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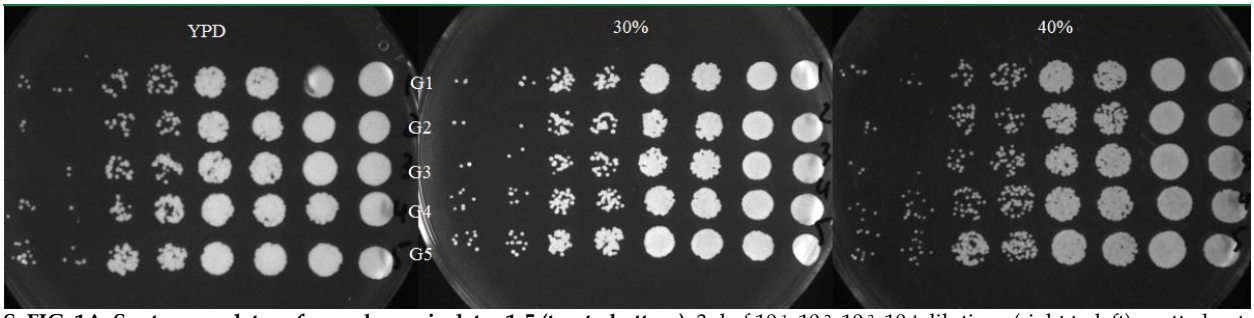
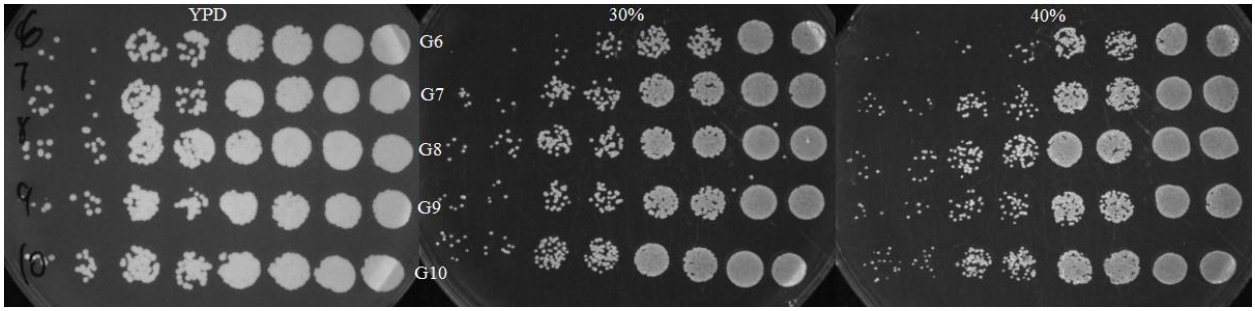
Yeasts from Greenhouse Grapes Show Less Phenotypic and Genetic Diversity than Yeasts from Vineyard Grapes when Isolated from Grape Crush Cultured in Liquid Media

Brittany Goldhawke, Manjot Kahlon, Jeremy Lotto, and Christoph M. Deeg
Department of Microbiology and Immunology, University of British Columbia

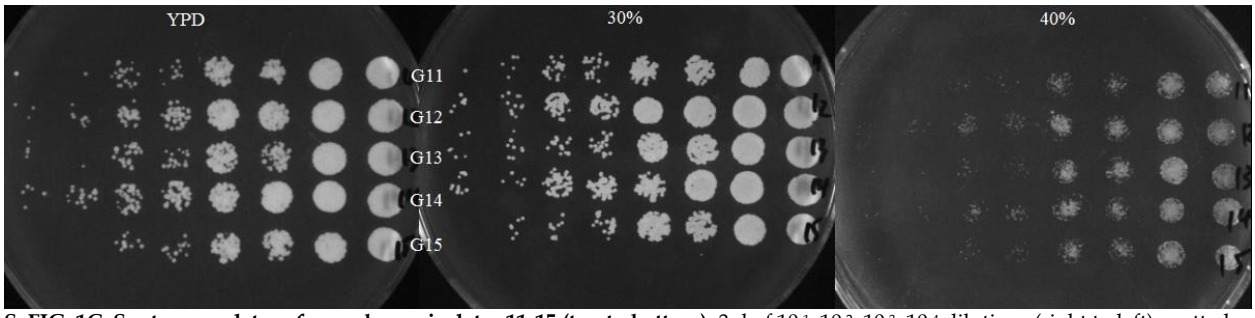
SUPPLEMENTAL MATERIAL



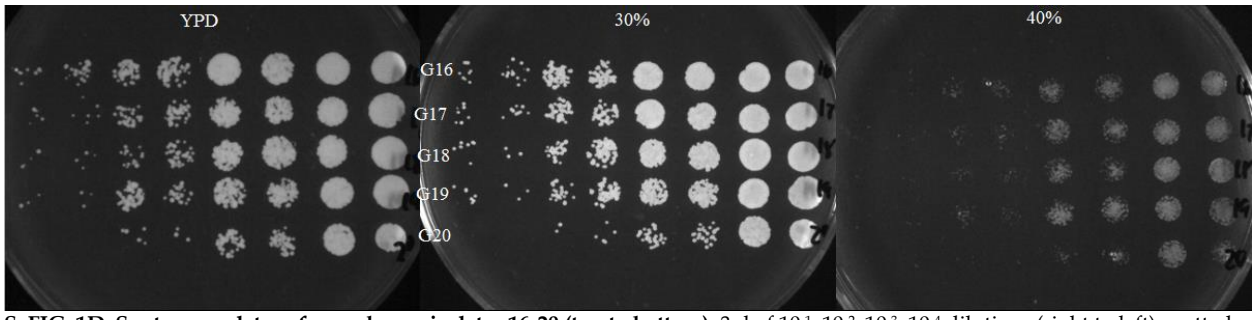
S. FIG. 1A. Spot assay plates of greenhouse isolates 1-5 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 30% Dextrose-YPD agar, 40% Dextrose agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.1, FIG.2).



