Improvement of fucosylated oligosaccharides synthesis by α -L-fucosidase from *Thermotoga maritima* in water-organic cosolvent reaction system

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Abstract

The effects of water activity (a_w), pH, and temperature on transglycosylation activity of α -L-fucosidase from *Thermotoga maritima* in the synthesis of fucosylated oligosaccharides were evaluated using different water - organic cosolvent reaction systems. The optimum conditions of transglycosylation reaction were the pH range between 7 and 10, and temperature 90–95°C. The addition of organic cosolvent decreased α -L-fucosidase transglycosylation activity in the following order: acetone > dimethyl sulfoxide (DMSO) > acetonitrile (0.51 > 0.42 > 0.18 mM/h). However, the presence of DMSO and acetone enhanced enzyme-catalyzed transglycosylation over hydrolysis as demonstrated by the obtained transglycosylation/hydrolysis rate ($r_{T/H}$) values of 1.21 and 1.43, respectively. The lowest $r_{T/H}$ was calculated for acetonitrile (0.59), though all cosolvents tested improved the transglycosylation rate in comparison to a control assay (0.39). Overall, the study allowed the production of fucosylated oligosaccharides in water-organic cosolvent reaction media using α -L-fucosidase from *Thermotoga maritima* as biocatalyst.

Introduction

Although highly abundant, human milk oligosaccharides (HMOs) are unique to human breast milk (10–15 g/L). They are widely known to play an important role in infant health by acting as prebiotics, modulators of gut motility, as well as they prevent enteric infections by blocking the adhesion of pathogenic bacteria [1, 2]. Chemical structures of HMOs follow a similar blueprint, for example, 3-fucosyllactose can be formed when the hydroxyl group at C-3 of glucose at the reducing end of a lactose molecule is substituted by fucose while the most abundant 2'-fucosyllactose is obtained by the substitution of the hydroxyl group at C-2 of galactose [3].

In vivo biosynthesis of fucosylated oligosaccharides is catalyzed by specific transferases found in the Golgi apparatus of the mammary alveolar cells [4]. Since these enzymes are difficult to express and purify, as well as require expensive nucleotide-activated substrates, which add to the high cost of their production, their usage for the *in vitro* synthesis of fucosylated oligosaccharides is limited [5, 6]. In contrast, glycosidases with transglycosylation activity like α -L-fucosidases (E.C. 3.2.1.51), are more available, relatively inexpensive with broad substrate specificity which is an advantageous characteristic in the synthesis of fucosylated oligosaccharides [7, 8]. Under conventional conditions, fucosidases inherently catalyze the glycosidic bond hydrolysis [6]. However, when an acceptor molecule other than water is present in the reaction media, it can attack the nucleophilic glycosyl-enzyme intermediate, and the transglycosylation reaction can proceed. The most recent reports have described successful protocols to obtain fucosylated oligosaccharides in the α -L-fucosidase assisted enzymatic reactions [9–12].

Owing to the fact that fucosidase-catalyzed synthesis of carbohydrates can be controlled kinetically, it has been possible to shift reaction equilibrium in favour of transglycosylation by manipulating reaction conditions [13]. The implemented strategies rely on the suppression of water activity (a_w) in the media by

either the addition of high concentrations of acceptor substrate [14] or the incorporation of organic cosolvents [15–17].

The main limitation to these approaches lies in the fact that enzymes may show lower activity in the presence of organic cosolvents compared to an aqueous medium while solubility of non-polar substrates and products is increased [13, 16]. However, successful applications of miscible organic solvents in enzymatic reactions, such as dimethyl sulfoxide (DMSO) [18, 19], acetone [20, 21] or acetonitrile [22] have been reported.

Thus, this work aimed at studying the effects of water-miscible organic solvents in the reaction system on transglycosylation activity of α -L-fucosidase from *Thermotoga maritima* to produce fucosylated oligosaccharides.

