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Genetic Diversity and Population Structure of Siberian fir (*Abies sibirica* LEDEB.) in Middle Siberia, Russia

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Abstract

The genetic diversity and population structure of Siberian fir (*Abies sibirica* Ledeb.) in Middle Siberia were studied on the basis of allozyme variation analysis at 20 loci. The vegetative buds collected from 260 trees in nine populations representing different geographical localities and altitudinal sites were used as materials for study. Horizontal starch gel electrophoresis was applied to analyze the isozyme patterns of 11 enzyme systems. 20% of the gene loci assayed were revealed to be polymorphic at 95%, and 35% at a 99% criterion. The mean number of alleles per locus, the mean observed heterozygosity, the mean expected heterozygosity, and the effective number of alleles were equal to 1.45, 0.0569, 0.0642 and 1.13, respectively. More than 95% of total genetic variation was within each population and only 5.24% ($F_{st} = 0.0524$) was among the populations. The mean value of Nei's genetic distance, D among the populations ranged from 0.0005 to 0.0098 and averaged 0.0040. The obtained data indicates a low level of genetic diversity and a weak differentiation among the *A. sibirica* populations studied in Middle Siberia. The most significant difference in structure was that between Kozulka and Western Sayan, 1000 ($D=0.0098$). Within mountain populations of Siberian fir from Western Sayan the most essential statistically significant differences were between low altitudinal (Western Sayan, 400) and high altitudinal (Western Sayan, 1500) populations ($D=0.0060$).

Key words: Middle Siberia, *Abies sibirica*, starch gel electrophoresis, genetic diversity, structure and differentiation

Introduction

Siberian fir (*Abies sibirica* Ledeb.) is one of the most widespread fir species in Russia. This species occupies a vast territory including the northeast of the European part of Russia, the Urals and the major part of the Siberia forest zone. *Abies sibirica* is one of the main forest-forming species of the dark-coniferous taiga, but it rarely forms pure stands. Usually *A. sibirica* forms mixed stands with other coniferous species (Siberian spruce, Siberian pine). Over the greater part of its area *A. sibirica* grows on the flatlands southward of the permafrost zone. In addition, *A. sibirica* also is present in the mountains where it extends to the upper limits of the range of forest (1200 - 2000 m above sea level) forming a considerable part of the mountain forests in Southern Siberia (Bobrov, 1978).

For conservation of *A. sibirica*, the genetic diversity, structure and differentiation of its populations are still poorly understood. The investigations of this species conducted on the basis of allozyme variation analysis are not numerous and have not covered the whole range of Siberian fir (Goncharenko and Padutov, 1995; Goncharenko *et al.*, 1998; Goncharenko, 1999; Larionova, Ekart, 2005; Semerikova, Semerikov, 2006). Virtually nothing is known about the genetic diversity and structure of *A. sibirica* populations in the vast region of Middle Siberia. This region is characterized by various topographical and ecological conditions,

which was obstructive to make investigation and so it is of interest for the study of *A. sibirica* genetic diversity.

In addition, there is very little information about the genetic diversity, structure and differentiation of Siberian fir mountain populations along an altitudinal gradient. Situated on various altitudinal levels, which are differentiated from one another with strongly pronounced gradients and diverse environmental factors, the mountain populations often reveal marked differences in genetic structure.

The previous studies conducted in mountain populations of various species of conifers showed that the degree of genetic differences between populations located at various altitudes above sea level depended on the character of the spreading species within the limits of the mountain-mass under investigation, the altitude of the local populations and their phenological isolation (Mitton *et al.*, 1980; Neale and Adams, 1985; O'Reilly *et al.*, 1985; Alden and Loopstra, 1987; Shuster, *et al.*, 1989; Ettl and Peterson, 2001). In *Abies lasiocarpa* (Ettl and Peterson, 2001), the most significant differences in allele frequencies were revealed among high altitudinal and low altitudinal sites. In others species investigated, for example of the genus *Abies* as represented by *A. balsamea* in particular (Neale and Adams, 1985) and *A. fraseri* (Diebel, Feret, 1991), the differences between various altitudinal sites were insignificant. In some species, for example *Picea abies*

(Carpathian Mountains) and *Pinus pallasiana* (Crimea) (Korshikov *et al.*, 2005 a), the high altitudinal populations demonstrated greater levels of heterozygosity in comparison to low altitudinal populations.

