**Effect of a single point mutation on the interaction of glucans with a glucansucrase from
Leuconostoc mesenteroides NRRL B-1118**

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Abstract

Our previous work showed that substitution of an amino acid that is coupled with the +2 subsite adjacent to the transition stabilizer of a glucansucrase, which produces a water-insoluble glucan, resulted in significant changes in the structures and yields of the water-insoluble glucans produced. We now describe how these changes affect the ability of the glucansucrase to bind to exogenous glucans, and how these glucans can influence the yield, product structures, and kinetics of the mutant glucansucrases. The activity of the wild-type enzyme, with threonine at position 654, is not significantly activated by added dextran, and the yield of water-insoluble glucan from sucrose is only slightly increased by dextran. Mutant T654Y is not affected at all by the addition of dextran. However, several mutant enzymes exhibit markedly lower yields of glucan relative to the wild type; these lower yields can be partially or completely overcome by the addition of water-soluble dextran. Although evidence indicates that the soluble dextran is incorporated into water-insoluble glucan, the increased yields cannot be accounted for solely by incorporation of the dextran into insoluble product. Furthermore, these DsrI mutants are significantly activated by exogenous glucans. The addition of dextran does not markedly change the K_M for sucrose in the mutant enzymes, but does increase the V_{max} of the reaction. These effects apparently depend on the presence of unbranched sequences of $\alpha 1 \rightarrow 6$ -linked D-glucose units in the glucan.

Introduction

Leuconostoc mesenteroides strain NRRL B-1118 produces at least two extracellular glucosyltransferases: a dextranucrase (**DsrS**, EC 2.4.1.5), which produces a water-soluble dextran linked predominantly $\alpha 1 \rightarrow 6$, and a glucansucrase (**DsrI**, EC 2.4.1.125), which produces a water-insoluble glucan with both $\alpha 1 \rightarrow 6$ -linked domains and $\alpha 1 \rightarrow 3$ -linked domains.¹ We have previously cloned and expressed these enzymes in *Escherichia coli*¹ as well as in *Lactococcus lactis*,² and demonstrated that certain mutations at threonine 654 in the latter enzyme, DsrI, significantly affect the yields and structures of the insoluble glucans produced. In particular, mutants T654I, T654K, T654R, T654Q and T654N produced insoluble glucans with higher percentages of $\alpha 1 \rightarrow 3$ linkages, albeit in much lower yields.³ Furthermore, these same DsrI mutants had increased activity in the presence of soluble dextran, while the native DsrI and most of the other mutant enzymes were unaffected. Similar activation by dextran has been described with numerous glucansucrases from *Streptococcus* and *L. mesenteroides* that include enzymes producing both soluble and insoluble glucan.⁴⁻¹¹ The mechanism of this activation is currently unknown, but many early theories originally postulated that dextran served as primer during the glucosyl transfer from sucrose to the growing glucan chain. The glucan binding domain for this dextran-stimulated transfer is critical as demonstrated by the complete loss of activation when the C-terminal glucan binding domain was removed from the *S. sobrinus* GTF-I (glucosyltransferase, insoluble-glucan-producing).⁷ In addition, replacing the glucan binding

domain of the primer-independent *S. mutans* GTF-I with that from the primer-dependant GTF-S (soluble glucan-producing) resulted in hybrids that were stimulated by dextran.¹²

Activation cannot be full explained by the primer model, since dextran treated with tripsyl chloride, which blocks the nonreducing ends of dextran and prevents further extension by glucansucrase, still activated GTF-I from *S. mutans*.¹³ It has been suggested that dextran activation is an allosteric effect or that dextran binding alters the catalytic site.^{8,13} However, one should not discount that dextran can also serve as an acceptor for the glucosyl transfer.^{6,8,14,15} The modified DsrI from *L. mesenteroides* provide a unique example to study this dextran activation since the full-length enzymes differing by only one amino acid have significant differences with this so-called “primer effect”. We now follow up on that work with experiments designed to investigate the underlying mechanism behind those findings, and to exploit those results with methods for the production of modified glucan structures in increased yields.

Experimental

Enzymes

Glucansucrases genes were cloned as previously described^{1,3} and expressed in *Lactococcus lactis*.² The *L. lactis* cultures were centrifuged to remove cells, and the cell-free culture fluid was concentrated and diafiltered against 20 mM sodium acetate buffer (pH 5.6) containing 2mM CaCl₂ and 1.5 mM NaN₃, using a tangential-flow apparatus with a 100,000 MW cutoff membrane cassette (Millipore, Billerica, MA, USA). The concentrated and diafiltered culture fluid was used without further purification.

