Studies on succinate dehydrogenase and its relationship with economic characters of silkworm *Bombyx mori* L.

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ABSTRACT

Four mulberry silkworm races viz., Pure Mysore, Nistari, NB4D2 & CSR2 and two hybrid (Pure Mysore × CSR2 and Nistari × NB4D2) silkworms were selected for the present study. The specific activity of succinate dehydrogenase (SDH) in the haemolymph as well as midgut tissue was estimated. The results of quantitative analysis were subjected for regression analysis against selected commercial characters viz., fecundity, larval weight, larval duration, single cocoon weight, single shell weight, shell ratio, filament length, denier and renditta, to know the correlation coefficient between them. The results of statistical analysis clearly showed that haemolymph and midgut SDH activity level has positive correlation with selected commercial characters except renditta. The qualitative analysis of SDH was carried out by Native-PAGE. The zymograms of succinate dehydrogenase also exhibited variation among the selected silkworm varieties.

Keywords: *Bombyx mori*, haemolymph, midgut, succinate dehydrogenase, Native -PAGE, commercial characters.

INTRODUCTION

The various aspects of metabolism have attracted the interest of many insect biochemists. Goldschmidt, Troland, Wright and many other investigators have given serious considerations to gene-enzyme hypothesis [1]. Enzymes are protein catalysts that accelerate the rates of biochemical reactions and regulate metabolic pathways. The succinate dehydrogenases (succinate: acceptor oxidoreductase; EC 1.3.99.1) is an enzyme complex, bound to the inner mitochondrial membrane of mammalian mitochondria, insects and many bacterial cells. It is the only enzyme that participates in both the citric acid cycle and the electron transport chain. In silkworm *Bombyx mori* most of the studies on the succinate dehydrogenase are limited to enzyme activity levels during cytoplasmic polyhedrosis [2] in F1 progeny raised from ethyl methanesulfonate treated larvae [3]; when silkworms exposed to organophosphorous insecticides [4] and during uzi infestation [5]. The analysis of enzymes like amylase, succinate dehydrogenase [6], alkaline phosphatase and alkaline protease [7] may help in the silkworm breeding programme for cocoon characters and disease resistance. However, studies combining biomolecules like succinate dehydrogenase with commercial characters of silkworm *Bombyx mori* are rather scarce. Hence, the present investigation was undertaken.

MATERIALS AND METHODS

Four mulberry silkworm races viz., Pure Mysore, Nistari, NB4D2 & CSR2 and two hybrid (Pure Mysore × CSR2 and Nistari × NB4D2) silkworms were used for the present investigation. The silkworm rearing was conducted in the laboratory following the method described by Krishnaswamy [8, 9]. The economic traits selected for present study included fecundity, weight of fifth instar larva, larval duration, single cocoon weight, single shell weight, shell ratio, filament length, denier and renditta. In each replication 500 larvae were kept after third moult.
The larvae from first day of fifth instar were collected daily with a regular interval of 24h till the end of fifth instar. The haemolymph was collected, centrifuged at 3000 rpm for 5 minutes in a cooling centrifuge at 5°C [6, 10, 11] and preserved in a deep freezer at -20°C as stock and it was used whenever required.

The midgut tissue was obtained from five larvae of fifth instar by dissecting the larvae in ice cold water and the gut contents were removed. The tissue was thoroughly washed in distilled water. A 10 % (w/v) homogenate of the midgut tissue was prepared in pre cooled distilled water using mortar and pestle. The homogenate was centrifuged at 3000 rpm for 10 minutes in a cooling centrifuge at 5°C. The clear supernatant was used for the enzyme analysis.

The total soluble protein present in the haemolymph as well as midgut tissue was estimated by following the method of Lowry et al. [12]. Bovine Serum Albumin was used as standard protein. Quantitative analysis of Succinate dehydrogenase activity was estimated by the method of Nachlas et al. [13]. The activity levels were expressed in micromoles of formazan formed/mg protein/min at 37°C.

The experimental data were statistically analyzed through SPSS by two way ANOVA [14], Scheffe’s post hoc test [15] and linear regression analysis [16] wherever they were applicable.