Unfortunately, the lack of data on the genetic variability of *A. sibirica* populations has made it difficult to estimate the genetic potential of this species, as well as the character of the distribution of genetic variability through the species' range and the degree of ecotypic and geographic differentiation among populations.

During the last two decades it has become possible to utilize for studies of genetic diversity in conifers various types of DNA markers. The successes of these new directions in research are so great that some researchers think it expedient to even forego the analysis of isozymes (the most often used gene markers) and concentrate all attention on DNA polymorphism. It is obvious that such an approach is erroneous even *a priori*. Polymorphisms of proteins and DNA naturally are related and one's focus will depend on the particular problems under investigation. Therefore, it is only by the combined use of these two methods that qualitatively new data about intra-species diversity and differentiation of conifer populations can be obtained (Altukhov and Salmenkova, 2002).

The objective of this first stage of investigation of *A. sibirica* species was to determine the genetic structure, the level of genetic diversity and the degree of differentiation of this species among populations located in Middle Siberia by means of the method of isozyme analysis.

Materials and Methods

Buds collected from single trees in nine fir stands (in this study referred to as populations) representing different geographical localities and altitudinal sites were sampled. The total number of trees sampled was

260. Designations and geographical coordinates of the populations investigated are shown in Table 1.

Buds from each individual (from 5 to 7 buds) were ground in two or three drops of an extracting buffer (0.05 M Tris-HCl, pH 7.7 with the addition of 3% PVP-40000, 0.05% β -mercaptoethanol, 0.02% EDTA- Na_2 , and 0.06% dithiothreitol). Then the extracts were absorbed onto filter-paper wicks and these were inserted into a 13-14% starch gel. Electrophoretic separation of the extracts was achieved by means of horizontal gel electrophoresis. Three buffer systems were used for electrophoresis: I - Morpholine-Citric acid, pH 7.0 (Clayton, Tretiak, 1972), II - Tris-Citric acid, pH 8.5 / Lithium hydroxide-Boric acid, pH 8.1 (Ridgway *et al.*, 1970), and III - Tris-EDTA-Boric acid, pH 8.6 (Markert and Faulhaber, 1965). Gel and electrode buffers were as recommended for these systems. Eleven enzyme systems were assayed for each tree. Standard methods of histochemical enzyme staining (Brewer, 1970; Vallejos, 1983; Cheliak and Pitel, 1984; Goncharenko and Padutov, 1988) were followed with minor modifications. The enzymes assayed, their abbreviations, and the buffer systems used are given in Table 1 (Appendix, Table 1).

Enzymes, loci, and alleles were designated as described by Prakash *et al.* (1969). Loci were numbered according to the electrophoretic mobility of the corresponding activity zones. The locus coding the most mobile zone was numbered 1 and locus coding a zone with more low mobility was labeled 2, etc. Within each locus, the most frequent allele was designated as 1.00. Other alleles of the locus were designated according to the electrophoretic mobility of the corresponding allozymes with reference to the allozyme value of 1.00. Alleles that coded as phenotypically undetectable allozymes were designated as null.

Allele frequencies were analyzed using the BIOSYS-1 (Swofford and Selander, 1981) and PopGen (Yeh *et al.* 1999) computer programs. For each

Table 1. Locality data for the 9 populations of *A. sibirica*.

Population name	Designation	Sample size	Latitude	Longitude	Elevation (m)
Eniseisk	1	20	58° 21'	91° 49'	100
Tyuchtet	2	30	56° 35'	89° 10'	200
Kozulka	4	30	56° 12'	91° 10'	300
Emelyanovo	2	30	56° 11'	92° 20'	420
Eastern Sayan, 200 m	3	30	55° 57'	92° 18'	200
Eastern Sayan, 640 m	5	30	55° 57'	92° 13'	640
Western Sayan, 400 m	5	30	53° 08'	92° 56'	400
Western Sayan, 1000 m	8	30	53° 00'	93° 13'	1000
Western Sayan, 1500 m	9	30	52° 50'	93° 15'	1500

population the mean number of alleles per locus (A), the percentage of polymorphic loci at the 95% (P_{95}) and 99% (P_{99}) criteria, the observed (H_o) and Hardy-Weinberg expected (H_e) heterozygosities and the effective number of alleles (n_e) were calculated. At the species level these indices were obtained by analyzing all the populations as one unit.