Endodextranase (Dextranase L) from *Chaetomium sp.* was a gift from Amano Enzymes USA (Elgin, IL), and contained approximately 30,000 U/mL.

Carbohydrates

¹⁴C-U-Sucrose was purchased from MP Biochemicals, LLC (Santa Ana, CA). Industrial-grade dextrans of various molecular weight ranges were purchased from Sigma-Aldrich, USA. Native, high-Mw NRRL B-1355, B-1498, B-1501, and B-21297 alternan was prepared as previously described.^{16,17} Alternan modified by endodextranase treatment was prepared as described by Leathers et al.,¹⁸ and NRRL B-742 and B-1355-L dextran fractions were prepared as previously described.¹⁹⁻²¹ Their respective molecular weights were as follows: Native high-Mw glucans were approximately 10⁷; alternan modified by endodextranase was bimodal, at 5x10⁶ and 4x10⁵; and endodextranase-treated B-742-S was 5x10³. All were readily soluble in water at the concentrations used. All other carbohydrates were reagent grade.

DsrS (soluble-dextran producing dextransucrase) from *L. mesenteroides* NRRL B-1118 was cloned and expressed in *E. coli* as previously described.²² This was used to synthesize ¹⁴C-labeled B-1118 soluble dextran. One mL of DsrS containing 0.67 U/mL was mixed with 0.4 mL of 0.3M ¹⁴C-U-sucrose (42 cpm/ μg) in 20 mM sodium acetate buffer, pH 5.5, with 2 mM CaCl₂ and 1.5 mM NaN₃. After the reaction was complete, the reaction mixture was mixed with 1.5 volumes of ethanol, and the precipitate was collected by centrifugation. This was redissolved in 3 mL of water and precipitated a second time with ethanol. A total of three dissolution-reprecipitation steps were performed to remove all low-Mw ¹⁴C sugars. The ¹⁴C-labeled water-soluble dextran (42 cpm/μg) was dissolved in buffer (14.5 mg/mL). Equal volumes of ¹⁴C-dextran solution, 1M sucrose, and DsrI mutant T654R enzyme (0.9 U/mL) were allowed to incubate at room temperature with mixing by gentle rocking. After 2.5 days, the mixture was centrifuged and the water-insoluble glucan product was separated from the soluble reaction products. The insoluble material was washed with water three times, followed by one wash in 50% ethanol and one wash in absolute alcohol, then dried in vacuo at 50°C. The dried water-insoluble material and the soluble supernatant solution were both analyzed for ¹⁴C content by liquid scintillation counting in Ecolume cocktails.

Analytical methods

Glucansucrase radioassays, NMR, dextranase susceptibility studies and thin-layer chromatography techniques have been described.^{1,3}

Results

Activation by soluble glucans

In our previous work, we showed that several DsrI mutants expressed in *E. coli* were activated by water-soluble dextran (2,000,000 Mw).³ We have now determined the kinetic constants K_M and V_{max} for those mutants of DsrI expressed and secreted extracellularly in *Lactococcus lactis*. Using a constant concentration of 0.83% (w/v) dextran and varying sucrose concentrations in the range of 7.5mM to 150 mM, Eadie-Hofstee plots were constructed, and K_M values for sucrose were calculated. V_{max} values were also calculated, but since these are dependent on enzyme concentrations present in the reaction mixtures, we present in Table 1 the V_{max} in the presence of dextran relative to V_{max} in the absence of dextran, as the ratio V_{max}^+/V_{max}^- . One-way ANOVA (Analysis of Variance) was performed (t-tests) to determine the significance of any differences in K_M and V_{max} values. In all cases tested, the absence or presence of dextran did not significantly alter the K_M for sucrose with any DsrI mutant. However, the V_{max} was significantly increased by the addition of dextran for T654 mutants K, I, R, N, and Q, whereas it was essentially unchanged for the wild type and mutant T654Y.