The qualitative analysis of SDH isozymes was carried out in Native Poly Acrylamide Gel Electrophoresis (PAGE) with the discontinuous buffer system containing 5% stacking and 8% separating gel. The vertical slab gel apparatus was used. The gels, soon after the removal, washed in running distilled water followed by the incubation in 100 ml of sodium phosphate buffer (50mM pH 7.0) containing sodium EDTA 400 mg, sodium succinate 250 mg, ATPNa2 50mg, NAD+ 70 mg, NBT 40mg and PMS 2mg at 37°C in rotary shaker in dark for 1 h or until the bands appeared [17]. Then the gels were scanned, analyzed and photographed in a gel scanner (Vilber Laurmat Bioprofil image analysis system).

RESULTS

The summary of the studied commercial characters are presented in the table1. From the table it is clear that the two bivoltine races are superior for productivity traits whereas multivoltines are superior for viability traits. The hybrids showed average values of their parents. The results of Two way ANOVA revealed that the variation in all commercial characters among the experimental batches are all significant at 0.1 % (P<0.001). The specific activity of succinate dehydrogenase (SDH) in haemolymph and midgut tissue samples is shown in the tables 2 & 3 respectively. The activity of SDH in haemolymph and midgut tissue samples showed significant changes in their levels at every 24 hours till the end of fifth instar. Almost similar trend was observed in both the tissues of all the experimental batches. The highest SDH activity in haemolymph was observed in PMXCSR2 (2.72 µM/mg/min at 37°C was the average during fifth instar), followed by NB4D2 (2.68 µM/mg/min at 37°C ), Pure Mysore ( 2.58 µM/mg/min at 37°C), CSR2 ( 2.57 µM/mg/min at 37°C), Nistari × NB4D2 ( 2.46 µM/mg/min at 37°C) and Nistari (1.87 µM/mg/min at 37°C). The specific activity of statistical analysis revealed that the variation among the experimental batches are all found to be significant at 0.1 % (P<0.001). In the case of midgut tissue, the highest activity of SDH was observed in NB4D2 (2.94 µM/mg/min at 37°C ), followed by CSR2 (2.90 µM/mg/min at 37°C), Nistari ( 2.86 µM/mg/min at 37°C), PMXCSR2 (2.83 µM/mg/min at 37°C), Pure Mysore ( 2.76 µM/mg/min at 37°C) and Nistari × NB4D2 ( 2.53 µM/mg/min at 37°C). The results of regression analysis between the haemolymph amylose activity levels and commercial characters are presented in figures 1-9. From the results of regression analysis, it is very clear that the activity of SDH in haemolymph with filament length (R²=0.415), denier (R²= 0.330), cocoon weight (R²=0.319), showed strong positive correlation. Also shell weight (R²=0.246), shell ratio (R²=0.214), larval weight (R²=0.19) and larval duration (R²=0.165) showed moderately positive relationships; whereas fecundity (R²=0.003) and renditta (Y= -6.297X+24.95) showed weak positive and negative relationships respectively. In the case of midgut tissue SDH and commercial characters, the results of regression analysis are presented in figures 10-18. From the results of statistical analysis, it is very clear that the correlation coefficient between the activity of SDH in midgut with denier (R²= 0.315) showed strong positive relationship. Also shell ratio (R²=0.188), shell weight (R²=0.183), fecundity (R²=0.112) and cocoon weight (R²=0.103) showed moderately positive relationships. On the other hand, larval weight (R²=0.066), filament length (R²=0.056) and larval duration (R²=0.004) showed weak positive relationships; whereas renditta (Y= -3.469+19.06) showed negative relationship.