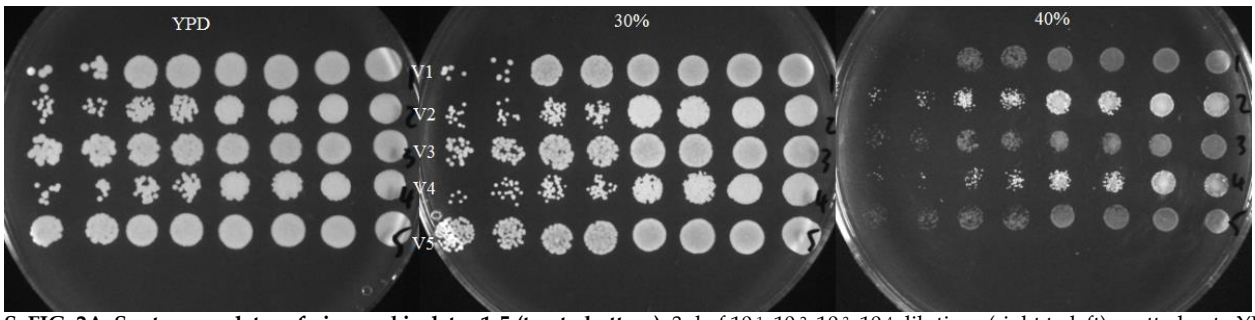
S. FIG. 1B. Spot assay plates of greenhouse isolates 6-10 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 30% Dextrose-YPD agar, 40% Dextrose agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.1, FIG.2).



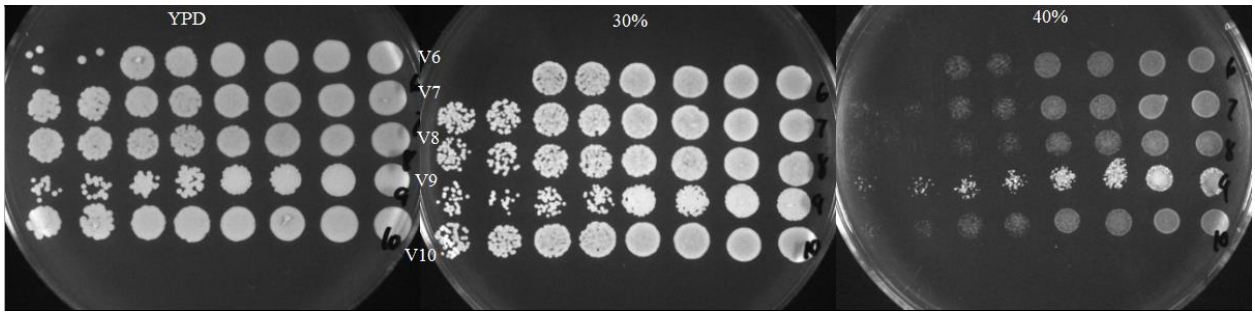
S. FIG. 1C. Spot assay plates of greenhouse isolates 11-15 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 30% Dextrose-YPD agar, 40% Dextrose agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.1, FIG.2).



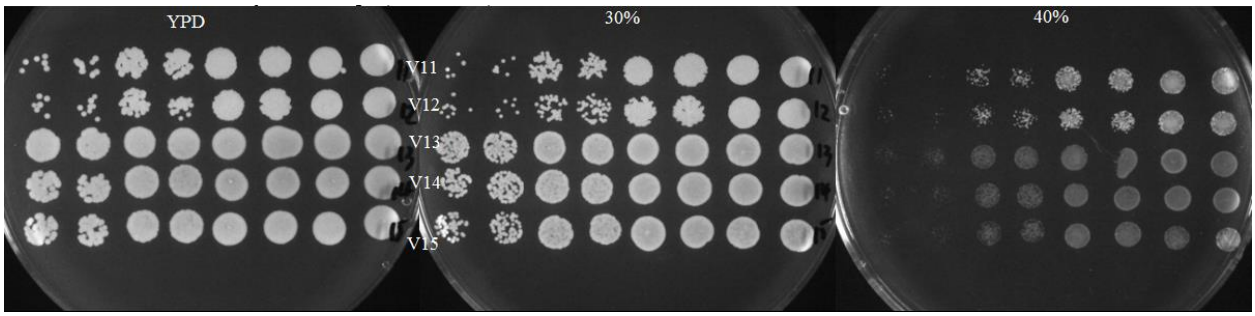
S. FIG. 1D. Spot assay plates of greenhouse isolates 16-20 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 30% Dextrose-YPD agar, 40% Dextrose agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.1, FIG.2).