Several glucans other than commercial dextran were also analyzed for their ability to activate the mutant enzymes. Soluble starch, levan and pullulan showed no effect, but native, high Mw

alternan from several sources (NRRL B-1355, B-1498, B-1501, and B-21297) caused significant activation of T654K, I, R, N and Q. Similarly, native dextran fraction NRRL B-742-S^{19,20} also activated these DsrI mutants. None of these glucans caused significant activation of the wild-type enzyme, nor of mutant T654Y. However, alternan modified by treatment with endodextranase¹⁸ showed no activation of any of the mutant enzymes, nor did endodextranase-limit B-742-S dextran^{20,21} (data not shown).

Increase in insoluble glucan yield

Yields of water-insoluble glucan were measured for all of the mutant enzymes mentioned above with 2,000,000 Mw dextran (Figure 1), and for mutant T654R with dextran fractions of 2,000,000 Mw, 200,000 Mw, and 20,000 Mw (Figure 2). Mutant T654R was selected for most of the yield enhancement studies because its yield was the most affected by dextran (Fig. 1). The yield of water-insoluble glucan from T654Y, already the highest in the absence of dextran, was not increased by addition of dextran. Yield from the wild-type was initially increased a small amount by dextran, but quickly reached a maximum. Mutant enzymes T654K, I, R, N and Q were all significantly affected by added dextran. In the absence of dextran, they each produced much less insoluble glucan than the wild type. However, soluble dextran was able to increase the yields to levels equal to or higher than either the wild type or T654Y.

In Figure 2, it is evident that the molecular weight of the added dextran must play a role in the yield of water-insoluble glucan formed, at least by mutant DsrI T654R. The highest Mw dextran tested, 2,000,000, increased the yield of water-insoluble product throughout the range tested. Higher concentrations could not be tested because the resulting reaction mixture was a semi-solid gel. The lowest Mw dextran tested, 20,000, initially increased insoluble yields to the same level as high-Mw dextran, but yields decreased slightly at higher concentrations. The intermediate Mw range dextran, 200,000, resulted in a yield curve intermediate to the other two.

In addition to commercial dextran fractions, the fraction L dextran produced by *Leuconostoc citreum* NRRL B-1355¹⁹ was also capable of increasing yields of insoluble glucan when tested with mutant T654R, as were native, high-Mw alternan fraction from *L. citreum* NRRL B-1501 and B-21297 (Fig. 3), although in none of these cases was the increase as great as it was with high-Mw (2,000,000) B-512F commercial dextran. Soluble starch or dextranase-treated alternan did not increase the yields of insoluble glucan formed by T654R.

Structures of insoluble products

In the case of T654R with added 2,000,000 Mw dextran, the resistance of the water-insoluble glucan to dextranase hydrolysis was 80% when synthesized in the absence of dextran, and decreased to 38% at the maximum amount of dextran added (Fig. 4). This indicates an increase

in the proportion of dextranase-susceptible $\alpha(1\rightarrow6)$ -linked glucan linkages in the insoluble product. Again using the example of insoluble glucan formed by T654R in the presence and absence of 2,000,000 Mw dextran, NMR analysis showed a large increase in the proportion of $\alpha(1\rightarrow6)$ -linkages when water-soluble dextran was present during the synthesis. As illustrated by Figure 5, the peak at ~97 ppM, which arises from anomeric carbon in $\alpha(1\rightarrow6)$ -linkage, is much larger than the peak at ~102 ppM, which arises from an anomeric carbon in $\alpha(1\rightarrow3)$ -linkage, when the insoluble glucan is formed in the presence of dextran. Likewise, peaks at ~61 and ~66 ppM, which arise from unlinked and linked C-6, respectively, reflect the same change.

¹⁴C Labeling experiment

Duplicate experiments were carried out with ¹⁴C-labeled dextran, as described in the Experimental section. After liquid scintillation counting of the ¹⁴C present in the soluble and insoluble phases after reaction with DsrI from T654R, it was found that >99% of the ¹⁴C recovered was in the water-insoluble glucan fraction, with negligible amounts in the water-soluble phase. No significant incorporation was measured with the wild-type enzyme.