A number of qualitative and quantitative variations were observed in the zymograms of haemolymph and midgut tissue SDH (figures 19-24). In the case of multivoltines, the haemolymph SDH isozymes in Pure Mysore exhibited entirely different pattern of banding when compared to Nistari silkworms. In the case of Pure Mysore larvae, an isozyme fraction with 0.399 R.F. exhibited more intensity during later days of fifth instar. Also, another band with R.F. 0.430 appeared prominently from 6th to 8th day; same band was paler on 1st and 2nd day and it was completely absent from 3rd to 5th day. However, in the case of Nistari three bands with R.F. 0.414, 0.452 and 489 were appeared
on 1st and 2nd days only. However, a band with R.F 0.452 was pale only on 6th day. Among the bivoltines, two bands with 0.509 and 0.873 R.F. in NB\textsubscript{D2}, one band with 0.391 R.F. in CSR\textsubscript{2} were more prominent. Of the hybrids, PMXCSR\textsubscript{2} showed one new band with 0.337 R.F. was appeared only on first day. Whereas in Nistari × NB\textsubscript{D2}, a band with 0.779 R.F. was more prominent. In the case of midgut SDH isozyme profiles of Pure Mysore and Nistari silkworms, the banding pattern was almost same. However, in Pure Mysore two bands with R.F. 0.326 and 0.413 showed variation in their intensities during fifth instar. In the case of Nistari silkworms an isozyme fraction with 0.423 R.F. exhibited gradual increment in their intensity as the age progressed. In the case of bivoltines, the isozyme profiles of NB\textsubscript{D2} showed two bands with 0.289 and 0.390 R.F. are more prominent in 3rd day. In the case of CSR\textsubscript{2} silkworms, a new isozyme fraction with 0.249 R.F. appeared only on the first day. Further, the intensity of two bands with 0.392 and 0.540 R.F. showed gradual reduction in their intensity from 1st day till the end of fifth instar. Among hybrids, PMXCSR\textsubscript{2} silkworms exhibited a new band with 0.300 R.F. on 5th and 6th days only. Whereas in the case of Nistari × NB\textsubscript{D2} larvae there is no significant variations. Among the silkworm varieties used in the present investigation the R. F. values and intensity/volume of the bands are different with each other.

**DISCUSSION**

Quantitative analysis of succinate dehydrogenase activity levels clearly indicated three types of correlations viz., positive, negative or neutral correlation between haemolymph and midgut SDH activity levels with commercial characters. In the present investigation, variation in the succinate dehydrogenase activity levels might be due to different genotype of the silkworm larvae [3, 18] used. Such alterations in the activity levels indicate the level of metabolism of a tissue or an organism. Enhanced rate of digestive and oxidizing enzymes in silkworm larvae might help in the utilization of more food material and efficient conservation of digested food material, ultimately leading to superior economic traits [3]. In contrast, during cytoplasmic polyhedrosis the SDH activity reduces significantly [2]. Observation on the SDH isozyme pattern has revealed that the banding pattern differs between pure races, between hybrids and between pure races and hybrids. The zymograms indicated the variation in R.F. and volume/intensity of the bands among the experimental silkworm varieties. The qualitative analysis of SDH indicated six types of changes i.e., the intensity of the bands either more or less, besides, some of the bands either present or absent. Some of the bands increased or decreased in their intensity as the age advances, in addition to altered R.F. value. Presence or absence of protein bands indicates either the non production or utilization or degradation of protein contents [3] when the studies are restricted within a particular race/breed. However, when the studies are concentrated between the races, it directly targets the genetic material as they are exactly determined by the genetic material of the organism. The variations in the activity levels and isozyme pattern clearly showed differences between the races. The variations in the activity levels of enzymes during present investigations clearly indicated both synthesis as well as utility of specific proteins.

![Figure 1: Correlation between haemolymph SDH activity level and fecundity](image)

y = 3.891x + 479.5

R\textsuperscript{2} = 0.003

SDH activity in haemolymph (µM/mg/min at 37°C) vs. Fecundity
Figure 2: Correlation between haemolymph SDH activity level and larval weight

Figure 3: Correlation between haemolymph SDH activity level and larval duration

Figure 4: Correlation between haemolymph SDH activity level and cocoon weight
Figure 5: Correlation between haemolymph SDH activity level and single shell weight

\[ y = 0.188x - 0.206 \]
\[ R^2 = 0.246 \]

Figure 6: Correlation between haemolymph SDH activity level and shell ratio

\[ y = 6.393x + 1.415 \]
\[ R^2 = 0.214 \]

Figure 7: Correlation between haemolymph SDH activity level and filament length

\[ y = 562.3x - 626.8 \]
\[ R^2 = 0.415 \]
**Figure 8:** Correlation between haemolymph SDH activity level and denier

**Figure 9:** Correlation between haemolymph SDH activity level and renditta

**Figure 10:** Correlation between midgut SDH activity level and fecundity
Figure 11: Correlation between midgut SDH activity level and larval weight

\[ y = 1.483x - 0.956 \]
\[ R^2 = 0.066 \]

Figure 12: Correlation between midgut SDH activity level and larval duration

\[ y = 18.18x + 539.8 \]
\[ R^2 = 0.004 \]
Figure 13: Correlation between midgut SDH activity level and cocoon weight

