

**Table 1. Utilization of carbon sources by *Rhizobium leguminosarum* BIHB 645 as recorded using BIOLOG**

Carbon source	Test	Carbon source	Test	Carbon source	Test
$\alpha$ -cyclodextrin	-	D-raffinose	-	$\beta$ -hydroxy butyric acid	-
Dextrin	+	L-rhamnose	-	$\gamma$ -hydroxy butyric acid	-
Glycogen	-	D-sorbitol	-	Glucose-6-phosphate	-
Tween 40	-	Sucrose	-	Itaconic acid	-
Tween 80	-	D-trehalose	-	$\alpha$ -keto butyric acid	-
N-acetyl-D-galactosamine	-	Turanose	-	$\alpha$ -keto glutaric acid	-
N-acetyl-D-glucosamine	-	Xylitol	-	$\alpha$ -keto valeric acid	-
Adonitol	-	Methyl pyruvate	-	DL lactic acid	-
L-arabinose	+	Mono-methyl-succinate	-	Malonic acid	-
D-arabitol	-	Acetic acid	-	Propionic acid	-
Cellobiose	-	Cis-aconitic acid	-	Quinic acid	-
Erthryritol	-	Citric acid	-	D-saccharic acid	-
D-fructose	-	D-psicose	-	Sebacic acid	-
L-Fucose	-	Formic acid	+	Succinic acid	-
D-galactose	-	D-galactonic acid lactone	+	Bromo succinic acid	-
Gentiobiose	-	D-galacturonic acid	-	Siccinamic acid	-
$\alpha$ -D-glucose	-	D-gluconic acid	-	Glucuronamide	-
m-inositol	-	D-glucosaminic acid	-	Alaninamide	-
$\alpha$ -D-lactose	-	D-glucuronic acid	-	D-alanine	-
Lactulose	-	$\alpha$ -hydroxy butyric acid	-	L-alanine	+
Maltose	-	$\beta$ -methyl D-glucoside	+	L-alanyl glycine	-

Mannitol	-	L-asparagine	-	L-pyroglutamic acid	-
Mannose	-	L-aspartic acid	-	D-serine	-
Melibiose	-	L-glutamic acid	-	L-serine	-
L-leucine	-	Glycyl-L-aspartic acid	-	L-threonine	-
L-ornithine	-	Glycyl-L-glutamic acid	-	DL-carnitine	-
L-phenyl alanine	-	L-histidine	-	$\gamma$ -amino butyric acid	-
L-proline	-	Hydroxyl L-proline	-	Urocanic acid	+
Thymidine	-	2-amino ethanol	+	Inosine	-
Phenyl ethylamine	-	2,3-butanediol	-	Uridine	-
Putrescine	+	Glycerol	+	Glucose-6-phosphate	-
p-hydroxy phenylacetic acid	-	DL $\alpha$ -glycerol phosphate	-		

**Table 2. Fatty acid methyl ester composition of *Rhizobium leguminosarum* BIHB 645**

Fatty acid composition			
Name	%	Name	%
10:00	0	16:00	6.61
10:0 3 OH	0	17:1 w8c	0
12:00	0	17:00	0
12:0 2OH	0	18:1 iso H	0
12:1 3 OH	0	18:1 w7c 11-methyl	2.34
14:00	0	18:00	6.01
15:0 iso	0	20:1 w7c	0
15:0 2OH	0.51		
16:0 iso	0		
16:1 w5c	0		

**Table 3. Specific growth rate of *Rhizobium leguminosarum* BIHB 645 .**

Medium	Specific growth rate ( $\mu$ )
Yeast Mannitol Broth (YMB)	0.28
YMB except K <sub>2</sub> HPO <sub>4</sub>	0.35
YMB except MgSO <sub>4</sub>	0.25
YMB except NaCl	0.26
YMB except CaCO <sub>3</sub>	0.28
YMB except Yeast extract	0.22
YMB with 7.5g/l Mannitol	0.28
YMB with 5.0g/l Mannitol	0.31
YMB with 2.5g/l Mannitol	0.28