Discussion

Two related phenomena have been demonstrated by this work. Firstly, we have shown that specific point mutations at threonine 654 of the cloned B-1118 DsrI are capable of altering the enzyme from one that is not activated by dextran to one that is activated by dextran. In these cases, activation is a result of changes in the $V_{\max}$ of the enzyme, with no alteration in the K_M for sucrose. This is similar to glucosyltransferases GtfK (primer stimulated), GtfL (primer independent), and GtfM (primer independent) from *S. salivarius*, where there was no change to the K_M for sucrose in the presence of T10 dextran.⁹ However, dextransucrase from *S. sanguis* showed more than a ten-fold decrease in K_M for sucrose in the presence of dextran T10.⁸ In addition, they found that V (normalized to V per unit of enzyme) was approximately two times higher in the presence of dextran. The results with DsrI implies that significant conformational changes probably do not occur at the sucrose binding site upon altering threonine 654. However, some changes must occur which enable an increase in initial reaction velocity upon binding to dextran.

Secondly, the increases in reaction rates are accompanied by increases in yields of water-insoluble glucan. The fact that the resulting insoluble glucan contains an increased proportion of $\alpha(1\rightarrow6)$ -linkages (Figs. 4 and 5) suggests that some of the added dextran may be incorporated into the water-insoluble product. When the ¹⁴C labeling results are taken into account, it becomes clear that the added water-soluble dextran must be incorporated into the water-insoluble product, most likely via acceptor reactions. Graft copolymer has been postulated by others for streptococcal enzymes,²³⁻³⁰ and must also be occurring here. However, additional phenomena

1 must also be accounted for. Firstly, the incorporation of dextran into water-insoluble product
2 does not by itself explain the increase in initial rates of synthesis by dextran. Secondly, the
3 incorporation of dextran into final insoluble product does not entirely explain the increases in
4 yield. For example, the addition of 50 mg of water-soluble dextran resulted in a 124 mg increase
5 in water-insoluble glucan synthesis by DsrI T654R (Figs. 1-3). Even if all of the dextran were
6 incorporated into water-insoluble product, that leaves 74 mg in increased yield unexplained.

7 To explain these results, one must bear in mind that, even though all of the sucrose is consumed
8 in these experiments, in the absence of added dextran, not all of the glucosyl units from sucrose
9 are incorporated into glucan. Instead, significant amounts are transferred to the fructose released
10 from sucrose, in what are known as acceptor reactions, yielding the disaccharide leucrose.⁶ In the
11 case of some mutants, such as T654I and T654K, these acceptor reactions account for the
12 majority of glucosyl transfer reactions when dextran is absent. Apparently, the addition of
13 dextran alters the rate of glucan synthesis and the proportion of glucosyl units going into glucan
14 synthesis instead of acceptor reactions is also increased. Again, this suggests the possibility that
15 dextran acts as both an allosteric activator and as an acceptor.

16 One possibility may involve the differences in acceptor binding (e.g., +1 and +2 subsites)
17 between the T654 mutants. It has been proposed that the amino acids following the transition
18 state stabilizer are involved in orientation of the acceptor molecule and influence the type of
19 glycosidic bond that is formed.^{31,32} Therefore, it would be expected that any alterations within
20 this region may also influence the affinity of acceptor binding and enzyme efficiency. As an
21 example, activity increased with the degree of polymerization with glucansucrase from *S.*
22 *sobrinus*⁶ and *S. sanguis*.⁸ We propose that T654 substitutions may result in either improved
23 binding of dextran acceptor or decreased binding of fructose through steric interference.
24 Alternatively, the results presented here can be interpreted as lending support to the types of
25 mechanisms described by Komatsu et al.⁶ for insoluble glucan synthesis by *Streptococcus*
26 *sobrinus* GtfI and by Leemhuis et al.³² for glucansucrases in general. In the latter model, there is
27 flexibility in the position of the glucan-binding domain relative to the rest of the glucansucrase.³³
28 The proximity and relative position of one to the other can influence the extent of polymer
29 formation vs. oligomer formation. Although there is no direct evidence, it is possible that in
30 some of our mutants, the change in threonine 654 has altered the conformation about this hinge
31 region. In the wild type and mutant T654Y, the two domains may be positioned such that binding
32 of dextran is not necessary for optimal synthesis of insoluble glucan. However, in mutants
33 T654K, I, R, N and Q, the two domains may be separated sufficiently to result in oligosaccharide
34 synthesis rather than polymer synthesis. Binding to dextran could bring the two domains back
35 into proper alignment for increased polymer synthesis. In the latter cases, the resulting glucan is
36 also more similar to wild-type product compared to the mutant products synthesized *de novo*
37 without dextran. Affinity binding studies with different acceptor molecules, including dextran,
38 may help determine the mechanisms for this enzyme activation, but this would likely require
39 removing the glucan-binding domain in order to limit the measurements within the active site. In

1 addition, three dimensional structural analyses may be used to elucidate whether altered
2 conformations associated with the hinge region are involved.

