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SHORT COMMUNICATIONS

Effects of Nitrogen Deficiency and Wheat Lectin on the Composition and Structure of Some Biopolymers of *Azospirillum brasilense* Sp245

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Widespread nitrogen-fixing rhizobacteria of the genus *Azospirillum* are exposed to various kinds of stress under natural conditions [1, 2]. Although interest in both fundamental and applied aspects of the interactions of these bacteria with plants has recently been increasing [1, 3], the metabolic characteristics of azospirilla under stress conditions have been studied to this point only in a few reports [1, 2].

The goal of the present work was to investigate cell responses of *A. brasilense* Sp245, a native endophyte of wheat, to nitrogen deficiency and to the presence of wheat germ agglutinin (WGA) in a medium under aerobic conditions.

The method of research was IR spectroscopy sensitive to changes in the composition and structure of biopolymers in intact cells [4, 5]. The interest in the effect of WGA, a plant lectin that is a factor of plant–microbial communication and a stress response protein [6], is also aroused by the fact that distinct cell responses of *A. brasilense* Sp245 to the presence of WGA have not been previously registered under aeration, in contrast to microaerobic conditions when nanomolar WGA concentrations induced quite a number of metabolic responses in the bacterium [7].

A. brasilense Sp245 (from the collection of Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences) was grown on a malate–salt medium (MSM, pH 6.8) [8] on a shaker (160 rpm). Nitrogen limitation stress conditions were achieved by exclusion of NH_4Cl from the medium. WGA preparation (Lectinotest, Ukraine) was introduced along with the inoculum to a final concentration of 0.2 μg/ml. In all experiments, the initial culture density was 2.0×10^7 cells/ml. For obtaining IR spectra, the biomass of cells grown under appropriate conditions (up to 70 h of growth) was separated from the culture liquid by centrifugation, washed three times with the physiological saline, dried at 105°C for 3 h, and pulverized. The spectra (in the diffuse reflectance mode, DR) were obtained using an accessory device for measuring DR infrared Fourier transform (DRIFT) spectra with a Nicolet spectrometer (United States), model Magna IR 750, with a total of 100 scans (resolution 4 cm^{-1}); the spectra were processed by the OMNIC 3.1 standard software package (Nicolet).

Although *A. brasilense* is considered a microaerophilic bacterium, its growth under aeration in the NH_4^+-containing medium was not worse than without stirring (OD_{595} of 70-h cultures, measured in 1-cm cuvettes, was 0.92 ± 0.01 and 0.81 ± 0.11, respectively). Thus, the control variant (NH_4^+-containing medium with stirring) may be considered as practically free from stress. Under these conditions, the IR spectrum of *A. brasilense* Sp245 dry biomass is generally close to the spectra of other bacteria [4] (the full spectrum is not presented). Figure a shows the spectrum of the control variant in the middle frequency range (2000–800 cm^{-1}) including, in particular, the typical amide I (about 1650 cm^{-1}) and amide II (about 1550 cm^{-1}) bands of proteins, bands of deformational vibrations of C–H bonds (1500–1300 cm^{-1}), and a series of bands at about 1100–950 cm^{-1}, mostly corresponding to cellular polysaccharides [4]. Note that the amide I band reflects the conformational state of proteins in a sample; this region (~1620–1690 cm^{-1}) is a superposition of bands corresponding to different components of the secondary structure of polypeptide chains [4, 9], with a maximum of about 1657 cm^{-1} corresponding to α-helix fragments.

Figure b shows the IR spectrum of the cells grown in the absence of NH_4^+ in the medium (nutritional stress); a decreased possibility of physical contact between the cells due to stirring in this case may also be considered an additional unfavorable factor that impedes adaptation of bacteria to the stress. This spectrum, essentially different from the spectrum presented

