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Organic acid mediated repression of sugar utilization in rhizobia

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ABSTRACT

Rhizobia are a class of symbiotic diazotrophic bacteria which utilize C_4 acids in preference to sugars and the sugar utilization is repressed as long as C_4 acids are present. This can be manifested as a diauxie when rhizobia are grown in the presence of a sugar and a C_4 acid together. Succinate, a C_4 acid is known to repress utilization of sugars, sugar alcohols, hydrocarbons, etc by a mechanism termed as Succinate Mediated Catabolite Repression (SMCR). Mechanism of catabolite repression determines the hierarchy of carbon source utilization in bacteria. Though the mechanism of catabolite repression has been well studied in model organisms like *E. coli*, *B. subtilis* and *Pseudomonas* sp., mechanism of SMCR in rhizobia has not been well elucidated. C_4 acid uptake is important for effective symbioses while mutation in the sugar transport and utilization genes does not affect symbioses. Deletion of *hpr* and *sma0113* resulted in the partial relief of SMCR of utilization of galactosides like lactose, raffinose and maltose in the presence of succinate. However, no such regulators governing SMCR of glucoside utilization have been identified till date. Though rhizobia can utilize multitude of sugars, high affinity transporters for many sugars are yet to be identified. Identifying high affinity sugar transporters and studying the mechanism of catabolite repression in rhizobia is important to understand the level of regulation of SMCR and the key regulators involved in SMCR.

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1. Introduction

Rhizobia are symbiotic diazotrophic bacteria that live in association with the legumes (Beringer et al., 1979). Currently, there are seven phylogenetically distinct genera of rhizobia which comprise of *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Ensifer*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* falling into two main classes of α proteobacteria and β proteobacteria. An important feature of rhizobia is the presence of large extrachromosomal DNA in the form of megaplasmids constituting major portion of the genome (Martinez et al., 1990; Martinez Romero, 1994). These plasmids

Abbreviations: ABC, ATP-binding cassette; N_2, nitrogen; DNP, 2,4-dinitrophenol; ED, Entner-Doudoroff; EMP, Embden Meyerhof Paranas; FBP, fructose bisphosphatase; FBPA, fructose bisphosphate aldolase; ICL, isocitrate lyase; KCN, potassium cyanide; PBP, periplasmic binding protein; PEPCK, phosphoenolpyruvate carboxykinase; PP, pentose phosphate; TRAP, tripartite; ATP, independent periplasmic; PTS, phosphotransferase system; SMCR, succinate mediated catabolite repression; TCA, tricarboxylic acid.

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may be pSymA, encoding genes important for symbiotic processes or pSymB which are not important for symbiosis. Both the types of plasmids are stably inherited from generation to generation (Mercado-Blanco and Toro, 1996). The *Rhizobium*-legume interaction is host specific wherein a rhizobial strain can associate with only a limited number of host plants (Spaink, 1994). During this *Rhizobium*- legume symbiosis, rhizobia enter the cells of the host plant and differentiates into N_2 fixing bacteroids (Oke and Long, 1999). Major factor limiting N_2 fixation by the *Rhizobium*-legume symbiosis is the carbohydrate supply (Hardy, 1977). Plant photosynthate (sucrose) produced in the shoot is converted to dicarboxylates like succinate, fumarate and malate (Day and Copeland, 1991) and is provided by the plant to the bacteroids as a source of energy for N_2 fixation. Bacteroids in turn provide fixed N_2 for plant uptake (Gordon et al., 1999; Lodwig et al., 2003). These dicarboxylates are oxidized to form ATP and reducing equivalents in the bacteroids (Day and Copeland, 1991; Stowers, 1985) which are then used for N_2 fixation. Studies indicate that this N_2 fixation by rhizobia is not only limited to the legumes but also extends to the cereals like rice, wheat, maize, etc (Cocking et al., 1994). Previous studies have shown symbiotic relationship of rhizobia with the non legumes like *Parasponia* sp. (Trinick, 1979). Rhizobia have also been isolated as natural endophytes from rice (Chi et al., 2005; Yanni et al., 1997), sweet corn, cotton (McInroy and Kloepper, 1995), maize, bean (Gutiérrez-Zamora and Martıínez-Romero, 2001), barley, wheat, canola (Lupwayi et al., 2004), etc. Further studies investigating the interaction of rhizobia with non legumes may enhance its use as a replacement to nitrogenous fertilizers, not only for the legumes but also for the non legumes.

In addition to providing fixed N_2 to the plants, bacteroids also function in amino acid cycling (Lodwig et al., 2003). In order to prevent the bacteroids from assimilating the ammonium produced by N_2 fixation, plants provide amino acids to the bacteroids. The ammonium produced via fixation is cycled back to the plants for their amino acid synthesis (Appels and Haaker, 1991; Rosendahl et al., 1992) which establishes a mutual dependence between the bacteroids and the plant; providing a selective pressure for the evolution of mutualism.