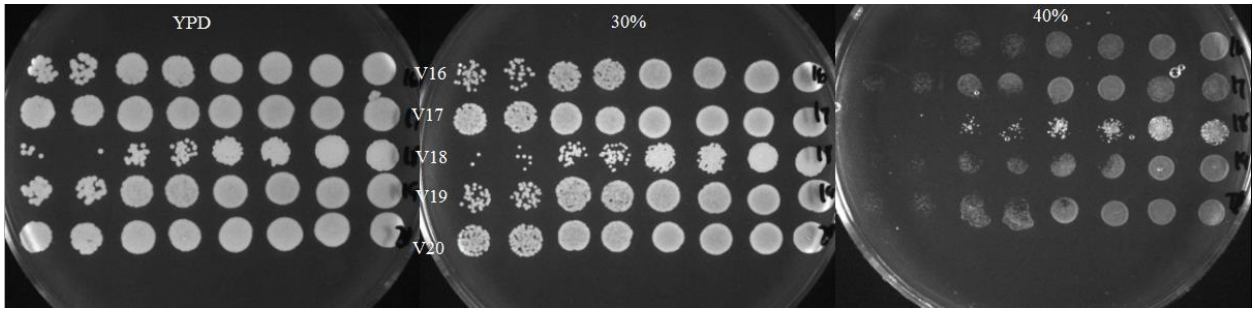
S. FIG. 2A. Spot assay plates of vineyard isolates 1-5 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 30% Dextrose-YPD agar, 40% Dextrose agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.2, FIG.2).



S. FIG. 2B. Spot assay plates of vineyard isolates 6-10 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 30% Dextrose-YPD agar, 40% Dextrose agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.2, FIG.2).



S. FIG. 2C. Spot assay plates of vineyard isolates 11-15 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 30% Dextrose-YPD agar, 40% Dextrose agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.2, FIG.2).



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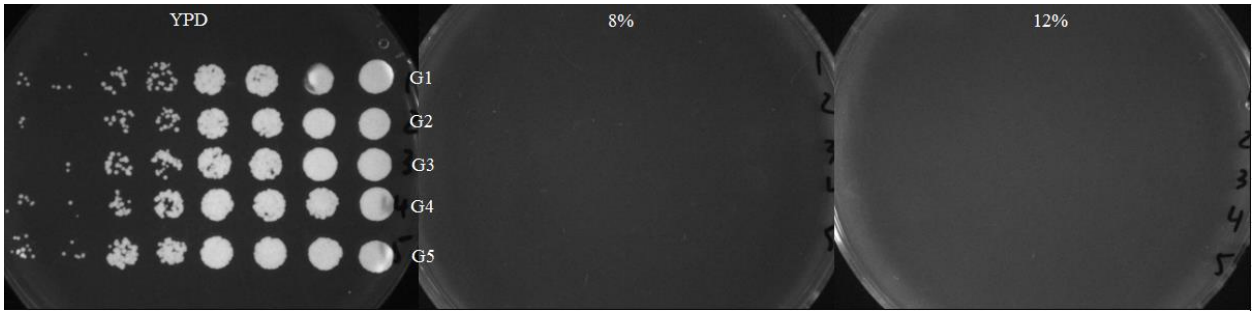
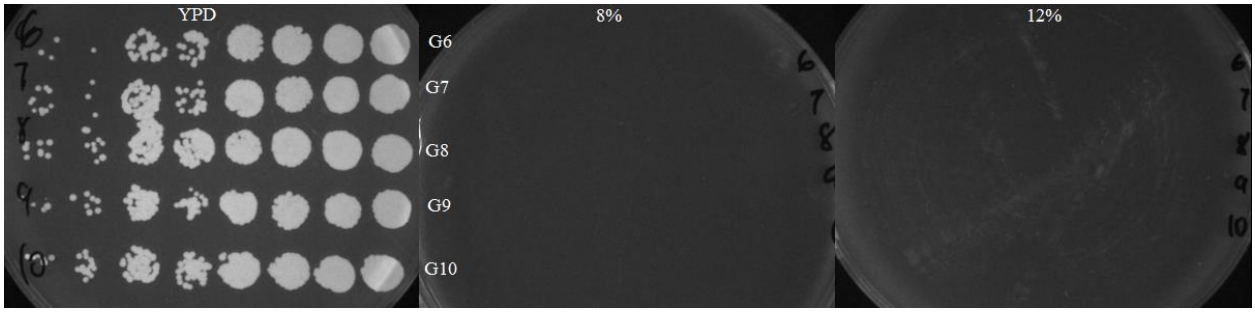


S. FIG. 3A. Spot assay plates of greenhouse isolates 1-5 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 8% ethanol-YPD agar, 12% ethanol-YPD agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.1, FIG.2)