Chi-square “goodness-of-fit” tests were used to determine if observed genotype frequencies were in accordance with expectations under Hardy-Weinberg equilibrium. Chi-square tests of homogeneity for allele frequencies were also conducted and Nei’s genetic distances (Nei, 1972) were calculated as well.

For assaying genetic structure fixation indices (F_{is} , F_{it} , F_{st}) were used (Guries, Ledig, 1982). F_{is} and F_{it} measure the deviation of genotype frequencies from Hardy-Weinberg proportions within populations and in *A. sibirica* as a whole, respectively, whereas F_{st} measures the degree of genetic differentiation among populations. The unweighted pair group method (UPGMA) of cluster analysis (Sneath and Sokal, 1973) was used to visualize differences in genetic distance among populations.

Results

Twenty nine allelic variants in the 20 loci were revealed in the course of the study of the 11 enzyme systems in natural populations of *A. sibirica* from Middle Siberia. Loci *Gdh*, *Lap-1*, *Lap-2*, *Mdh-1*, *Mdh-2*, *Mdh-4*, *Pgi-1*, *Pgi-2*, *Pgm-1*, *Pepca*, *Fe-3*, *Idh-1*, and *Idh-2* were monomorphic in all populations. The other loci (*Got-1*, *Got-2*, *Mdh-3*, *Pgm-2*, *Skdh-1*, *6-Pgd-1*, and *6-Pgd-2*) were polymorphic at least in one population. The polymorphic loci had two (*Got-1*, *Got-2*, *Skdh-1*, *6-Pgd-1*, *6-Pgd-2*) or three (*Mdh-3*,

Pgm-2) alleles. At locus *Got-2* a null allele was detected. The most common alleles of the polymorphic loci were the same in all populations studied. The allele frequencies for each population and each gene loci are listed in Table 2 (Appendix, Table 2). A maximum of 5 loci were polymorphic in any single population.

On the basis of allelic frequencies the main parameters of genetic variability were calculated. As seen in Table 2 the percentage of polymorphic loci at the 95% criterion (P_{95}) ranged from 10% to 20% and at the 99% criterion (P_{99}) ranged from 15% to 25%. The mean number of alleles per locus (A) varied from 1.20 to 1.30. The mean value of observed heterozygosity (H_o) ranged from 0.0400 to 0.0733 and the mean expected heterozygosity (H_e) ranged from 0.0485 to 0.0717. The effective number of alleles (n_e) was from 1.08 to 1.14. As a whole for the nine populations of *A. sibirica* studied the percentage of polymorphic loci at the 99% polymorphism criterion represented 35%, with the mean number of alleles per locus, the mean observed heterozygosity, the mean expected heterozygosity and the effective number of alleles being equal to 1.45, 0.0569, 0.0642 and 1.13, respectively. The population Western Sayan, 1500, had the highest values of observed and expected heterozygosities while the population Tyuchtet had the smallest values of these two measures of genetic variability.

In all populations except Western Sayan, 1500, and Eastern Sayan, 200 ($H_o > H_e$), the mean observed heterozygosity was lower than that expected under Hardy-Weinberg equilibrium. The most substantial deficit in heterozygous genotypes was observed in Eastern Sayan, 640. In this population, a statistically significant departure from the expected distribution of genotypes was revealed at locus *Pgm-2*.

Table 2. Genetic variability at 20 loci in 9 populations of *A. sibirica* (standart deviation in parentheses).

Population	Percentage of polymorphic loci		Mean number of alleles per locus	Mean heterozygosity		Effective number of alleles
	P_{95}	P_{99}		Observed	Expected	
			A	H_o	H_e	n_e
Eniseisk	15.00	25.00	1.30	0.0500	0.0506	1.08
Tyuchtet	10.00	20.00	1.25	0.0400	0.0485	1.10
Kozulka	15.00	20.00	1.25	0.0533	0.0621	1.13
Emelyanovo	15.00	15.00	1.20	0.0567	0.0598	1.12
Eastern Sayan, 200	20.00	25.00	1.30	0.0683	0.0666	1.13
Eastern Sayan, 640	15.00	20.00	1.25	0.0550	0.0722	1.14
Western Sayan, 400	15.00	25.00	1.30	0.0458	0.0501	1.08
Western Sayan, 1000	20.00	20.00	1.30	0.0667	0.0701	1.13
Western Sayan, 1500	20.00	20.00	1.30	0.0733	0.0717	1.14
In total for the species	20.00	35.00	1.45 (0.686)	0.0569 (0.144)	0.0642 (0.159)	1.13 (0.369)