![Graph showing the correlation between midgut SDH activity level and cocoon weight with the equation $y = 0.343x - 0.703$ and $R^2 = 0.183$]

Figure 14: Correlation between midgut SDH activity level and shell weight

![Graph showing the correlation between midgut SDH activity level and shell weight with the equation $y = 12.70x - 18.34$ and $R^2 = 0.188$]

Figure 15: Correlation between midgut SDH activity level and shell ratio

![Graph showing the correlation between midgut SDH activity level and shell ratio with the equation $y = 0.343x - 0.703$ and $R^2 = 0.183$]
Figure 16: Correlation between midgut SDH activity level and filament length

\[ y = 438.1x - 460.4 \]
\[ R^2 = 0.056 \]

Figure 17: Correlation between midgut SDH activity level and denier

\[ y = 2.022x - 3.412 \]
\[ R^2 = 0.318 \]

Figure 18: Correlation between midgut SDH activity level and renditta

\[ y = -3.469x + 19.06 \]
\[ R^2 = 0.034 \]
Figure 19: Native PAGE analysis of haemolymph SDH of Pure Mysore and Nistari silkworms. Lanes: 1-8 days in fifth instar.

Figure 20: Native PAGE analysis of haemolymph SDH of NB4D2 and CSR2 silkworms. Lanes: 1-6 days in fifth instar.
Figure 21: Native PAGE analysis of haemolymph SDH of Pure Mysore X CSR2 and Nistari X NB4D2 silkworms. Lanes: 1-7 days in fifth instar.

Figure 22: Native PAGE analysis of midgut SDH of Pure Mysore and Nistari silkworms. Lanes: 1-8 days in fifth instar.
Figure 23: Native PAGE analysis of midgut SDH of NB, D2 and CSR2 silkworms. Lanes: 1-6 days in fifth instar.

Figure 24: Native PAGE analysis of midgut SDH of Pure Mysore X CSR2 and Nistari X NB, D2 silkworms. Lanes: 1-7 days in fifth instar.

Table 1: Mean values ± SD of nine commercial characters in six races of silkworm, Bombyx mori

<table>
<thead>
<tr>
<th>RACES</th>
<th>FECUNDITY</th>
<th>LARVAL WEIGHT (g)</th>
<th>LARVAL DURATION (h)</th>
<th>SINGLE COCOON WEIGHT (g)</th>
<th>SINGLE SHELL WEIGHT (g)</th>
<th>SHELL RATIO (%)</th>
<th>FILAMENT LENGTH (m)</th>
<th>DENIER</th>
<th>RENDITTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PURE MYSORE</td>
<td>467.22±4.10.96</td>
<td>2.0±0.06</td>
<td>660±10.39</td>
<td>1.02±0.75</td>
<td>0.12±0.01</td>
<td>12.57±0.49</td>
<td>426±44±19.83</td>
<td>1.77±0.09</td>
<td>11.77±0.82</td>
</tr>
<tr>
<td>NISTARI</td>
<td>485.11±5.30</td>
<td>2.83±0.06</td>
<td>564.88±10.01</td>
<td>1.14±0.71</td>
<td>0.15±0.01</td>
<td>13.41±0.87</td>
<td>435.66±17.21</td>
<td>1.78±0.07</td>
<td>13.2±0.24</td>
</tr>
<tr>
<td>CSR₂</td>
<td>509.10±16.58</td>
<td>4.07±0.05</td>
<td>578.88±6.45</td>
<td>1.8±0.47</td>
<td>0.43±0.01</td>
<td>24.02±0.18</td>
<td>1011.99±12.34</td>
<td>2.93±0.22</td>
<td>5.78±0.23</td>
</tr>
<tr>
<td>NB₂D₂</td>
<td>520.53±16.46</td>
<td>4.1±0.05</td>
<td>576.67±11.08</td>
<td>1.76±0.30</td>
<td>0.35±0.01</td>
<td>20.27±0.15</td>
<td>1026±29.96</td>
<td>2.48±0.06</td>
<td>8.34±0.47</td>
</tr>
<tr>
<td>PURE MYSORE X CSR₂</td>
<td>466.66±11.52</td>
<td>2.68±0.07</td>
<td>610±11.10</td>
<td>1.67±0.23</td>
<td>0.28±0.01</td>
<td>17.29±0.21</td>
<td>910±18.74</td>
<td>2.75±0.06</td>
<td>7.6±0.12</td>
</tr>
<tr>
<td>NISTARI X NB₂D₂</td>
<td>490.77±6.81</td>
<td>3.46±0.04</td>
<td>557±10.21</td>
<td>1.47±0.22</td>
<td>0.23±0.01</td>
<td>16.0±0.85</td>
<td>805.99±12.36</td>
<td>1.83±0.02</td>
<td>9.22±0.85</td>
</tr>
</tbody>
</table>

Values are the mean ± SD of Per monoos, Monsoon and post monsoon observations.