2. Carbon metabolism pathways in rhizobia

Though free-living rhizobia can grow on a variety of carbon sources like the sugars, amino acids, organic acids and aromatic compounds (Stowers, 1985), organic acids form the major energy source for the bacteroids (Finan et al., 1981; Gardiol et al., 1982; Glenn et al., 1984; Ronson and Primrose, 1979). Free living rhizobia can metabolize carbon compounds by variety of pathways like ED (Entner-Doudoroff), PP (pentose phosphate), Gluconeogenesis and TCA (Tricarboxylic acid) cycle (Fuhrer et al., 2005) while EMP (Embden Meyerhof Paranas) pathway is virtually absent (Ronson and Primrose, 1979). However, one report suggests the presence of both ED and EMP pathway (Mulongoy and Elkan, 1977) and absence of PP pathway in *B. japonicum* (Martinez de Drets and Arias, 1972). Based on their growth rates, rhizobia have been subdivided into slow growing (i.e *Bradyrhizobium* sp.) and fast growing (i.e *Sinorhizobium* sp. and *Rhizobium* sp.) (Stowers, 1985).

Complete PP pathway exists in fast growing rhizobia in contrast to the slow growing rhizobia (Martinez de Drets and Arias, 1972). Presence of transketolase, transaldolase (Cervenansky and Arias, 1984) and ribose 5-phosphate epimerase (Djordjevic, 2004) has been shown in *S. meliloti*. Gluconeogenesis in *R. leguminosarum* occurs through two derepressible enzymes, PEPCK (phosphoenolpyruvate carboxykinase) and FBPA (fructose bisphosphate aldolase) (Mckay et al., 1985) whereas in *S. meliloti*, it occurs through PEPCK (Dunn, 1998) and through the combined action of pyruvate orthophosphate dikinase and malic enzyme (Osteras et al., 1997). Substantial expression of ICL (isocitrate lyase) was seen in *S. meliloti* (Duncan and Fraenkel, 1979) and *Bradyrhizobium* (Mandal and Chakrabartty, 1993) when grown on acetate indicating the presence of glyoxylate pathway in rhizobia.

In rhizobia, ED pathway is the widely used pathway for sugar metabolism (Stowers, 1985) along with the participation of PP pathway (Romanov et al., 1994). TCA cycle and other anaplerotic enzymes are imperative for the normal growth of rhizobia in the free living state as well as in the bacteroids for energy generation from the dicarboxylates (Dunn, 1998). ED pathway is the exclusive pathway of glucose metabolism whereas the pentoses are synthesized by the PP pathway (Fuhrer et al., 2005). High activities of the key enzymes of ED and PP pathway have been shown in sucrose, fructose and glucose grown *R. tropici* CFN299 whereas when grown on C_4 acids, activities of the ED enzymes were only about 30–38% of that of sucrose grown cells. When grown on malate or glutamate, invertase activity was 3–4.5 folds less as compared to that in glucose but the levels of PEPCK and FBPA increased significantly (Romanov et al., 1994). 40–50% decreased activity of ED enzymes were obtained when cells were grown on succinate as compared to glucose in *R. meliloti* (Irigoyen et al., 1990) and *R. leguminosarum* (Glenn et al., 1984). Undetectable levels of the carbon metabolism enzymes were obtained in *Bradyrhizobium* strain 32H1, when grown in succinate (Stowers and Elkan, 1983). Activities of the key enzymes of PP and ED pathways had 3–18 folds higher activity in glucose as compared to succinate in *Rhizobium* sp. NGR234 (Saroso et al., 1986) and *R. meliloti* (Finan et al., 1988; Finan et al., 1991). High activities of PEPCK, FBPA and FBP (Fructose bisphosphatase) indicating the operation of gluconeogenesis was seen in *R. tropici* bacteroids (Romanov et al., 1994) and *R. leguminosarum* MNF3841 (Mckay et al., 1985). Mutants with defective dicarboxylic acid transport are generally incapable of N_2 fixation (Arwas et al., 1985; Finan et al., 1983; Glenn and Brevin, 1981; Ronson et al., 1981) whereas hexose uptake mutants can form effective nodules (Arias et al., 1982; Duncan, 1981; Ronson and Primrose, 1979; Stowers and Elkan, 1983) indicating that efficient hexose transport is not important for functional symbiosis. Effect of different carbon metabolism gene mutations on the ability of rhizobia to grow on different C sources as well as to induce effective symbiosis has been shortlisted in Table 1.

3. Sugar transport in rhizobia

Though several reports have emphasized on the hexose metabolism enzymes in rhizobia (Ronson and Primrose, 1979), very little is known about the rhizobial hexose transport systems. In rhizobia, the transport of organic acids (Finan et al.1981; McAllister and Lepo, 1983), sugars and sugar alcohols (Mulongoy and Elkan, 1978; Arias et al., 1982; De Vries et al., 1982; Glenn et al., 1984; Stowers and Elkan, 1983) proceeds by an active process. *Rhizobium* is different from the other gram negative bacteria like *E. coli*, *S. typhimurium* and *K. pneumoniae* as it does not transport carbon compounds by means of phosphoenolpyruvate phosphotransferase system (PTS) (De Vries et al., 1982; Glenn et al., 1984; Mulongoy and Elkan, 1978; Stowers and Elkan, 1983).

Though *S. meliloti* has a PTS system, it is incomplete as it lacks the transport related PTS proteins and the proteins present as a part of an incomplete PTS system are not involved with sugar transport (Pinedo and Gage, 2009). Only fructose uptake is known to use PTS system in *Azospirillum* sp. (Gupta and Ghosh, 1984). In *R. meliloti*, D-mannose enters the cell in a phosphorylated form actively through the mannose uptake system, inhibited by azide and 2,4 dinitrophenol (DNP) (Arias et al., 1982) whereas uptake of fructose by *R. leguminosarum* occurs in a nonphosphorylated form