3 It is notable that the glucan binding domain in this enzyme is apparently selective for
4 uninterrupted sequences of $\alpha(1\rightarrow6)$ -linkages, even though the enzyme is characterized by the
5 synthesis of sequences of $\alpha(1\rightarrow3)$ -linkages. Germaine et al.⁴ found that neither glycogen,
6 amylose, inulin, nor isomaltose were sufficient for stimulation of the primer dependent *S. mutans*
7 dextranucrase. Several observations bear this out. Firstly, our mutants and the wild type all
8 exhibit similar binding to Sephadex (cross-linked dextran)², demonstrating that they all have
9 intact and functional glucan-binding domains. Secondly, native alternan and B-742-S dextran
10 activate the enzyme and give increased yields, but upon treatment with dextranase to eliminate
11 uninterrupted and unsubstituted $\alpha(1\rightarrow6)$ -linked regions,^{18,20} activation and increased yields
12 disappear. Shah et al.³⁴ found that alternan was capable of binding to glucan-binding domains of
13 several glucansucrases, and interpreted their results to indicate that these regions could bind a
14 variety of linkage sequences. However, they did not study binding to the same glucans after
15 treatment with endodextranase, so it is possible that the binding they observed was due to the
16 small stretches of $\alpha(1\rightarrow6)$ -linked regions known to be present in native alternan.¹⁸ The results
17 described here complement those of Walker and Schuerch²⁷, who found that the degree of
18 stimulation of *Streptococcus mutans* GTF-I by dextrans was inversely proportional to the degree
19 of branching, and concluded that unsubstituted $\alpha(1\rightarrow6)$ -linked regions were responsible for the
20 activation effect. They also found increases in yields upon addition of soluble dextrans, with
21 differences in the molecular weight of the added dextran showing little or no effect.

22 In addition to lending support to current ideas regarding the mechanism of glucansucrases,^{32,35}
23 this work also provides information that can be exploited for the enhanced production of custom-
24 tailored water-insoluble glucans. The increased yields induced by the addition and incorporation
25 of water-soluble dextran into water-insoluble products is in itself a valuable property.
26 Furthermore, the properties of the products may differ, depending on which water-soluble glucan
27 is used. For example, the products formed in the experiment summarized in Figure 2 differ in
28 their solubility and dispersibility. We have reason to believe that the yields of water-insoluble
29 glucan formed in the presence of 20,000 Mw dextran reach a maximum and then decrease
30 because of differences in the relative proportions and chain lengths of $\alpha(1\rightarrow6)$ -linked regions
31 and $\alpha(1\rightarrow3)$ -linked regions; the decrease in water-insoluble glucan appears to be accompanied
32 by an increase in water-soluble glucan. This was suggested by our observation that the reaction
33 medium seemed to increase in viscosity. These graft copolymers apparently comprise a wide
34 range of molecular weights and/or linkage ratios. When the case of alternan copolymers is
35 considered, the possibilities increase. Finally, in Figure 3, we show the formation of water-
36 insoluble graft copolymer with NRRL B-1355-L dextran. This dextran fraction is currently being
37 investigated for its ability to prevent corrosion on steel.³⁶ Formation of an insoluble graft
38 copolymer could render it less likely to wash off steel surfaces.

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1 This study, by showing that a single amino acid residue can change the effect of added dextran
2 on both enzyme activity and yields, provides the first example of a native enzyme in which both
3 effects are induced by a single residue, and provides an excellent model system for further
4 studies on the molecular mechanism of a glucansucrase.