Table 2: Succinate dehydrogenase (SDH) activity levels (µ moles of formazan formed/mg protein/min at 37°C) in the haemolymph during fifth instar

<table>
<thead>
<tr>
<th>RACES</th>
<th>1st Day</th>
<th>2nd Day</th>
<th>3rd Day</th>
<th>4th Day</th>
<th>5th Day</th>
<th>6th Day</th>
<th>7th Day</th>
<th>8th Day</th>
<th>AVERAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>2.39</td>
<td>2.45</td>
<td>2.52</td>
<td>2.65</td>
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<td>2.51</td>
<td>2.73</td>
<td>2.90</td>
<td>2.58</td>
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<tr>
<td>NISTARI</td>
<td>1.52</td>
<td>1.87</td>
<td>2.18</td>
<td>1.82</td>
<td>2.21</td>
<td>1.82</td>
<td>1.82</td>
<td>1.82</td>
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</tr>
<tr>
<td>CSR₂</td>
<td>2.62</td>
<td>2.25</td>
<td>2.86</td>
<td>2.38</td>
<td>2.31</td>
<td>2.37</td>
<td>2.37</td>
<td>2.37</td>
<td>2.37</td>
</tr>
<tr>
<td>NB₂D₂</td>
<td>2.57</td>
<td>2.70</td>
<td>2.86</td>
<td>2.38</td>
<td>2.31</td>
<td>2.37</td>
<td>2.37</td>
<td>2.37</td>
<td>2.37</td>
</tr>
<tr>
<td>PM x CSR₂</td>
<td>2.43</td>
<td>2.74</td>
<td>2.94</td>
<td>2.65</td>
<td>2.52</td>
<td>2.52</td>
<td>2.52</td>
<td>2.52</td>
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</tr>
<tr>
<td>NISTARI x NB₂D₂</td>
<td>1.87</td>
<td>1.98</td>
<td>2.38</td>
<td>2.60</td>
<td>2.98</td>
<td>2.97</td>
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</tr>
</tbody>
</table>

The variation between the races is statistically significant at 0.1% (P<0.001).

Values within parentheses represent percent change over previous day.

Table 3: Succinate dehydrogenase (SDH) activity levels (µ moles of formazan formed/mg protein/min at 37°C) in the midgut tissue during fifth instar

<table>
<thead>
<tr>
<th>RACES</th>
<th>1st Day</th>
<th>2nd Day</th>
<th>3rd Day</th>
<th>4th Day</th>
<th>5th Day</th>
<th>6th Day</th>
<th>7th Day</th>
<th>8th Day</th>
<th>AVERAGE</th>
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<td>2.57</td>
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<td>2.59</td>
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<td>2.63</td>
<td>2.63</td>
<td>2.63</td>
<td>2.63</td>
</tr>
<tr>
<td>NISTARI</td>
<td>2.93</td>
<td>3.33</td>
<td>3.27</td>
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<td>3.27</td>
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<tr>
<td>NB₂D₂</td>
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<tr>
<td>PM x CSR₂</td>
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</tr>
<tr>
<td>NISTARI x NB₂D₂</td>
<td>2.37</td>
<td>2.47</td>
<td>2.47</td>
<td>2.47</td>
<td>2.47</td>
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</tbody>
</table>

The variation between the races is statistically significant at 0.1% (P<0.001).

Values within parentheses represent percent change over previous day.

CONCLUSION

The present results clearly indicated that the haemolymph SDH activity levels exhibited moderately strong positive correlation with cocoon and filament traits. The midgut SDH activity exhibited moderately strong positive correlation with denier only. Hence, by studying the silkworm SDH with commercial characters, it is possible to have a clear picture about the level and kind of relationship between them. An understanding of such relationships will help us to identify and exploit the marker molecule during the evolution of new races of silkworm Bombyx mori with improved economic traits.

Acknowledgments

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