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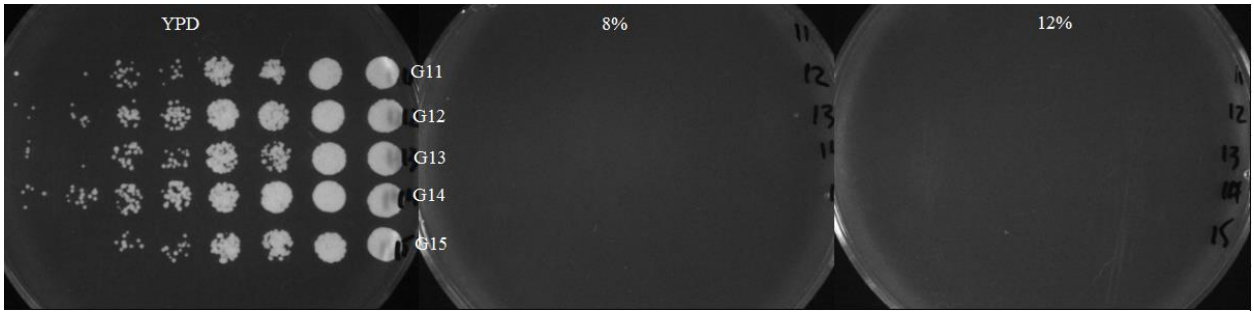

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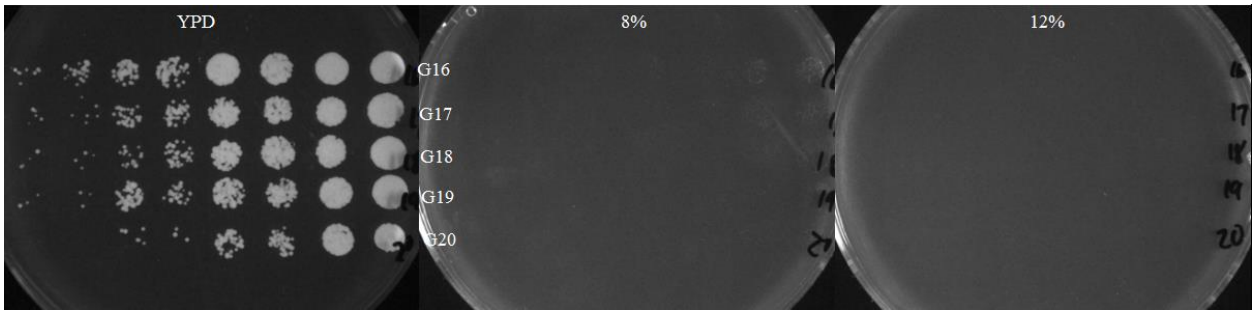

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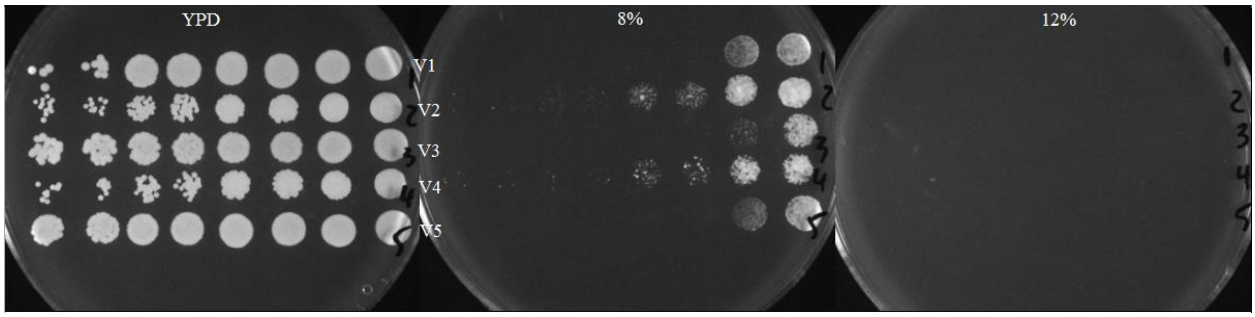
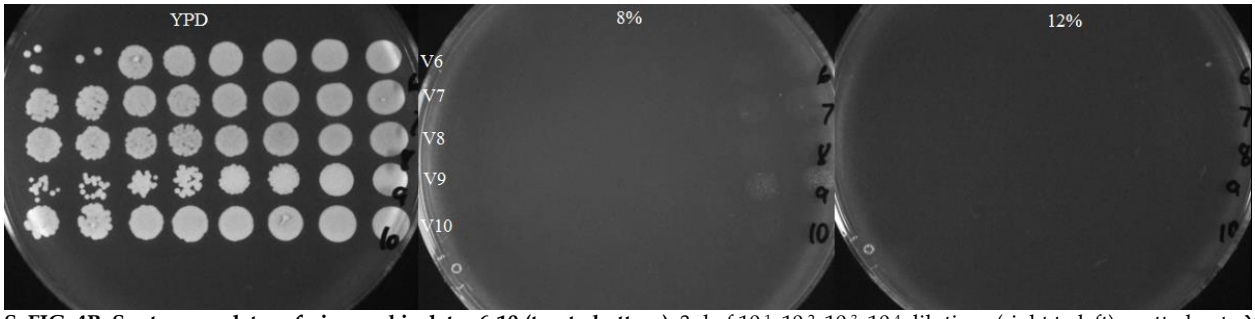
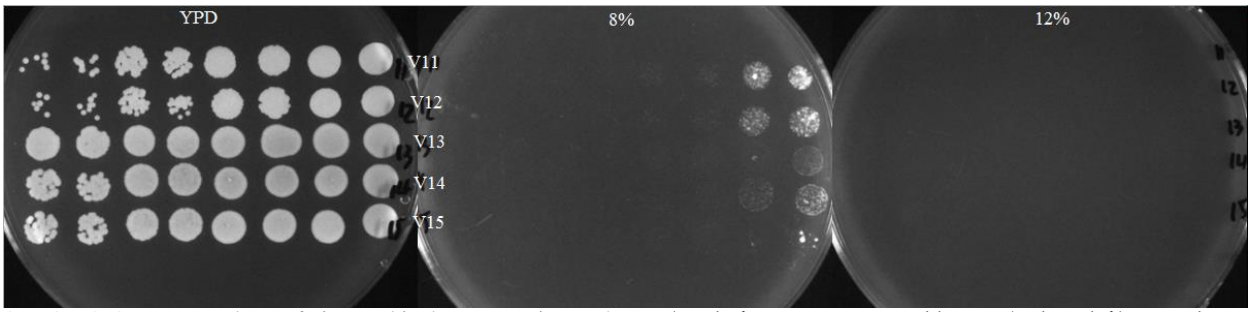