Materials And Methods

Material

4-nitrophenyl- α -L-fucopyranoside (*p*NP-Fuc), 4-nitrophenol (*p*NP), 2'-fucosyllactose, D-lactose and L-fucose were purchased from Sigma-Aldrich (St. Louis MO, USA), sodium monophosphate, sodium diphosphate, NaOH, acetone, acetonitrile and DMSO were purchased from J.T. Baker (Mexico State, Mexico). Recombinant α -L-fucosidase from *Thermotoga maritima* (E.C. 3.2.1.51) was acquired from Megazyme (Leinster, Ireland) at a concentration of 5 U/mL (2.6 U/mg) in 3.2 M ammonium sulphate and diluted 1:1000 (v/v) in 0.1 M phosphate buffer at the corresponding pH. Milli-Q[®] water (Merck, Germany) was used throughout all experiments.

α -L-Fucosidase activity assay

α -L-Fucosidase activity assay was carried out for 10 min at 60 °C. Briefly, 50 μ L of α -L-fucosidase from *T. maritima* was added to 450 μ L of *p*NP-Fuc (3.5 mM) in phosphate buffer (0.1 M, pH 8) and the reaction progress was analyzed every min. The reaction was stopped by adding 50 μ L of NaOH (1 M). Released *p*NP was quantified by measuring absorbance (Shimadzu UV-1800, Tokyo, Japan) at 410 nm. One unit of α -L-fucosidase is defined as the amount of enzyme required to release 1 mmol of *p*NP under the assay conditions.

Synthesis of fucosylated oligosaccharides

Enzymatic synthesis of fucosylated oligosaccharides was performed in phosphate buffer (0.1M, pH 8) for 120 min at 90 °C. The reaction mixture consisted of α -L-fucosidase (0.0065 U/mL), *p*NP-Fuc (3.5 mM) as a donor substrate, and D-lactose (438 mM) as an acceptor substrate. The reaction progress was analyzed every 30 min until stopped by adding 50 μ L of NaOH (1 M). The synthesized fucosylated oligosaccharides were quantified as described in section “Carbohydrate quantification”. Released *p*NP was quantified by measuring absorbance (Shimadzu UV-1800, Tokyo, Japan) at 410 nm.

Effects of pH and temperature on transglycosylation activity of α -L-fucosidase

The effect of pH on transglycosylation activity was determined in the pH range from 5 to 10 for 1 h at 60 °C. Briefly, 50 μ L of α -L-fucosidase (0.0065 U/mL) from *T. maritima* was added to 400 μ L of phosphate buffer (0.1 M) containing *p*NP-Fuc (3.5 mM) and D-lactose (438 mM). The reaction progress was analyzed at 10, 20, 40 and 60 min until stopped by adding 50 μ L of NaOH (1 M). The synthesized fucosylated oligosaccharides were quantified as described in section "Carbohydrate quantification". The transglycosylation activity was reported as the initial enzyme rate (V_o), expressed as concentration of the synthesized fucosylated oligosaccharides (mM) per minute. In a similar way, the effect of temperature on transglycosylation activity was determined. Experiments were carried out in a temperature range from 60 to 95 °C. The conversion yield was defined as the ratio of the synthesized fucosylated oligosaccharides to the initial concentration of donor substrate (*p*NP-Fuc)

Estimation of a_w

The a_w values of the reaction system consisting of organic cosolvent and water at different ratios were estimated by a combination of the models reported by Bell *et al.* [23] and García-Garibay *et al.* [24]. The solvents employed were DMSO, acetone and acetonitrile.

Effect of a_w on the stability and hydrolytic activity of α -L-fucosidase

The effect of a_w on enzyme stability was evaluated by the addition of organic cosolvent to the reaction system during 180 min at 60 °C. The test solvents were acetone (a_w 0.97), acetonitrile (a_w 0.96) or DMSO (a_w 0.99). Briefly, 50 μ L of α -L-fucosidase was mixed with 10 μ L of cosolvent and 40 μ L of phosphate buffer (0.1 M, pH 8). Reaction progress was monitored every 30 min. The enzyme activity was measured spectrophotometrically through the release of *p*NP as already described. Residual hydrolytic activity was determined as the percentage of enzyme activity compared to control.