The values of F_{is} varied among the loci (Appendix, Table 3) from -0.0345 for Got-2 to 0.1534 for Skdh-1 with an overall mean of 0.0610 . This indicates that within populations there was a 6.1% deficiency of heterozygotes relative to Hardy-Weinberg expectations. F_{it} values at loci Got-1, Got-2 and 6-Pgd-2 were negative and at the Mdh-3, Pgm-2, Skdh-1, and 6-Pgd-1 loci were positive, reaching 0.1917 at 6-Pgd-1. The weighted mean of F_{it} over all loci was 0.1102 . The positive F_{it} value is indicative of an 11.02% deficiency of heterozygotes in *A. sibirica* as a whole.

The value F_{st} ranged among the polymorphic loci from 0.0148 to 0.0878 . The highest contribution to the variability among populations was imparted by the 6-Pgd-1 and Skdh-1 loci, while the contribution of the 6-Pgd-2 locus was the smallest. The average value of F_{st} for all loci was equal to 0.0524 . Thus, about 95% of total genetic variability resided within populations and only 5.24% among populations (Appendix, Table 3).

Heterogeneity analysis of allelic frequencies by means of χ^2 -tests showed that the observed differences in allelic frequencies among populations were insignificant only for three polymorphic loci (Got-1, Got-2, 6-Pgd-2). For the remaining loci (Mdh-3, Pgm-2, Skdh-1, 6-Pgd-1) and for the totality of polymorphic loci as a whole the interpopulation differences in allelic frequencies were statistically significant (Appendix, Table 3).

The mean value of Nei's genetic distance, D (Nei, 1972) between the studied populations of *A. sibirica* ranged from 0.0005 to 0.0098 and equaled, on the average, 0.0040 (Appendix, Table 4). The obtained estimates of D are indicative of a low level of differentiation in *A. sibirica* populations in Middle Siberia. The maximum values of D were found between Kozulka and Western Sayan, 1000 and also between Kozulka and Emelyanovo; the minimum value was detected between Eniseisk and Western Sayan, 400. Among mountain populations of Siberian fir from Western Sayan the most significant differences were revealed between low altitudinal (Western Sayan, 400) and high altitudinal (Western Sayan, 1500) populations.

A dendrogram constructed by the unweighted pair

group method (UPGMA) of cluster analysis and Nei's (Nei, 1972) distance values depicts a fairly distinct division among the fir populations studied (Fig.1). The nine populations are grouped into three clusters. The low altitudinal Western Sayan, 400 united with the flatland populations (Eniseisk, Emelyanovo, and Tyuchtet) to form the first cluster. Western Sayan, 1000 and Western Sayan, 1500 formed the second cluster. The third cluster linked Kozulka, Eastern Sayan, 640 and Eastern Sayan, 200. The unification of the three clusters occurred at a distance of 0.005 .

Discussion

The obtained values of the main parameters of genetic diversity in our study indicate a not very high level of genetic variability in *A. sibirica* in Middle Siberia. The estimates of the observed mean and expected mean for heterozygosities for *A. sibirica* populations from this region were somewhat lower than those established by Semerikova and Semerikov (2006) for populations of *A. sibirica* from other parts of its range ($H_o = 0.057$, $H_e = 0.064$ and $H_o = 0.081$, $H_e = 0.083$, respectively). The percentage of polymorphic loci and the mean number of alleles per locus in total were higher for fir populations from Middle Siberia ($P_{99} = 35\%$, $A = 1.45$ and $P_{99} = 19.3\%$, $A = 1.32$, respectively). In another study of *A. sibirica* populations (Goncharenko, Savitskii, 2000) a higher percentage of polymorphic loci and higher mean values of the observed and expected heterozygosity were obtained ($P_{99} = 50\%$, $H_o = 0.108$, $H_e = 0.129$).

Among the currently investigated species of the genus *Abies*, low levels of genetic variability have been observed also in *A. balsamea* (Shea, Furnier, 2002), *A. guatemalensis*, *A. religiosa*, *A. flinckii* (Aguirre-Planter et al., 2000), and *A. semenovii* (Goncharenko et al., 1998). The highest genetic diversity was observed in *A. cephalonica*, *A. bornmulleriana* (Fady, Conkle, 1993) and *A. sachalinensis* (Goncharenko et al., 1998). The percentage of polymorphic loci in these species reached 60% and heterozygosity was on the average 20% and over.

