytic technologies. Anyway, these data indicate that although the molar yield of H⁺ is lower with arabinose than with mannitol, the net rate of H⁺ passage from cytoplasm to periplasm per mole of carbon source oxidized still might be around 50% higher with arabinose. Since growth rates in minimal medium with arabinose or mannitol are similar [6], energy consumption rates for growth should also be similar, and according to the above estimates, a higher PMF may remain available for maintenance functions when arabinose is the carbon and energy source.

Perspectives

We could envisage that the cell senses the conditions in which PMF is sufficient for ATP synthesis and motion of both flagella systems at the same time and only if these conditions are met, lateral flagella expression is allowed. The conditions need not necessarily involve high viscosity of the medium because the induction of the lateral flagellar system by arabinose was observed in liquid medium. If arabinose is present in the root exudates near the infection sites [3], the expression of lateral flagella in response to this carbohydrate might be useful for the bacteria to stabilize their swimming direction towards such sites [20]. To respond to the cell energy status, the regulator(s) of lateral flagella expression should perform some measure of the PMF. There exist some ways of measuring PMF in connection with motility. For instance, a group of chemoreceptors specialized in sensing the energy status of the cell is known. These chemoreceptors bind FAD and are able to sense the redox state of the electron transport chain to elicit energy taxis, i.e. the orientation of the bacterial cell swimming towards an energy-rich environment [21]. Another candidate is the Phosphotransferase System (PTS), which also participates in chemotaxis [22]. Despite these systems being known as sensors of energy status in relation with motility, they do not display a clear relationship with the control of transcription or translation. Whether these systems, or a yet unknown signal transduction system, play a role in the control of lateral flagellar expression in response to the carbon and energy source is a research issue that might provide new knowledge about regulation of energy use in bacteria.

This issue is of special importance in the *Bradyrhizobium*-soybean symbiosis. For instance, in Argentina more than 20 million hectares are cultivated with soybean, and 94% of producers use *Bradyrhizobium*-based inoculants to achieve N-nutrition through biological N₂ fixation in their crops [23]. Motility of *Bradyrhizobium* bacteria in the soil is essential to achieve a successful symbiotic interaction [5] and therefore, understanding the control of motility and its stimulation by root-exuded compounds is one key for the development of improved inoculants for agriculture.

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