THE EFFECT OF EXOGENOUS RHIZOBIAL LIPOPOLYSACCHARIDE ON SYMBIOSIS OF *RHIZOBIUM LEGUMINOSARUM* BV. *TRIFOLII* WITH RED CLOVER

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ABSTRACT

The effectivity of symbiosis of *Rhizobium leguminosarum* bv. *trifolii* with red clover in the presence of exogenous lipopolysaccharide (LPS) preparation was measured as a yield of green mass of infected plants. The addition of complete LPS that had been obtained from homologous *Rhizobium* strains influenced significantly the growth of plants. In the presence of defective LPS of *Rhizobium* mutant the effectivity of symbiosis did not change.

KEY WORDS : *Rhizobium leguminosarum* bv. *trifolii*, lipopolysaccharide mutant, symbiotic efficiency.

INTRODUCTION

Nitrogen is one of the most limiting factors for growth of plants. It can be supplied to crops by chemical fertilization as well as by biological nitrogen fixation. Estimation of biological fixation is difficult and data in this respect vary from 100 to 180x10⁶ metric tons per year, but it is agreement that biological fixation is two or more times greater than chemical fertilization (Burris, Roberts 1993; Hardarson 1993; Lorkiewicz 1994).

Symbiosis of legume plants with rhizobia is the most beneficial system of biological nitrogen fixation. This process is the major naturally occurring mechanism by which the atmospheric nitrogen is reduced to form utilized by plant. Its importance from the point of view of ecological aspects and reducing the cost of crop production has been main reason of intensive studies on this plant-microbe interaction. Symbiosis of bacteria of genera *Rhizobium*, *Bradyrhizobium* and *Azorhizobium* results in formation of unique plant organs, nodules, in which bacteria convert the atmospheric nitrogen into ammonia which is utilized by the plant as a nitrogen source. Development of mature nitrogen fixing nodules involves recognition of micro- and macrosymbiont, penetration of root hair wall, invasion of plant by a newly formed infection thread growing in direction of dividing cortical cells, bacterial release from the infection thread into the cytoplasm of host cell and differentiation into nitrogen fixing bacteroids (Franssen et al. 1992; Vance 1991; Mylona et al. 1995).

Rhizobium-plant interaction is very specific and usually the defined species *Rhizobium* can form nodules on limited number of hosts. In several *Rhizobium* species the genetic control of nodulation (nod genes) and nitrogen fixation (nif genes) as well as the mechanisms of recognition has been known (Fisher and Long 1992). The rhizobial genes essential for in-

fection and nodule formation include also genes controlling synthesis of surface polysaccharides: exopolysaccharides (EPS), lipopolysaccharides (LPS) and β -glucans. Bacterial mutants defective in synthesis of these compounds are mostly also defective in symbiotic properties. Surface components of *Rhizobium* can play a role as a signal molecule on different steps of nodulation process as well as in protection of bacteria from host defence mechanism. Most of mutants defective in surface polysaccharides form ineffective nodules with no or abortive infection thread (Kannenberg and Brewin 1994; Carlson et al. 1992).

It has been also observed, that isolated LPS preparations of *Rhizobium leguminosarum* bv. *trifolii* in low doses promote the early steps of nodulation in homologous interactions of *Rhizobium* and white clover (Dazzo et al. 1991). In this work we show the effect of complete and defective LPS on symbiosis of *Rhizobium leguminosarum* bv. *trifolii* with red clover.

MATERIALS AND METHODS

Bacterial strains

Rhizobium leguminosarum bv. *trifolii* 24.1 and its derivatives AR5 and 24.12 were used in this work. The strains 24.1 and AR5 are similar, both form effective nodules on clover roots and produce analogical lipopolysaccharides (Głowacka et al. 1989; Lorkiewicz et al. 1993). The derivative 24.12 is a transposon Tn5 mutant of 24.1 that has been obtained in triparental mating with p5011 and vehicle vector p2013 (Simon et al. 1983). The selective medium has been 79CA (Vincent 1970) supplemented with rifampicin 40 μ g/ml and neomycine 30 μ g/ml. This strain named earlier Rt 12 cannot form nodules on clover roots, usually small deformations are only observed (Głowacka 1992).

LPS isolation

It was performed according to Westphal and Jann (1965) and analysed by polyacrylamide gel electrophoresis (Krauss et al. 1988).

Plant assays

Surface sterilized seeds of *Trifolium pratense* var Hruszowska were placed on nitrogen free agar slants for plant cultivation (Vincent 1970). After five days the seedlings were treated with low dose (2 µg per seedling) of LPS preparations of effective strains 24.1 and AR5 as well as with LPS isolated from 24.12 strain that produced defective LPS. After 1h the seedlings were inoculated with 24.1 or AR5 strains in range 10⁸ cells per one seedling. The plants were incubated during 5 weeks in 10/14 dark/light period. Symbiotic properties were estimated as nodulation rate and the weight of green part of plants.