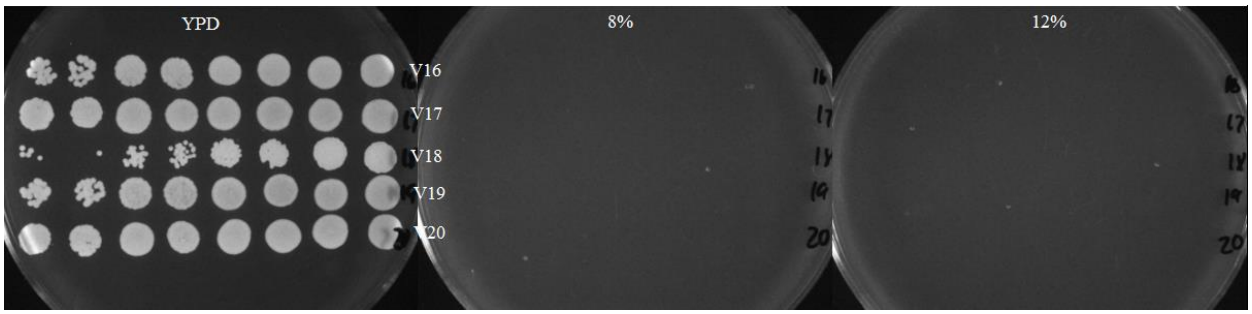
S. FIG. 4A. Spot assay plates of vineyard isolates 1-5 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 8% ethanol-YPD agar, 12% ethanol-YPD agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.2, FIG.2)



S. FIG. 4B. Spot assay plates of vineyard isolates 6-10 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 8% ethanol-YPD agar, 12% ethanol-YPD agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE2, FIG.2)



S. FIG. 4C. Spot assay plates of vineyard isolates 11-15 (top to bottom). 3µl of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} dilutions (right to left) spotted onto YPD agar, 8% ethanol-YPD agar, 12% ethanol-YPD agar (left to right) in duplicate, grown at 30°C for 24 hours. Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively. No growth denoted as "0" (S.TABLE.2, FIG.2)



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S.TABLE 1 Growth response of greenhouse isolates in dextrose and ethanol YPD media

	8% * ethanol	12% * ethanol	30%* glucose	40%* glucose
G1	0	0	4	4
G2	0	0	4	4
G3	0	0	4	4
G4	0	0	4	4
G5	0	0	4	4
G6	0	0	4	4
G7	0	0	4	4
G8	0	0	4	4
G9	0	0	4	4
G10	0	0	4	4
G11	0	0	4	3
G12	0	0	4	3
G13	0	0	4	3
G14	0	0	4	3
G15	0	0	3	3
G16	0	0	4	3
G17	0	0	4	3
G18	0	0	4	3
G19	0	0	4	3
G20	0	0	3	2