The effect of a_w on the α -L-fucosidase hydrolytic activity was evaluated by varying the volume of cosolvent in phosphate buffer (0.1 M, pH 8). The reactions were carried out at 60 °C for 10 min. In brief, 50 μ L of α -L-fucosidase from *T. maritima* was added to the reaction mixture containing 3.5 mM *p*NP-Fuc, and different concentrations of test cosolvents in the reaction volume of 450 μ L as specified in Table 1. The reaction was monitored every min until stopped by adding 50 μ L of NaOH (1 M). The hydrolytic activity was measured spectrophotometrically through the release of *p*NP as already described. Residual hydrolytic activity was determined as the percentage of enzyme activity compared to control.

Effect of organic media on the transglycosylation reaction

The effect of acetone (a_w 0.97), acetonitrile (a_w 0.96) and DMSO (a_w 0.99) on the α -L-fucosidase transglycosylation activity was evaluated for 180 min at 60 °C. Reaction volume of 450 μ L was kept constant while varying the organic cosolvent concentration in phosphate buffer (0.1 M, pH 8) as specified in Table 1. The reaction mixture consisted of 50 μ L of α -L-fucosidase from *T. maritima* (0.0065 U/mL),

*p*NP-Fuc (3.5 mM) and D-lactose (438 mM). The reaction progress was analyzed every 30 min until stopped by adding 50 μ L of NaOH (1 M). The synthesized fucosylated oligosaccharides were quantified by HPLC as described in section “Carbohydrate quantification”. Released *p*NP was quantified by measuring the absorbance at 410 nm. The rate of transglycosylation to hydrolysis ($r_{T//H}$) was expressed as the concentration ratio of the synthesized fucosylated oligosaccharides to the released *p*NP.

Carbohydrate Quantification

The quantification of the synthesized fucosylated oligosaccharides and the released fucose was performed on the HPLC (LabAlliance, State College, PA, USA) with an ion-exclusion column Rezex RNO-Oligosaccharides Na⁺ (4%) (60 x 10 mm; particle size 12 μ m) (Phenomenex; Amstelveen, Netherlands) and oven temperature of 75 °C. The HPLC was equipped with a SOFTA 300S light scattering detector (Chrom Tech, Minnesota, USA) with a nitrogen flow of 62.5 psi, spray chamber temperature of 10 °C and a drift tube temperature of 45 °C.

Samples were filtered through 0.22 μ m Millipore Durapore membranes prior to assay by HPLC and were eluted with Milli-Q water at a flow rate of 0.3 mL/min. A calibration curve of 2'-fucosyllactose was used to estimate the amount of the product formed. Additionally, a standard 2'-fucosyllactose, D-lactose and L-fucose were used to determine their retention times and identify the reaction products.

Statistical Analysis

All experiments were performed in triplicate and results are reported as a mean value $\pm$ standard deviation. For statistical analysis, the statistical software IBM SPSS Statistic version 25.0 for Windows (IBM, New York, USA) was used to carry out a one-way analysis of variance (ANOVA) followed by the Tukey's test for comparing all pairs of groups. A *p* value of 0.05 was considered statistically significant.

Results And Discussion

Synthesis of fucosylated oligosaccharides

The synthesis of fucosylated oligosaccharides was carried out using *p*NP-Fuc and D-lactose as donor and acceptor substrates, respectively. Fig 1a shows a typical HPLC chromatogram of transglycosylation products. Retention time of the obtained fucosylated oligosaccharide (7.7 min) coincided with that of 2'-fucosyllactose standard (Fig 1b). In addition, Fig 1a reveals the presence of fucose and fucosylated oligosaccharides which is indicative that the enzyme catalyzed both the hydrolysis of *p*NP-Fuc and transglycosylation, respectively.