RESULTS AND DISCUSSION

Rhizobial lipopolysaccharides have been for a long time believed to play an important role in the development of mature nodules on legume plant roots. The isolation of defined one gene mutants (transposon mutants) with altered LPSs proved the direct relationship between the structure of LPS of *Rhizobium* and its symbiotic properties. Lipopolysaccharides of Gram negative bacteria consist of three regions: most external polysaccharide part of O-antigen specificity (O- side polysaccharide) that is linked to the core oligosaccharide and lipid part (lipid A) embedded in the outer membrane. Most transposon mutants of *Rhizobium leguminosarum* lacking O-polysaccharide part were unable to form effective nitrogen fixing nodules. So it was concluded that LPS is involved in releasing of bacteria from the infection thread into cytoplasm of the host cell (Carlson et al. 1992).

In effective nodules induced by wild type strains the endosymbiotic forms of *Rhizobium*, bacteroids contain much less LPS with low electrophoretic mobility (polysaccharide part) compared to free living bacteria. There were studies showing that LPS was needed in the stage of releasing of bacteria. During endocytosis of the bacteria into host plant cell cytoplasm O-antigen containing part of LPS disappeared that had been studied by monoclonal antibodies (Goosen de Roo et al. 1991). But LPS can also participate in early stages of nodulation. Numerous papers concerning the interaction of LPSs with plant lectins have been published in earlier studies (Kato et al. 1979). It was also observed that preinoculation treatment of clover roots with *Rhizobium leguminosarum* bv. *trifolii* LPS at low doses promoted early stages of infection. This effect concerned various preparations of LPS that had been isolated from wild type *Rhizobium leguminosarum* bv. *trifolii* strains. But infection was neither increased by preinoculation treatment with LPSs from *Rhizobium* strains of different species nor bacteria of another family (Dazzo et al. 1991).

We tested the effect of exogenous LPSs on symbiotic interaction of *Rhizobium leguminosarum* bv. *trifolii* strains with red clover. LPSs were prepared from wild type effective strains 24.1 and AR5 as well as from the transposon mutant 24.12. The latter strain formed LPS deprived of O-side polysaccharide part (LPS I) (Głowacka 1992). During polyacrylamide gel electrophoresis only the fast migrating fraction (LPS II) was observed while LPS of parental strain 24.1 contained also slow migrating polysaccharide part (Fig. 1). LPS of AR5 is similar in composition to LPS of 24.1 (Lorkiewicz et al.

Fig. 1. Electrophoretic migration patterns of LPS of *Rhizobium leguminosarum* bv. *trifolii*.

1993; Głowacka et al. 1989). LPS preparations suspended in sterile water were added to five days old clover seedling one hour before inoculation with strain 24.1 or AR5. In both cases the addition of LPSs resulted in slightly changes of average root nodules on clover roots (Table 1). The green mass of plants cultivated with complete LPS preparation differed significantly from green mass of control plants that had been infected with *Rhizobium* strains without addition of complete LPSs. Also, the presence of defective LPS deprived of high molecular weight polysaccharide (isolated from 24.12 strain) did not influence significantly the effectivity of symbiosis measured as a yield of green mass of plants (Table 1). This observations are analogical with earlier results of Dazzo et al. (1991). They observed the promotion of infection thread formation in root hairs by preinoculation treatment of roots with

TABLE 1. Symbiotic properties of *Rhizobium leguminosarum* bv. *trifolii* strains in presence of exogenous LPS (2 µg/plant)*

Inoculant	LPS source	Average nodule number per plant after 5 weeks	Average green mass in mg
24.1	none	14.5	83.4
	24.1	19.0	132.0**
	AR5	14.4	109.8**
	24.12	18.7	93.4
AR5	none	20.2	98.7
	24.1	13.4	129.2
	AR5	15.8	129.6**
	24.12	17.8	108

* Data from 2 series of plants

** Green mass of plants differed significantly from control plants, P < 0.05 (Student test)

Rhizobium trifolii LPS isolated from wild type strains. Our results suggest that infection-modulating biological activity of LPS is restricted to complete homological LPS, because the defective LPS that had been isolated from the derivative of 24.1 (i.e. 24.12) have not influenced significantly the growth of plants.

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WPLYW EGZOGENNEGO LIPOPOLISACHARYDU NA SYMBIOZĘ *RHIZOBIUM LEGUMINOSARUM* BV. *TRIFOLII* Z KONICZYNĄ

STRESZCZENIE

Efektywność symbiozy *Rhizobium leguminosarum* bv. *trifolii* z koniczyną czerwoną w obecności egzogen-nych preparatów lipopolisacharydu (LPS) była oznaczana jako masa części zielonych zakażonych roślin. Obecność kompletnego LPS, otrzymanego z homologicznych szczepów *Rhizobium* wpływała w istotny sposób na wzrost roślin. W obecności LPS defektywnego, izolowanego z mutantu *Rhizobium*, efektywność symbiozy nie uległa zmianie.

SŁOWA KLUCZOWE: *Rhizobium leguminosarum* bv. *trifolii*, mutant lipopolisacharydowy, efektywność symbiozy.