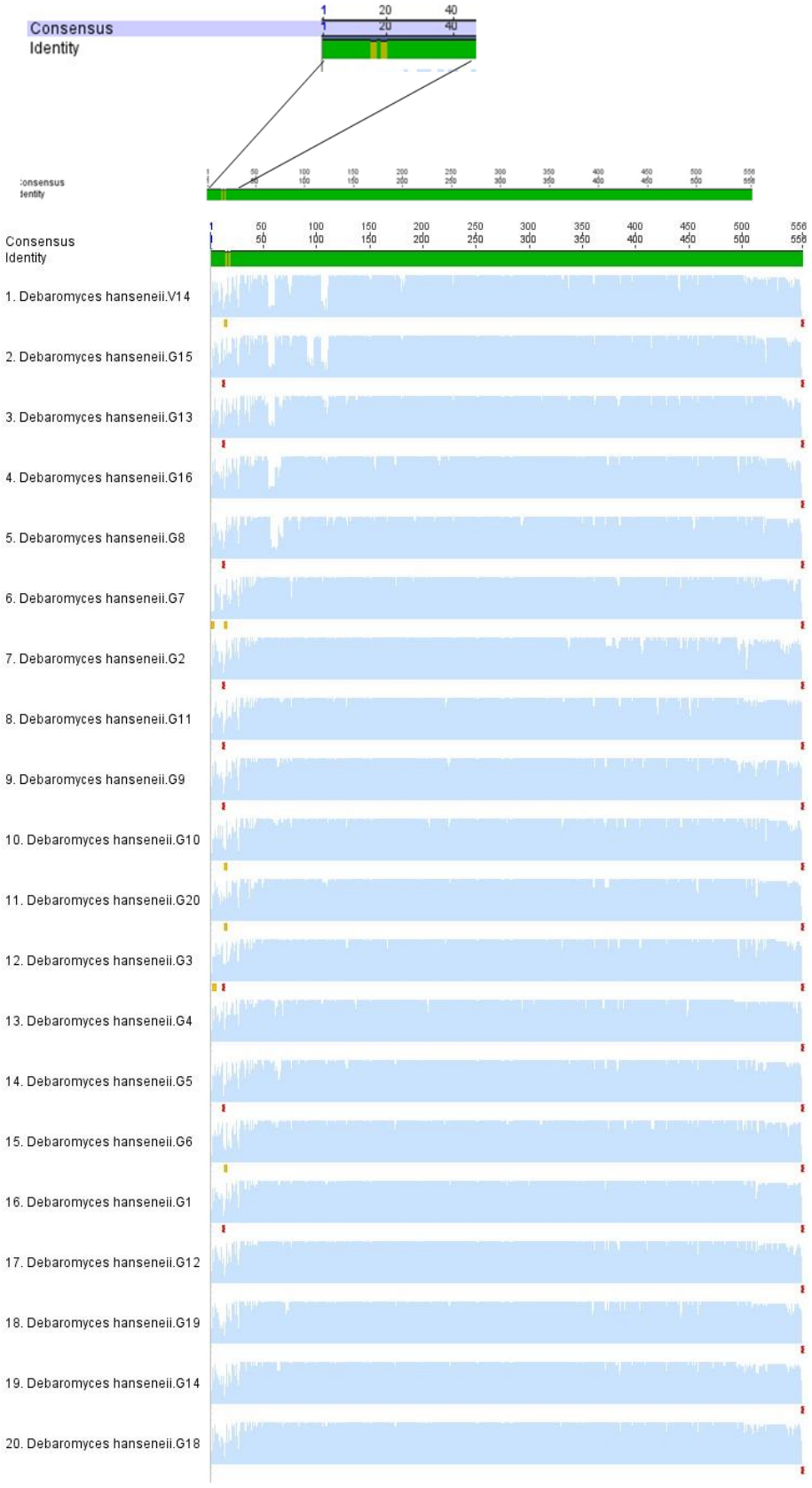
*8%, 12%, 30%, 40% was the final concentration of ethanol or dextrose in YPD media
Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively.
No growth denoted as "0" (S.FIG.1-4, FIG.2)

S.TABLE 2 Growth response of vineyard isolates in dextrose and ethanol

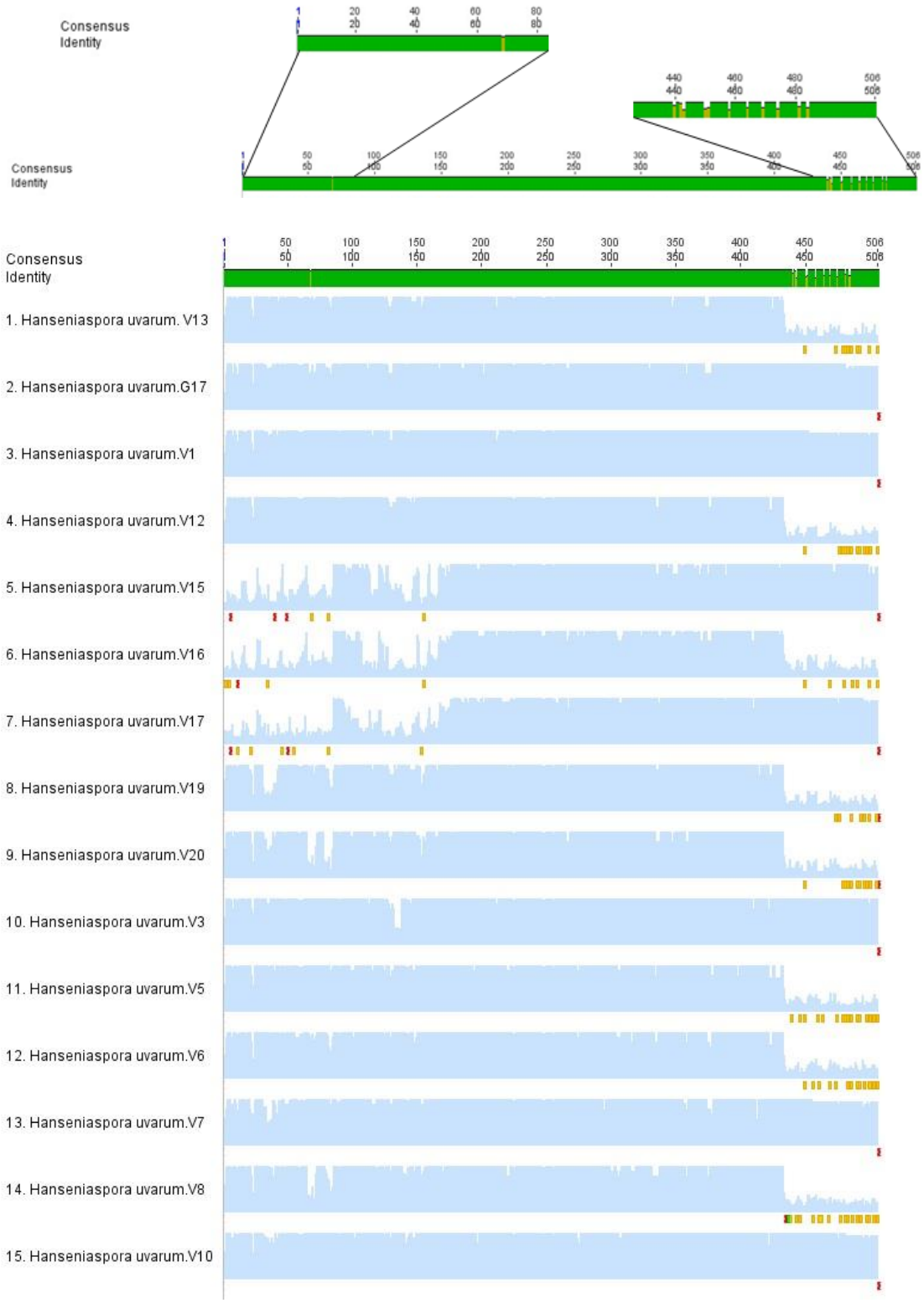
	8% * ethanol	12%* ethanol	30%* dextrose	40%* dextrose
V1	1	0	4	3
V2	2	0	4	4
V3	1	0	4	3
V4	2	0	4	3
V5	1	0	4	3
V6	0	0	3	3
V7	0	0	4	3
V8	0	0	4	3
V9	0	0	4	4
V10	0	0	4	4
V11	1	0	4	3
V12	1	0	4	3
V13	0	0	4	3
V14	1	0	4	3
V15	0	0	4	3
V16	0	0	4	3
V17	0	0	4	4
V18	0	0	4	3
V19	0	0	4	3
V20	0	0	4	4