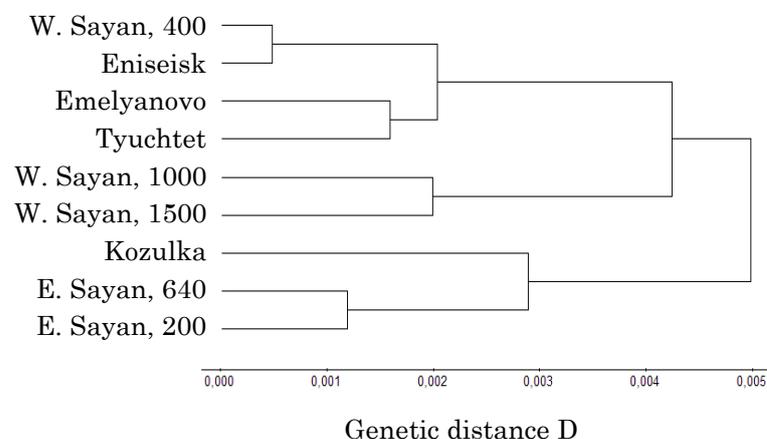


Fig. 1. UPGMA dendrogram based on genetic distances D [Nei, 1972] between *A. sibirica* populations.

In this study we found that the genetic diversity among populations of *A. sibirica* situated along an altitudinal gradient in the Western Sayan Mountains differed. Our results showed that the values of heterozygosity increased with increasing altitude above sea level. The highest values of both observed and expected heterozygosity were revealed in Western Sayan, 1500, which is situated near the tree line in the Western Sayan Mountains. The low-altitudinal population situated in Western Sayan, 400 had the smallest values of these indices. The estimates of the heterozygosity for this population were comparable with those obtained for populations on flat land. In the Eastern Sayan Mountains where the altitudinal gradient was far less (440 m) than in the Western Sayan Mountains (1100 m), the expected heterozygosity also increased with increasing altitude.

Clinal patterns in the levels of heterozygosity depending on elevation gradients suggest that the differences in genetic diversity among mountain populations of *A. sibirica* are associated with the action of local differentiating selection in heterogeneous environments caused by altitudinal zonation. The greater heterozygosity in populations situated in instable and harsh ecological conditions at the timberline in comparison with much lower altitudinal populations could be a result of selection in favor of heterozygotes which have greater adaptational advantages under such conditions.

Higher values of heterozygosity for high-altitudinal populations in comparison to low-altitudinal populations were also found in a study of *Picea abies* in the Ukrainian Carpathian Mountains and *Pinus pallasian* in the Crimea (Korshikov *et al.*, 2005a).

A deficiency of heterozygotes has been reported for populations of many species of genus *Abies*: *A. lasiocarpa* (Shea, 1990) – 34.1%; *A. guatemalensis*, *A. religiosa*, *A. hickelii*, and *A. flinckii* (Aguirre-Planter *et al.*, 2000) – 23.5%, 21.6%, 12.1%, and 7.4%, respectively; *A. cephalonica* (Fady, Conkle, 1993) – 23.4%; *A. balsamea* (Neale, Adams, 1985; Shea, Furnier, 2002) – 14.9%, 15.4%; and *A. alba* (Longauer *et al.*, 2002; Korshikov *et al.*, 2005b) – 2.5–11.3%, 2.6%. The average values of F_{is} and F_{it} obtained for *A. sibirica* in our study were close to the minimum values of these indices established for species of genus *Abies*.

The value of F_{st} (0.0524) was far less than that (F_{st} = 0.1016) reported by Semerikova and Semerikov (2006) for *A. sibirica* populations from different regions of its range. It is indicative of a low subdivision among *A. sibirica* populations in Middle Siberia. Among other fir species investigated a significant subdivision of populations was found only for *A. flinckii* (F_{st} = 0.271), *A. religiosa* (F_{st} = 0.250) and *A. guatemalensis* (F_{st} = 0.122) (Aguirre-Planter *et al.*, 2000) from Southern Mexico and Guatemala. The lowest value of F_{st} was reported for populations of *A. fraseri* (F_{st} = 0.002) (Diebel, Feret, 1991).

