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COMPARATIVE ANALYSIS OF THE ELECTROPHORETIC COMPOSITION OF PROTEINS FROM NATIVE AND TRANSFORMED STRAINS OF THE MUNG-BEAN *Bradyrhizobium* sp. *Vigna radiata* L.

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An experiment on the transfer into the mung-bean rhizobia gene of a recombinant plasmid containing an insect toxin gene (pCaVItoxneo) was performed in order to protect the root nodules from parasitic insects. Transformed strains (MTL) are obtained. The frequency of transformation was 10^{-6}. A study of the protein composition showed that the transformed strain MTL 12 contained an additional three polypeptides with MM 83, 93, and 98 kDa; the transformed strain MTL 17, one additional polypeptide with MM 83 kDa.

The transfer of specific genes in order to endow plants with the ability to repel insect pests is one of the most urgent problems of modern genetic engineering [1]. This problem is successfully solved by transfering the gene for insect resistance from the genome of *Bacillus* sp.

We transfered into the gene of mung-bean rhizobia a recombinant plasmid containing the gene of an insect toxin (pCaVItoxneo) in order to protect mung-bean root nodules from parasitic insects. Transformed strains (MTL) were obtained. A comparative study of the protein composition of the rhizobia and their transformed counterparts is essential to determining the effect of the transformation.

Comparative electrophoretic analysis of the protein composition of the native (M 12 and M 17) and transformed (MTL 12 and MTL 17) strains of mung-bean rhizobia is shown in Fig. 1. Visual analysis of the electrophoregrams of proteins from the native strains (M 12 and M 17) shows 27 discreet bands (paths 2 and 3), among which are strongly, moderately, and weakly colored polypeptides evenly distributed along the whole length of the polyacrylamide gel. The distribution spectrum revealed 4 strongly, 16 moderately, and 7 weakly colored polypeptides. The electrophoretic mobility (R_f) of these components varied from 0.93 to 0.19 with molecular masses (MM) from 1.0 to 79 kDa, respectively.

Figure 1 shows that protein extracts from strains MTL 12 and 17 contain 28 and 30 discreet bands (paths 4 and 5), respectively. The R_f values of the protein components for MTL 12 varied from 0.92 to 0.11. The MM of these components was 1.0-98 kDa. The electrophoretic mobility of the polypeptides from MTL 17 fell in the range 0.92-0.17 with MM from 1.0 to 83 kDa. The protein compositions of these strains were significantly different both quantitatively and qualitatively from the studied native strains and between themselves. It is interesting that weakly colored polypeptides were not found in the transformed strains. In our opinion, this is due to increased transcription and translation of the genome of the transformed strains owing to the incorporation into their cells of the recombinant plasmids. The protein composition of the extract from MTL 12 had 14 strongly and 16 moderately colored polypeptide bands whereas that from MTL 17 showed 12 strongly and 16 moderately colored polypeptide bands. The results indicate that the protein components of the transformed strains differ quantitatively and qualitatively.

The transformed strains MTL 17 and 12 differed from the native rhizobia strains by the presence of 1-3 additional polypeptides. Their MM from MTL 17 were 83 kDa; from MTL 12, 83, 93, and 98 kDa. Furthermore, polypeptides from transformed strains with electrophoretic mobilities 0.67, 0.48, and 0.43 and MM 21, 35.5, and 41 kDa were present in larger quantities than in the native strains.

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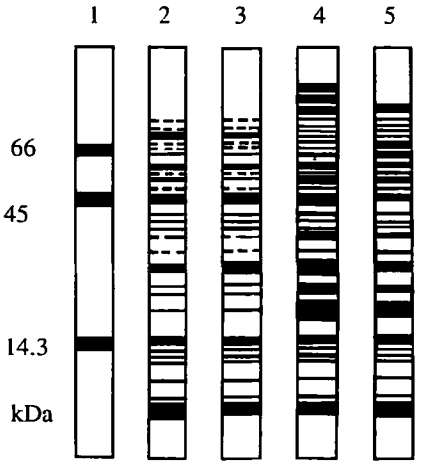

Fig. 1. Electrophoregrams of mung-bean rhizobia *Bradyrhizobium* sp. *Vigna radiata* L.: Markers (1), starting native strains M 12 and M 17, respectively (2, 3), native transformed laboratory strains MTL 12 and MTL 17, respectively (4, 5).

Analogous results were obtained for transformation of *Rhizobium* by a DNA fragment containing the *Bacillus thuringiensis* Sub. sp. *tenebrionis* endotoxin gene [2]. The transformed cells synthesized an additional two polypeptides with MM 68 and 73 kDa.

Transgenic plants that produce σ-endotoxin in a quantity sufficient to repel caterpillars of scaly winged insects have already been grown [3, 4]. Recombinant proteins were produced in experiments on the transformation of tobacco plants with σ-endotoxin genes from *B. thuringiensis kurstaki* [5].

The literature data and our results suggest that the differences in the protein composition of the native and transformed strains of mung-bean rhizobia can be explained as follows. First, the additional polypeptides may result from the translation of plasmogenes introduced into the rhizobia cells. Second, this phenomenon may result from a mutation caused by the invasion of the rhizobia cells by the recombinant plasmid or the uneven assimilation of the plasmid fragments in the recipient cell genome.

The experimental results on the comparative characteristics of the mung-bean rhizobia proteins in general confirm that the native strains isolated by us (M 12 and 17) have different protein compositions than the transformed strains (MTL 12 and 17), both quantitatively and qualitatively. The transformed strains MTL 12 and 17 have 1-3 additional polypeptides.

EXPERIMENTAL

Mung-bean rhizobia (*Bradyrhizobium* sp. *Vigna radiata* L.) were transformed according to the literature method [6]. The transformation introduced the specially constructed recombinant vector plasmid pCaVItoxneo, which contained a toxin gene from *B. thuringiensis* var. *kurstaki* HD-1 and a gene for resistance to antibiotics.

Transformed colonies were selected in a selective medium containing kanamycin (150 μg/ml). Only the transformed colonies can survive on this selective nutrient medium owing to a selective marker for resistance to kanamycin that was transfered to this strain of rhizobia by the constructed plasmid. The transformation frequency was 10^{-6}. The biomass of single colonies of transformed strains increased enormously after seeding in No. 79 liquid medium.

Isolation of Proteins from Rhizobia. Cells grown in No. 79 liquid medium were precipitated by centrifugation at 3000 rpm for 20 min. The precipitate was washed twice with 0.01 M Tris-HCl buffer (pH 8.0). The cells were disrupted by ultrasonic irradiation (UZDN-1) at 0.6 A and 35 kHz for 3 min with intervals of 10 sec on ice. The destroyed cell mass was centrifuged at 5000 rpm for 20 min at 4°C. Then the precipitate was lyophilized. The protein concentration was determined using the

Lawry method [7].

Protein extract was prepared by dissolving protein powder in buffer (Tris-HCl, 0.062 M, pH 7.4; 2.3% SDS, 10% glycerin, 1% β-mercaptoethanol, 0.05% BFS). Samples were boiled for 5 min. The supernatant from the centrifugation was used for the electrophoretic analysis.

Electrophoresis of the proteins was performed in 10-17% polyacrylamide gradient gel PAAG according to Laemmli [8] using reagents from Sigma. Marker proteins were bovine serum albumin, egg albumin, and lysosyme of MM 66, 45, and 14.3 kDa, respectively.

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