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References

1. Côté, G. L.; Skory, C. D. *Appl. Microbiol. Biotechnol.*, **2012**, 93, 2387-2394.
2. Skory, C. D.; Côté, G. L. *Appl. Microbiol. Biotechnol.*, **2015**, (in press, DOI 10.1007/s00253-015-6854-0)
3. Côté, G. L.; Skory, C. D. *Appl. Microbiol. Biotechnol.*, **2014**, 98, 6651-6658.
4. Germaine, G. R.; Chludzinski, A. M.; Schachtele, C. F. *J. Bacteriol.*, **1974**, 120, 287-294.
5. Germaine, G. R.; Harlander, S. K.; Leung, W.-L. S.; Schachtele, C. F. *Infect. Immun.*, **1977**, 16, 637-48.
6. Komatsu, H.; Abe, Y.; Eguchi, K.; Matsuno, H.; Matsuoka, Y.; Sadakane, T.; Inoue, T.; Fukui, K.; Kodama, T. *FEBS J.*, **2011**, 278, 531-540.
7. Konishi, N.; Torii, Y.; Yamamoto, T.; Miyagi, A.; Ohta, H.; Fukui, K.; Hanamoto, S.; Matsuno, H.; Komatsu, H.; Kodama, T.; Katayama, E. *J. Biochem.*, **1999**, 126, 287-295.
8. Mayer, R. M.; Matthews, M. M.; Futerman, C. L.; Parnaik, V. K.; Jung, S. M. *Arch. Biochem. Biophys.*, **1981**, 208, 278-287.
9. Simpson, C. L.; Cheetham, N. W. H.; Giffard, P. M.; Jacques, N. A. *Microbiol.*, **1995**, 141, 1451-1460.
10. Stringer, C. S.; Tsuchiya, H. M. *J. Am. Chem. Soc.*, **1958**, 80, 6620-6625.

11. Tsuchiya, H. M.; Hellman, N. N.; Koepsell, H. J.; Corman, J.; Stringer, C. S.; Rogovin, S. P.; Bogard, M. O.; Bryant, G.; Feger, V. H.; Hoffman, C. A.; Senti, F. R.; Jackson, R. W. *J. Am. Chem. Soc.*, **1955**, 77, 2412-2419.
12. Nakano, Y. J.; Kuramitsu, H. K. *J. Bacteriol.*, **1992**, 174, 5639-5646.
13. Robyt, J. F.; Corrigan, A. J. *Arch. Biochem. Biophys.*, **1977**, 183, 726-731.
14. Mukasa, H.; Shimamura, A.; Tsumori, H. *Infect. Immun.*, **1979**, 23, 564-570.
15. Robyt, J. F.; Yoon, S. H.; Mukerjea, R. *Carbohydr. Res.*, **2008**, 343, 3039-3048.
16. Côté, G. L. *Carbohydr. Poly.*, **1992**, 19, 249-252.
17. Côté, G. L. Chapter 13 in *Biopolymers*, Vol. 5. A. Steinbüchel, Ed. Wiley-VCH, Weinheim, Germany. **2002**. Pp. 323-350.
18. Leathers, T. D.; Nunnally, M. S.; Côté, G. L. *Biotechnol. Lett.* **2009**, 31, 289-293.
19. Wilham, C. A.; Alexander, B. H.; Jeanes, A. *Arch. Biochem. Biophys.*, **1955**, 69, 61-75.
20. Côté, G. L.; Robyt, J. F. *Carbohydr. Res.*, **1983**, 119, 141-156.
21. Côté, G. L.; Leathers, T. D.; Ahlgren, J. A.; Wyckoff, H. A.; Hayman, G. T.; and Biely, P. Chapter 8. In: *Chemistry of Novel Foods*, Okai, H., Mills, O., Spanier, A. M., and Tamura, M., (eds.), ACS Symposium Series, **1997**, pp. 95-109.
22. Côté, G. L.; Skory, C.D. In *Green Polymer Chemistry: Biobased Materials and Biocatalysis III*; Cheng, H.N., Gross, R. A., Smith, P.B., Eds.; ACS Symposium Series 1191; American Chemical Society: Washington, DC, **2015**; Chapter 7.
23. Hare, M. D.; Svensson, S.; Walker, G. J. *Carbohydr. Res.*, **1978**, 66, 245-264.
24. Fukui, K.; Moriyama, T.; Miyake, Y.; Mizutani, K.; Tanaka, O. *Infect Immun.*, **1982**, 1-9.
25. Koga, T.; Sato, S.; Inoue, M.; Takeuchi, K.; Furuta, T.; Hamada, S. *J. Gen. Microbiol.*, **1983**, 129, 751-754.
26. Hanada, N.; Takehara, T.; Itoh, M.; Saeki, E. *FEMS Microbiol. Lett.*, **1986**, 36, 173-175.
27. Walker, G. J.; Schuerch, C. *Carbohydr. Res.*, **1986**, 146, 259-270.

