

FREE AMINO ACIDS OF *Sinorhizobium meliloti*, BIOMASS AND ROOTS OF *Medicago sativa* AFTER INOCULATION

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UDC 579.841.31:581.19:633.31

The amino-acid composition of active symbiotic nitrogen-fixing Sinorhizobium meliloti in addition to the biomass and roots of Medicago sativa after inoculation was studied. The bacterium contains in its cells and releases to the substrate 13-16 free amino acids. The greatest amount of free amino acids appears in 10-day cultures of strains 125 and 131 (203.26 and 255.15 mg%). Inoculation of seeds helps to increase the content of free amino acids and protein in biomass and roots of alfalfa, which significantly increases its value and improves the soil condition.

Key words: nodule bacteria, strain, alfalfa, inoculation, amino acids.

Free amino acids from various types of microorganisms were well studied. However, little is known about amino-acid synthesis by *Sinorhizobium meliloti* [1, 2].

In the present article, the free amino acids in cells and culture medium of *S. meliloti* and in the biomass and roots of inoculated *Medicago sativa* growing in Tashkent District are reported. Our results indicate that the cells of alfalfa tuber bacteria contain 14-16 free amino acids (Table 1). The dicarboxylic acids aspartic and glutamic; monoaminoacids with an unbranched C chain alanine and glycine; with a branched chain valine; and diaminoacids lysine and arginine dominate. Cystine is observed in smaller quantities in all cultures. Tryptophan was found in strains 125 and 131.

The amino-acid concentration, except for that of cystine, increases in 10-day cultures. The total free amino acids increases during growth for strain 415 by three times and for strain 131 by 1.5 times owing to the increase in cells of aspartic and glutamic acids and alanine. Tyrosine was found in 10-day cultures of strains 125, 1, and 131 (0.06, 0.02, and 0.16 mg%, respectively).

During the study of the amino-acid composition in the culture medium, it has been determined that alfalfa nodule bacteria release into the substrate 12-16 amino acids. Strain 131 is the most active in this respect (Table 2). The culture medium of this strain compared with that of strain 1 contains four times more aspartic and glutamic acids; three times, phenylalanine, isoleucine, valine, and methionine; and 1.5 times, alanine and arginine.

A comparison of the quantities of amino acids in cells and culture medium has been shown that *S. meliloti* can both accumulate amino acids in cells and release them to the substrate. The culture activity is greater, the greater the amino-acid content in the cells and culture medium.

Inoculation of alfalfa seeds has a positive influence on the plants (Tables 3 and 4).

A study of the amino-acid composition of alfalfa biomass and roots has been shown that inoculation of seeds improves nitrogen exchange and increases the content of free amino acids (Table 3). Biomass and roots of control and inoculated plants contain 16 free amino acids. The total free amino acids for strains 125 and 131 increased in biomass by 37.5-42.5%; in roots, by 34.1-43.1%, respectively; including unsubstituted amino acids by 1.5-2 times; total nitrogen in leaves, by 0.3-0.36%; in roots, by 0.3-0.65%.

TABLE 1. Free Amino Acids in *Sinorhizobium meliloti* Cells, mg% of Dry Weight

Amino acid	Strain							
	1		125		131		415	
	3-day	10-day	3-day	10-day	3-day	10-day	3-day	10-day
Cystine	0.9	0.4	1.5	1.1	1.4	1.1	1.3	1.1
Lysine + arginine	1.8	2.6	3.7	6.7	3.2	5.4	1.3	2.2
Histidine	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
Aspartic acid	30.3	50.4	18.3	96.4	66.3	112.2	21.3	63.4
Serine + glycine	2.6	3.0	3.1	3.8	3.7	4.8	1.5	2.3
Glutamic acid	20.5	31.4	41.3	61.7	54.5	88.7	18.6	63.7
Threonine	Tr.	Tr.	Tr.	Tr.	0.02	0.09	Tr.	Tr.
Alanine	6.2	12.7	5.4	19.8	9.1	28.4	7.6	16.3
Tyrosine	-	0.02	-	0.06	0.1	0.16	-	-
Valine + methionine	2.5	3.8	4.9	6.7	6.3	8.9	2.4	3.7
Tryptophan	-	-	5.1	2.6	Tr.	Tr.	-	-
Phenylalanine + isoleucine	0.05	1.3	1.49	4.7	2.5	5.4	0.41	1.18
Total free amino acids	64.95	105.62	144.79	203.26	147.22	255.15	54.41	153.88

TABLE 2. Free Amino Acids in *Sinorhizobium meliloti* Culture Medium, mg% of Dry Weight