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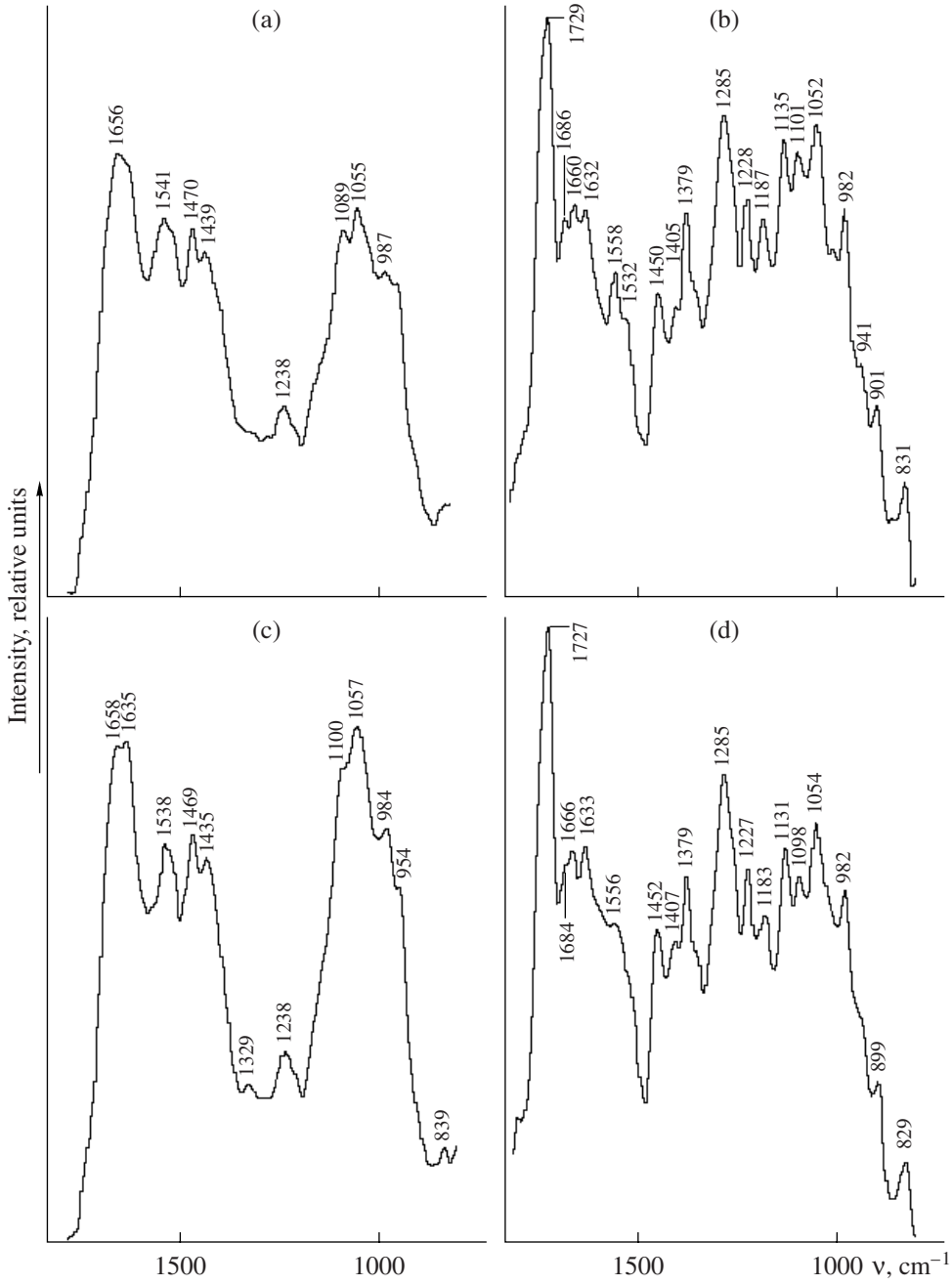

IR spectra of dry biomass of *A. brasilense* Sp245 cells grown in malate medium in the presence of 3 g/l NH_4Cl (a, c), in the same medium without ammonium salts (b, d), and in the presence of 0.2 μg/ml wheat germ agglutinin (c, d).

in figure, a has distinct major bands corresponding to poly-3-hydroxybutyrate (PHB), with maxima at about 1730 cm^{-1} (stretching vibrations of C=O polyester groups), 1450 and 1379 cm^{-1} (deformational vibrations of groups $–CH_2–$ and $–CH_3$), and at about 1285, 1135, and 1052 cm^{-1} (different types of vibrations of fragments C–C–O) [4, 10]. Induction of PHB biosynthesis and accumulation in a number of soil bacteria, including *A. brasilense*, is known to be a specific cell response to some types of stresses [2, 5].

It should be noted that, in spite of the deficiency of bound nitrogen and ample aeration, intensive growth of azospirillum was observed under these conditions (after 66 h of incubation, the CFU value increased more than 30-fold, up to 6.7×10^8 cells/ml). This may be explained by enhanced biosynthesis of extracellular polysaccharides [11] (besides PHB), protecting nitrogenase from inhibition by oxygen.

The spectrum (see figure, b) also shows a splitting of the amide I band (about 1660 cm^{-1}); there are additional

bands at 1632 and 1686 cm^{-1}, corresponding to an increased fraction of β-structures among proteinaceous secondary structure components [4, 9]. A similar splitting of the amide I band (as compared with the control in figure, a) was also observed in the IR spectra of cells grown in the presence of WGA, both under the optimal conditions (figure, c) and under nitrogen deficiency (figure, d); the presence of WGA did not reduce PHB accumulation (cf. figure, b and d). Although nutritional stress was reported to result in a decrease of total protein content in *A. brasilense* cells [12] and in a change in the composition of *A. lipoferum* surface proteins [13], there are no data on the conformational changes of cell proteins under the influence of external factors.

For some of the characterized proteins of *A. brasilense* cell surface, including lectin (hemagglutinin) and the major outer membrane protein (MOMP, an adhesin homologous to bacterial porins) [1, 14], their involvement in stress resistance and/or colonization of plant roots has been proved (colonization also contributes to the overcoming of stress by bacteria). Since both bacterial hemagglutinins and porins are rich in β-structures [15–17], one may believe that the revealed increase of the content of β-structure fragments of cell proteins of *A. brasilense* Sp245 under stress conditions as well as in the presence of WGA (see above) is associated with induction of the biosynthesis of one or more cell surface (glyco)proteins: hemagglutinin and/or porin. In particular, in the case of WGA, azospirilla may respond to this signal of "plant presence" [6, 7] by readiness for colonization, synthesizing biopolymers involved in the latter.

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