*8%, 12%, 30%, 40% was the final concentration of ethanol or dextrose in YPD media
Growth at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} is denoted "1", "2", "3", and "4" respectively.
No growth denoted as "0" (S.FIG.1-4, FIG.2)

A.


Consensus
Identity
1 20 40
1 20 40
Consensus
Identity
1 50 100 150 200 250 300 350 400 450 500 556
1 50 100 150 200 250 300 350 400 450 500 556
1. Debaromyces hanseneii.V14
2. Debaromyces hanseneii.G15
3. Debaromyces hanseneii.G13
4. Debaromyces hanseneii.G16
5. Debaromyces hanseneii.G8
6. Debaromyces hanseneii.G7
7. Debaromyces hanseneii.G2
8. Debaromyces hanseneii.G11
9. Debaromyces hanseneii.G9
10. Debaromyces hanseneii.G10
11. Debaromyces hanseneii.G20
12. Debaromyces hanseneii.G3
13. Debaromyces hanseneii.G4
14. Debaromyces hanseneii.G5
15. Debaromyces hanseneii.G6
16. Debaromyces hanseneii.G1
17. Debaromyces hanseneii.G12
18. Debaromyces hanseneii.G19
19. Debaromyces hanseneii.G14
20. Debaromyces hanseneii.G18

B.


Consensus
Identity
Consensus
Identity
Consensus
Identity
1. Hanseniaspora uvarum. V13
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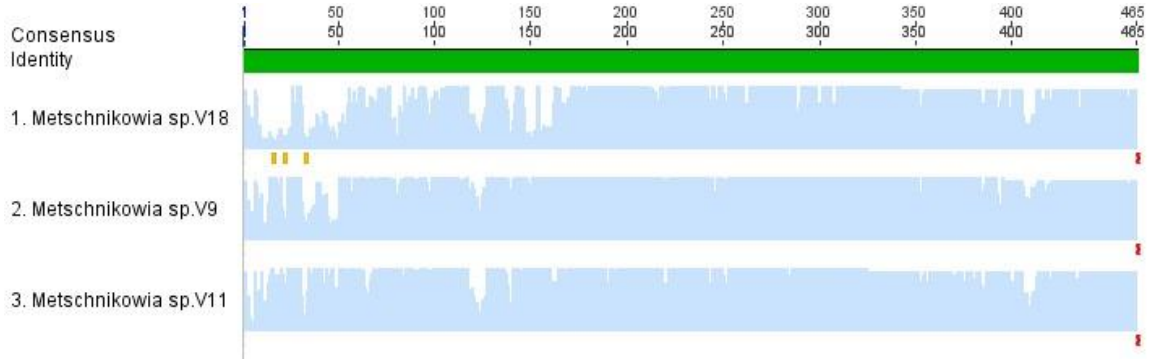
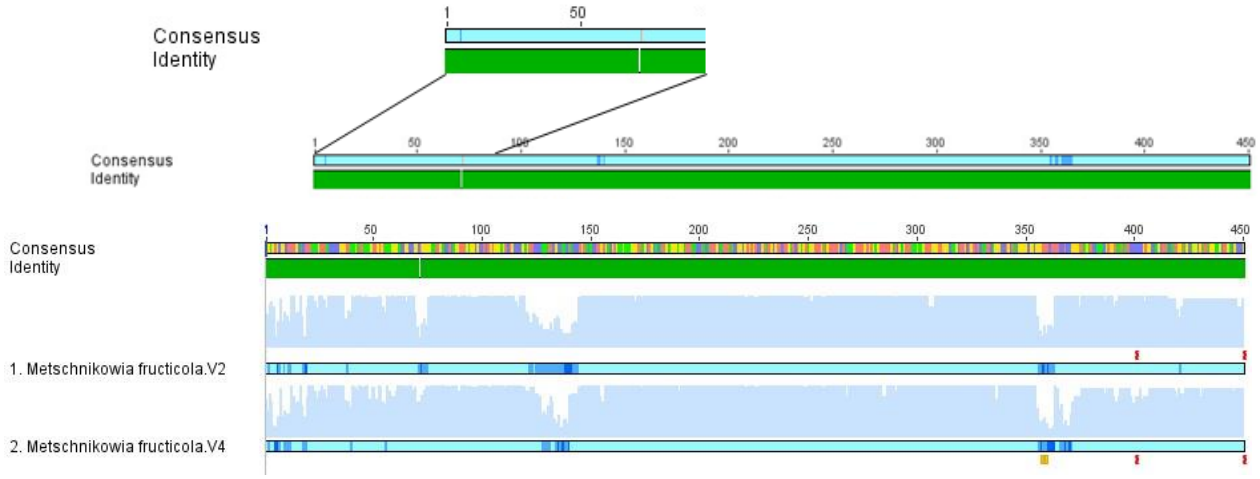
3. Hanseniaspora uvarum.V1
4. Hanseniaspora uvarum.V12
5. Hanseniaspora uvarum.V15
6. Hanseniaspora uvarum.V16
7. Hanseniaspora uvarum.V17
8. Hanseniaspora uvarum.V19
9. Hanseniaspora uvarum.V20
10. Hanseniaspora uvarum.V3
11. Hanseniaspora uvarum.V5
12. Hanseniaspora uvarum.V6
13. Hanseniaspora uvarum.V7
14. Hanseniaspora uvarum.V8
15. Hanseniaspora uvarum.V10