**Table 4. Actual and predicted values of biomass for *Rhizobium leguminosarum* BIHB 645 in the medium with mannitol as a carbon source in experimental setup using Response Surface Methodology**

Run No.	Biomass (g/l)	
	Actual	Predicted
<b>1</b>	0.400	0.352
<b>2</b>	0.330	0.420
<b>3</b>	1.200	1.110
<b>4</b>	1.700	1.748
<b>5</b>	0.369	0.503
<b>6</b>	0.960	0.956
<b>7</b>	0.000	0.044

<b>8</b>	0.391	0.257
<b>9</b>	0.660	0.573
<b>10</b>	1.730	1.686
<b>11</b>	0.000	0.004
<b>12</b>	0.930	1.016
<b>13</b>	1.730	1.628
<b>14</b>	1.690	1.628
<b>15</b>	1.300	1.628
<b>16</b>	1.720	1.628
<b>17</b>	1.700	1.628

**Table 5. The coefficients of regression for the biomass production of *Rhizobium leguminosarum* BIHB 645 in the medium with mannitol using Response Surface Methodology**

<b>Term</b>	<b>Coefficient</b>
Constant	-7.262***
Mannitol	0.953***
Yeast extract	1.030*
Temperature	0.424***
Mannitol*Mannitol	-0.090***
Yeast extract* Yeast extract	-0.285*
Temperature*Temperature	-0.008***

Mannitol*Yeast extract	0.076 *
Mannitol*Temperature	-0.002
Yeast extract*Temperature	-0.005
<b>R<sup>2</sup></b>	<b>96.9%</b>

\* $P \leq 0.5$ ; \*\*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.005$

**Table 6. Validation of the model used for the biomass production of *Rhizobium leguminosarum* strain BIHB 645 in the medium with Mannitol using Response Surface Methodology**

<b>Replicates</b>	<b>Biomass (g/l)</b>
I	1.8
II	1.7
III	1.9
IV	2.0
V	1.9
<b>Mean</b>	<b>1.86</b>
<b>Standard error of mean</b>	<b>0.065</b>

**Table 7. Actual and predicted values of biomass for *Rhizobium leguminosarum* BIHB 645 in medium with molasses as a carbon source in experimental setup using Response Surface Methodology**

RUN No.	Biomass (g/l)	
	Actual	Predicted
1	0.320	0.429
2	1.640	1.763
3	0.564	0.441
4	1.960	1.851
5	0.300	0.306
6	1.400	1.393
7	0.530	0.537
8	2.200	2.194
9	0.232	0.117
10	0.100	0.217
11	0.800	0.683
12	0.567	0.683
13	1.300	1.422
14	1.500	1.422
15	1.350	1.422
16	1.560	1.422
17	1.400	1.422

**Table 8. The coefficients of regression for the biomass production of *Rhizobium leguminosarum* BIHB 645 in the medium with molasses using Response Surface Methodology**

<b>Term</b>	<b>Coefficient</b>
Constant	4.193*
Molasses	- 0.109*
Yeast extract	1.836***
Temperature	0.350***
Molasses* Molasses	0.008**
Yeast extract * Yeast extract	- 0.874***
Temperature* Temperature	- 0.006***
Molasses* Yeast extract	0.005
Molasses* Temperature	0.003 *
Yeast extract * Temperature	- 0.004 ***
<b>R<sup>2</sup></b>	<b>97.7%</b>

**\* $P \leq 0.5$ ; \*\*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.005$**

**Table 9. Validation of the model used for the biomass production of *Rhizobium leguminosarum* BIHB 645 in the medium with molasses using Response Surface Methodology**

<b>Replicates</b>	<b>Biomass (g/l)</b>
I	2.1
II	2.2
III	2.1
IV	2.3
V	2.3
<b>Mean</b>	<b>2.2</b>
<b>Standard error of mean</b>	<b>0.057</b>