Amino acid	Strain			
	1	125	131	415
Cystine	1.63	5.85	1.2	5.9
Lysine	1.12	4.8	4.33	3.85
Arginine	2.2	0.58	3.94	0.83
Histidine	-	Tr.	Tr.	Tr.
Aspartic acid	2.1	6.7	8.01	7.8
Serine + glycine	0.33	3.08	3.43	2.75
Glutamic acid	2.1	6.83	8.6	6.08
Threonine	-	0.30	0.26	0.35
Alanine	10.3	15.7	17.9	17.3
Tyrosine	0.77	0.96	1.2	1.5
Valine + methionine	3.07	10.0	10.91	11.68
Tryptophan	-	Tr.	Tr.	Tr.
Phenylalanine + isoleucine	5.2	12.1	19.55	16.72
Total free amino acids	28.82	66.70	84.33	74.76

The chemical composition of alfalfa after inoculation has been shown that it contains during flowering a high percentage of raw protein with a moderated content of cellulose, fat, and ash. This is indicative of higher quality alfalfa for animal feed (Table 4). Alfalfa of strains 125 and 131 contained the highest quantity of protein (125.16%). Using both these cultures increases the ash content and decreases the cellulose content compared with the control.

Thus, *S. meliloti* actively produces free amino acids. Inoculation of alfalfa seeds with nodule bacteria helps to accumulate proportionately free amino acids and protein, improves its nitrogen exchange, and increases the soil fertility.

The nodule bacteria strains that we used are preserved in the laboratory collection of microorganisms at the IM AS RUz.

TABLE 3. Free Amino Acids (mg%) and Total Nitrogen (%) in Biomass and Roots of Alfalfa after Inoculation

Amino acid	Alfalfa biomass					Alfalfa roots				
	Control	Strain inoculated				Control	Strain inoculated			
		1	125	131	415		1	125	131	415
Cystine	80	146	146	156	97	105	128	134	1.36	128
Lysine	30	42	32	63	96	60	76	70	89	66
Arginine + histidine	100	160	135	160	158	216	220	320	340	305
Aspartic acid	361	530	440	501	484	858	892	991	1006	923
Serine + glycine	512	629	642	680	720	303	396	433	494	420
Glutamic acid + threonine	411	501	600	607	531	564	645	621	736	613
Alanine	503	511	525	534	433	365	624	644	611	526
Tyrosine	85	83	102	101	85	96	141	137	145	174
Valine + methionine	206	438	471	445	365	209	243	317	363	311
Tryptophan	115	191	194	175	190	115	153	164	175	114
Phenylalanine + isoleucine	138	203	208	199	198	116	204	205	211	190
Total free amino acids	2541	3413	3495	3621	3357	3007	3722	4036	4306	3760
Total N ₂ content	3.86	4.12	4.17	4.18	4.12	4.1	4.41	4.62	4.75	4.51

TABLE 4. Chemical Composition of Alfalfa, % of Dry Mass, as a Function of Inoculation (Flowering Stage)

Specimen	Raw protein	Raw cellulose	Total fat content	Ash
Control (not inoculated)	13.2	34.26	2.15	7.63
Strain inoculated:				
1	19.32	27.75	2.11	7.50
125	24.21	24.26	2.19	9.58
131	24.86	24.31	2.16	9.51
415	21.45	26.83	2.17	9.38
Based on strains 125 and 131	25.16	25.83	2.24	10.29

EXPERIMENTAL

Cultures of *S. meliloti* strains 1, 125, 131, and 415 were isolated from pink tubers of alfalfa during budding and flowering and were selected according to nitrogen-fixing activity. Nodule bacteria were grown in Ehrlenmeyer flasks in Rigo medium for 3 and 10 days on a rocker (190 rpm) at 28°C. Two-day cultures of bacteria (0.5% by volume) were used for inoculation.

The effect of inoculation on the amino-acid and chemical composition of alfalfa was determined after setting up the vegetative experiment in containers with typical gray desert soil (12 kg). Seeds were innoculated with lyophilized cultures of nodule bacteria using 2×10^4 cells per seed.

The biomass was stabilized with ethyl alcohol (10 times the volume, 96%).

Quantitative and qualitative contents of amino acids in cells of *S. meliloti* culture medium, biomass, and roots of alfalfa were determined by paper chromatography [3, 4]. The chemical composition of alfalfa raw protein was studied by the Kjeldahl method [5]; total fat content, by the literature method [6]; ash, by ignition according to GOST-23637-70; raw cellulose, as before [6].

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