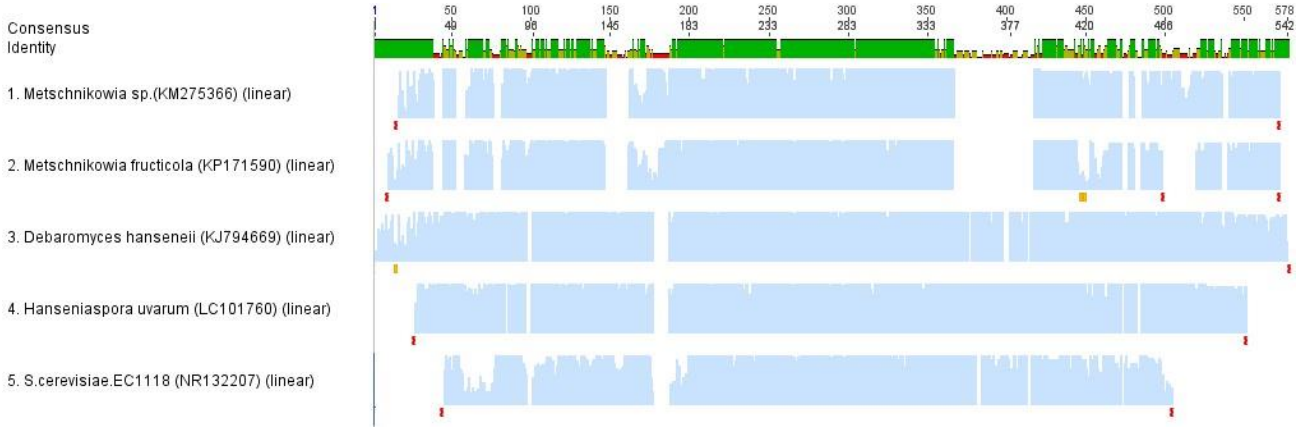
C.



D.



S.FIG 5. Geneious 9.0.4 trimmed chromatograms and MUSCLE multiple alignment of sequences. Mean pairwise identity over all pairs in the column is provided. Green indicates 100% identity, green-brown indicates 30%-100%, and red indicates below 30% identity (A) *D. hanseneii* isolates G1-G20, and V14. Length: 556, Sequences: 20, Identical Sites: 552 (99.3%), Pairwise % Identity: 99.9%. (B) *H.uvarum* isolates V1, 3, 5-8, 10, 13, 15-17, 19-20. Length: 506, Sequences: 15, Identical Sites: 494 (97.6%), Pairwise % Identity: 99.1%. (C) *Metschnikowia sp.* isolates V9, 11, 18. Length: 465, Sequences: 3, Identical Sites: 465 (100.0%), Pairwise % Identity: 100.0%. (D) *M.fructicola* isolates V2, V4. Length: 450, Sequences: 2, Identical Sites: 449 (99.8%), Pairwise % Identity: 99.8%.



S.FIG 6. Geneious 9.0.5 trimmed chromatograms and MUSCLE multiple alignment of 5 sequences chosen with a high HQ%, used with RAxML to construct the phylogenetic tree in FIG.4. Mean pairwise identity over all pairs in the column is provided. Green indicates 100% identity, green-brown indicates 30%-100%, and red indicates below 30%. D. hanseneii isolate G14, H.uvarum isolates V10. *Metschnikowia* isolate V11, *M.fructicola* isolate V4. Length: 578, Sequences: 5, Identical Sites: 328 (58.2%), Pairwise % Identity: 71.5%