28. Takehara, T.; Ansai, T.; Yamashita, Y.; Itoh-Andoh, M.; Hanada, N.; Kunimori, A. *Oral Microbiol. Immunol.*, **1992**, 7, 155-158.
29. Robyt, J. F.; Martin, P. J. *Carbohydr. Res.*, **1983**, 113, 301-315.
30. Mukasa, H.; Tsumori, H.; Shimamura, A. *Carbohydr. Res.*, **2001**, 333, 19-26.
31. Leemhuis, H.; Pijning, T.; Dobruchowska, J. M.; Dijkstra, B. W.; Dijkhuizen, L. *Biocatal. Biotransform.*, **2012**, 30, 366-376.
32. Leemhuis, H.; Pijning, T.; Dobruchowska, J. M.; van Leeuwen, S. S.; Kralj, S.; Dijkstra, B. W.; Dijkhuizen, L. *J. Biotechnol.*, **2013**, 163, 250-272.
33. Pijning, T.; Vujičić-Žagar, A.; Kralj, S.; Dijkhuizen, L.; Dijkstra, B. W. *FEBS J.*, **2014**, 281, 2159-2171.
34. Shah, D. S. H.; Joucla, G.; Remaud-Simeon, M.; Russell, R. R. B. *J. Bacteriol.*, **2004**, 186, 8301-8308.
35. Moulis, C.; Joucla, G.; Harrison, D.; Fabre, E.; Potocki-Veronese, G.; Monsan, P.; Remaud-Simeon, M. *J. Biol. Chem.*, **2006**, 281, 31254-31267.
36. Finkenstadt, V. L.; Côté, G. L.; Willett, J. L. *Biotechnol. Lett.*, **2011**, 33, 1093-1100.

Table 1. Effect of dextran (2,000,000 Mw) on kinetic parameters K_M and V_{max} for DsrI mutants expressed in *Lactococcus lactis*.

DsrI mutant	K_M without dextran (mM sucrose, $\pm$ std. dev.)	K_M with 0.8% dextran (mM sucrose, $\pm$ std. dev.)	V_{max+}/V_{max-}
Wild type	39 ± 3.5	33 ± 3.5	0.9
T654Y	46 ± 1.4	46 ± 16	0.9
T654Q	36 ± 5.8	38 ± 9.2	1.3*
T654R	43 ± 12	44 ± 1.4	1.5*
T654N	36 ± 0.6	46 ± 14	1.7*
T654I	46 ± 8.5	46 ± 13	2.1*
T654K	27 ± 7.6	34 ± 0.8	3.4*

*These values represent statistically significant increases in V_{max} with added dextran.

Figure legends.

Figure 1. Yields of water-insoluble glucan synthesized by various mutant forms of DsrI in the presence of increasing amounts of water-soluble dextran (Mw=2,000,000). Reactions consisted of 2 mL of 1M sucrose, 2 mL of DsrI containing approximately 1-2 U/mL, and 2 mL of dextran of various concentrations, dissolved in 20 mM pH 5.5 sodium acetate buffer with 2 mM CaCl₂ and 1.5 mM NaN₃. All reactions were allowed to go to completion as judged by disappearance of sucrose when analyzed by TLC. *Top*: Wild type and mutant T654Y. *Bottom*: Mutants T654I, K, R, N and Q.

Figure 2. Yields of water-insoluble glucan formed by DsrI mutant T654R in the presence of various molecular weight fractions of dextran. T20=20,000 Mw; T200=200,000 Mw; T2000=2,000,000 Mw. Reaction conditions same as in Fig. 1.

Figure 3. Yields of water-insoluble glucan formed by DsrI mutant T654R in the presence of various water-soluble glucans. Conditions same as in Fig. 1.

Figure 4. Dextranase resistance of water-insoluble glucan produced by DsrI mutant T654R in the presence of various amounts of 2,000,000 Mw dextran. Resistance is expressed as percent of glucan remaining water-insoluble after exhaustive hydrolysis by endodextranase.

Figure 5. Proton-decoupled ¹³C-NMR spectra of water-insoluble glucans formed by DsrI mutant T654R in the absence (top) and presence (bottom) of 0.83% (w/v) dextran, Mw=2,000,000. Peak at ~102 ppm arises from anomeric carbon in α(1→3)-linkage, and peak at ~97 ppm from anomeric carbon in α(1→6)-linkage. Likewise, peaks at ~61 and ~66 ppm arise from unlinked and linked C-6, respectively.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.