Although, F_{st} did not show a high mean value for the studied populations of *A. sibirica*, the populations were unlike in genetic structure. Of the 36 possible comparisons between pairs of *A. sibirica* populations,

twenty one produced statistically significant differences in allelic frequencies of the 20 loci tested. Quantitative estimates of genetic differences showed that the degree of differentiation of the *A. sibirica* populations studied in Middle Siberia did not display a close connection with the geographical distance between individual populations. The populations from Eniseisk and Western Sayan, 400 situated far apart geographically had very similar genetic structure (D = 0.0005). On the other hand, Kozulka was substantially divergent in genetic structure both in terms of closely located populations and geographically removed populations (D = 0.0070–0.0098). Substantial differences in allelic frequencies were also observed between populations situated along the altitudinal gradient in the Western Sayan Mountains.

The comparative analysis of *A. sibirica* populations located at various elevations above sea level showed that the degree of genetic differentiation of these populations depends on their position. The most considerable statistically significant differences in allele frequencies were revealed between high-altitudinal (Western Sayan, 1500) and low-altitudinal (Western Sayan, 400) populations (D = 0.0060). The differences between low and middle (Western Sayan, 400; Western Sayan, 1000) altitudinal populations and also between high and middle (Western Sayan, 1500; Western Sayan, 1000) altitudinal populations were insignificant (D = 0.0046 and 0.0020, respectively).

Stronger genetic differentiation between high and low altitudinal populations of *A. sibirica* in the Western Sayan Mountains may be the result of selection acting on the genetic structure of populations. Environmental heterogeneity generates genetic heterogeneity by causing differing selection pressures and creating significant barriers to gene flow through differences in phenology (Linchart and Grant, 1996).

Conclusion

The study conducted showed that *A. sibirica* growing in Middle Siberia was characterized by lower genetic diversity, weak subdivision and a low level of genetic differentiation among populations. As a whole for the studied populations of this species the percentage of polymorphic loci at a 99% polymorphism criterion amounted to 35%, with the mean number of alleles per locus and the mean observed and expected heterozygosities being equal to 1.45, 0.0569, and 0.0642, respectively. The highest values of heterozygosity among the populations studied were found in Western Sayan, 1500, situated at the upper limits of the range of *A. sibirica* in the Western Sayan Mountains. The analysis of genetic diversity among populations of *A. sibirica* along an altitudinal gradient in the Western and Eastern Sayans showed that the values of heterozygosity increased with increasing altitude above sea level.

It was established that the populations of *A. sibirica* located in Middle Siberia display insignificant deficiency of heterozygous genotypes. On the whole for populations studied the inbreeding of individuals relative to the species was equal to 11.02% (F_{it} =

0.1102). About 95% of the total genetic variability resided within populations and only 5.24% ($F_{st}=0.0524$) was distributed among populations. The genetic distances D (Nei, 1972) between populations varied from 0.0005 to 0.0098 and averaged 0.0040. In spite of the weak differentiation among populations on the whole, most of the populations studied displayed statistically significant differences in the allelic frequencies of the loci tested. The data obtained from the investigated area through analysis did not reveal a close connection between geographical distances among populations and the degree of genetic differentiation.

The clusterization of *A. sibirica* populations at genetic distances D showed that the populations studied grouped for the most part according to whether they belonged to flatland or mountain populations. It was shown also that the heterogeneity of ecological conditions in various altitudinal zones of the Sayan Mountains makes an essential contribution to the genetic differentiation of *A. sibirica* populations.

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Appendix

Table 1. List of enzyme systems tested.

Enzyme	Abbreviation	Enzyme Commission number (EC)	Buffer
Malate dehydrogenase	MDH	1.1.1.37	I
Shikimate dehydrogenase	SKDH	1.1.1.25	I
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	I
Isocitrate dehydrogenase	IDH	1.1.1.42	I
Phosphoenolpyruvate carboxylase	PEPCA	4.1.1.31	I
Glutamate-oxaloacetate transaminase	GOT	2.6.1.1	II
Leucine aminopeptidase	LAP	3.4.11.1	II
Phosphoglucosomerase	PGI	5.3.1.9	II
Phosphoglucosomutase	PGM	2.7.5.1	III
Fluorescent esterase	FE	3.1.1.1	III
Glutamate dehydrogenase	GDH	1.4.1.2	III