More detailed observations of the reaction progress of typical transglycosylation reaction are shown in Fig 2. The highest concentration of fucosylated oligosaccharide (0.76 mM) was detected after 60 min. As can be observed, in parallel to the *p*NP-Fuc hydrolysis, some of the released fucose was enzymatically transferred to lactose leading to the formation of fucosylated oligosaccharide while the remaining fucose

was observed as free fucose in the reaction medium. Similar results have been described by other authors Lezyk *et al.* [10] reported a conversion rate of 0.23 mM/h in the synthesis of 2'-fucosyllactose using α -L-fucosidase from *T. maritima* at a concentration of 0.51 μ g/mL, using 20 mM *p*NP-Fuc and 25 mM D-lactose at pH 5.0 and 30 °C. In another study, α -L-fucosidase from *T. maritima* was employed at a concentration of 0.13 μ g/mL to catalyze the synthesis of fucosyllactose, using 3.5 mM *p*NP-Fuc and 584 mM D-lactose at pH 5.9 and 60 °C. The highest conversion rate achieved in these conditions was 0.29 mM/h [9]. The conversion yield obtained in this study (0.76 mM/h) was higher than those reported by the aforementioned authors and using lower concentration of α -L-fucosidase (0.013 μ g/mL).

Effect of pH and temperature on the α -L-fucosidase transglycosylation activity

Fig 3a shows the transglycosylation activity of α -L-fucosidase from *T. maritima*. The highest activity was found in the pH range from 7 to 10 since no statistically significant difference was calculated for this pH interval ($p < 0.05$). Additionally, the enhanced transglycosylation activity was observed with the increasing pH from 5 to 8, which resulted in the improved yields from 6.07 to 40.67%, respectively. The obtained results are in accordance with other studies that investigated optimum reaction conditions of glycosidases from thermophilic sources. Wu *et al.* [25] reported improved yields in galactooligosaccharides (GOS) synthesis catalyzed by β -glycosidase from *Sulfolobus solfataricus* by changing pH from 4 to 6. Likewise, two recombinant β -glycosidases expressed in *E. coli* (F441Y and F359Q) performed better at pH 6.5 than 4. In another study, Ji *et al.* [26] also observed that pH 6 was optimum for the GOS production catalyzed by a recombinant β -galactosidase from *T. maritima* expressed in *E. coli* in contrast to pH above 7 at which the enzyme activity was remarkably reduced. In another study, the improved yields of GOS were linked to the pH rise from 5 to 5.5 using β -mannosidase from *Pyrococcus furiosus* as a catalyst [27].

It is noteworthy that conversion yields of enzymatic transglycosylation increase in alkaline media. Sulzenbacher *et al.* [28] and Tarling *et al.* [29] suggested that pH changes of the reaction medium affected the pK_a of a key amino acid involved in the catalysis. Abdul Manas *et al.* [13] concluded that ionization of this key amino acid was a determinant factor in favoring interactions with either water, which would lead to hydrolysis, or sugar acceptor resulting in the improved yield of transglycosylation product as observed in the present work.

As for the effect of temperature, the highest transglycosylation activity was observed in the range of 90 °C to 95 °C (Fig. 3b). The reaction yield at 95 °C was three times higher than that obtained at 60 °C (32.01 and 10.58% respectively). Similar effect was observed by Zeuner *et al.* [30] who reported a sixfold increase of the reaction yield (from 0.9 to 5.4%) in the *N*-acetylglucosamine synthesis catalyzed by β -galactosidase from *Pyrococcus furiosus* when the temperature was raised from 40 to 90 °C. In another work, the conversion yield of GOS in the presence of β -galactosidase from *Bacillus circulans* was three times greater at 60 °C than at 25 °C (5 and 15 g/L, respectively) [31]. Moreover, the research group of Fourage *et al.* [32] reported a 2.6-fold increase of reaction yield in the *p*NP-Fuc-Fuc synthesis (from 16 to

42%) catalyzed by β -glycosidase from *Thermus thermophilus*, in response to the temperature rise from 37 to 75 °C.

Estimation of a_w in the reaction system

Since water distribution in the reaction medium of enzymatic process tends to maintain balance with all the components of such system, the thermodynamic parameter a_w is the best variable to determine water availability in mixed-solvents systems. The a_w values of media containing test cosolvents were evaluated, according to a model previously described for enzymatic reactions in organic media [23, 24] and reported in Fig. 4 and Table 1. As can be seen, the test solvents affected the a_w of reaction system differently. Since the obtained isotherms followed the same pattern as those reported by Bell *et al.* [23] and Cruz-Guerrero *et al.* [20] the data were deemed valid.

