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SHORT COMMUNICATION

Nod Bj-V ($C_{18:1}$, MeFuc) production by *Bradyrhizobium japonicum* (USDA110, 532C) at suboptimal growth temperatures

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Summary

Nod factors (Lipo-chitooligosaccharides, or LCOs) act as bacteria-to-plant signal molecules that modulate early events of the *Bradyrhizobium*–soybean symbiosis. It is known that low root zone temperature inhibits the early stages of this symbiosis; however, the effect of low soil temperature on bacteria-to-plant signaling is largely uninvestigated. We evaluated the effect of low growth temperatures on the production kinetics of Nod factor (LCO) by *B. japonicum*. Two strains of *B. japonicum*, 532C and USDA110, were tested for ability to synthesize Nod Bj-V ($C_{18:1}$, MeFuc) at three growth temperatures (15, 17 and 28 °C). The greatest amounts of the major Nod factor, Nod Bj-V ($C_{18:1}$, MeFuc), were produced at 28 °C for both strains. At 17 and 15 °C, the Nod factor production efficiency, per cell, of *B. japonicum* 532C and USDA110 was markedly decreased with the lowest Nod factor concentration per cell occurring at 15 °C. Strain 532C was more efficient at Nod factor production per cell than strain USDA 110 at all growth temperatures. The biological activity of the extracted Nod factor was unaffected by culture temperature. This study constitutes the first demonstration of reduced Nod factor production efficiency (per cell production) under reduced temperatures, suggesting another way that lower temperatures inhibit establishment of the soybean N_2 fixing symbiosis.

Abbreiviations: BM, Bergerson minimal media; CFU, colony forming units; HPLC, high pressure liquid chromatography; LCO, Lipo chitooligosaccharide; RZT, root zone temperature

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Introduction

Legume nodulation is a multi-step process requiring the exchange of specific signaling molecules between plant and bacterial symbiotic partners. The first step involves the secretion of phenolic compounds, mainly flavonoids, by the plants. These compounds activate the expression of *nod* genes in rhizobia, culminating in the biosynthesis of bacterial Nod factor (chemically these are lipo-chitooligosaccharides—LCOs) (Schultze and Kondorosi, 1998). All Nod factors have a similar composition: a chitooligosaccharide (a linear chain of 3–5 β-1,4-linked *N*-acetylglucosamines) linked to an acyl side chain (Mergaert et al., 1997). In addition, there can be modifications to both reducing and non-reducing ends of the chitin backbone. For example, *Bradyrhizobium japonicum*, specificity is determined, in part, by the presence of a 2-*O*-methylfucose residue attached to the terminal reducing sugar. Nod factors are able to invoke root hair deformations in host plant roots (Sanjuan et al., 1992). In response to plant secreted flavonoid signals, the appropriate rhizobia multiply in the rhizosphere, followed by chemotaxis toward plant exudates, adhesion of rhizobia to root hairs and infection leading to the formation of nitrogen fixing nodules (Hungria and Stacey, 1997; Broughton et al., 2003). *B. japonicum* USDA110 produces one major Nod factor (Sanjuan et al., 1992) and several less abundant LCOs (Spaink et al., 1992). In a recent study Souleimanov et al. (2002) extracted the most abundant Nod factor from *B. japonicum* 532C and it was shown to be Nod Bj-V ($C_{18:1}$, MeFuc).

The symbiotic association between *B. japoniucm* and soybean is affected by a number of environmental factors, including low root zone temperature (RZT), which can limit soybean production in short season areas such as Eastern Canada (Whigham and Minor, 1978). Soybean requires a 25–30 °C RZT for optimal growth and N_2 fixation activity (Jones and Tisdale, 1921). Besides its effect on growth and nodule function, low RZTs also inhibit inter-organismal signaling between the two symbiotic partners. It is known that low RZTs inhibit the biosynthesis and rhizosecretion of plant-to-bacteria signal molecules (for example genistein) from soybean roots that are necessary for induction of the *nod* genes of *B. japonicum* (Zhang and Smith, 1996). Low RZTs also inhibit the induction of bacterial nodulation genes that are necessary for the biosynthesis of bacteria-to-plant signaling molecules, the Nod factors (Zhang et al., 1996). McKay and Djordjevic (1993) reported that Nod factor production/excretion by *Rhizobium leguminosarum* bv. *trifolii* was disrupted at low incubation temperatures and this was related to effects on transcription, translation, or post-translation modification events involved in Nod factor production.

Taken together, data of previous studies show that low RZTs suppress signaling between symbiotic partners. Low temperature also affects the growth of rhizobial cells and Nod factor production. However, the effect of low RZTs on the efficiency of signaling from the bacteria (LCOs) is largely uninvestigated. Thus, the objective of this study is to evaluate the effects of low growth temperatures on the production of the major Nod factor [Nod Bj-V ($C_{18:1}$, MeFuc)] produced by cells of two *B. japonicum* strains (532C and USDA110).

Materials and methods

Bacterial strains and culture conditions

B. japonicum strains 532C and USDA110 were obtained from Liphatech Inc. (Milwaukee, WI, USA). These strains were selected because strain 532C is currently used in most Canadian and US inoculants, and strain USDA110 was used in US inoculants. *B. japonicum* cultures were grown in Bergersen Minimal (BM) medium as described by Spaink et al. (1992).

Growth temperature and production of Nod Bj-V ($C_{18:1}$, MeFuc)

This experiment was conducted to study the effect of three growth temperatures (15, 17, and 28 °C) on Nod factor production. The two strains tested were *B. japonicum* 532C and USDA110. The treatments consisted of factorial combinations of the two strains and three growth temperatures. There were five replicates of each treatment.

The cultures were grown in 200 mL of BM medium at 28 °C, originating from starter cultures of the same OD (A_{620} 0.08) values for the two strains tested. On the sixth day of culture, five replicates of each strain were transferred to 17 °C, and five to 15 °C incubation temperatures; five were also left at 28 °C. Throughout the experiment, the cultures were held in glass flasks and shaken at 150 rpm on an incubator orbital shaker (model 4580, refrigerated console, Forma Scientific Inc., USA). After 24 h of acclimatization to the new growth temperatures, the cultures were induced with 5 μM genistein since this concentration shows maximum induction of the *nod* genes (Kosslak et al., 1987; Zhang et al., 1996). After five days of induction,