Effect of a_w on the hydrolytic activity and stability of α -L-fucosidase

By changing a_w of the reaction medium, it is possible to increase production yield of fucosylated oligosaccharides. Prior to the experiment, enzyme stability was examined in a test medium with a variable a_w value obtained by the addition of a water-miscible cosolvent. The three organic solvents and the corresponding a_w of the reaction medium were acetone (a_w 0.97), acetonitrile (a_w 0.96), and DMSO (a_w 0.99). As shown in Fig. 5a, no difference in enzyme activity was observed for the reaction systems tested ($p < 0.05$) during the reaction course, and the enzyme retained around 76% of its activity after 180 minutes in comparison to control. Pyeon *et al.* [33] reported that the recombinant β -glycosidase from *Microbulbifer thermotolerans* displayed 93, 79 and 20% of its activity after incubation in acetone, (a_w 0.97), DMSO (a_w 0.98) and acetonitrile (a_w 0.96), respectively. In their previous study [34] a recombinant chitinase MtCh509 from *Microbulbifer thermotolerans* was reported to retain up to 100% its activity in media with DMSO as an additive at a_w of 0.99 and 0.96, whereas in the presence of acetone and acetonitrile the enzyme activity decreased remarkably. As a general rule, it is assumed that thermophilic enzymes possess rigid structures that directly contribute to their apparent resistance to organic solvents [35]. In this context, the preserved enzymatic activity of α -L-fucosidase used in this work in the presence of the three cosolvents can be explained in a similar manner.

As can be seen in Fig. 5b, residual hydrolytic activity of α -L-fucosidase exceeded 100% in the presence of acetone (a_w 0.97, 0.95, 0.93) and acetonitrile (a_w 0.96, 0.93, 0.91). Nevertheless, at the a_w values lower than 0.91 the enzyme activity declined. In case of DMSO as cosolvent in the reaction system at a_w 0.99, no significant improvement of enzymatic activity was observed in comparison to the control assay ($p < 0.05$), but at lower a_w the α -L-fucosidase performance was negatively affected. Conversely, Mallek-Fakhfakh and Belghith [36] described no changes in the activity of β -glucosidase from *Talaromyces thermophilus* in the presence of DMSO at a_w of 0.97 while further drop of a_w to 0.91 caused remarkable enzyme deactivation. Furthermore, Jiang *et al.* [37] reported that the activity of xylanase B from *T. maritima* was unaffected by the addition of DMSO (a_w 0.96). The solvent impact on enzyme activity is

mostly correlated with Log P or another measure of its polarity rather than a combination of factors such as the dielectric constant, the dipole moment, the ability to form hydrogen bonds, as well as denaturation capability. Taken together, these properties determine how the solvent affects the way reactants interact with the active site of the enzyme [38]. The extremophile α -L-fucosidase from *T. maritima* employed in this work displayed higher resistance in the presence of different concentrations of organic solvents. This is an attractive feature for their usage in many industrial processes.

Effect of organic media on the α -L-fucosidase transglycosylation activity

Based on the results from previous experiments, α -L-fucosidase from *T. maritima* showed high stability and activity in the reaction media containing acetone (a_w 0.97), acetonitrile (a_w 0.96) and DMSO (a_w 0.99). Thus, these cosolvents were studied in the enzymatic synthesis of fucosylated oligosaccharides. Fig. 6a demonstrates the reaction course of transglycosylation using different cosolvent in a reaction system. The highest conversion rate was achieved in the medium with the addition of acetone followed by DMSO and acetonitrile (acetone>DMSO>acetonitrile = 0.51>0.42>0.18 mM/h).

On the contrary, Trincone *et al.* [39] reported improved yields in the synthesis of β -D-glucopyranoside disaccharides and trisaccharides catalyzed by a mutant β -glycosidase from *S. solfataricus* (Ss- β -glyE387G) when acetonitrile was added to the reaction system. In other studies, a hyperthermophilic β -glycosidase was used in the synthesis of GOS in water – acetone system at a_w of 0.47 and the highest conversion rate was 0.051 μ mol/mL [20]. In the case of porcine liver α -L-fucosidase employed to produce fucopyranosides in the reaction system with DMSO, the extended reaction time was needed, by approximately 9%, to afford the same yield as in conventional media [19].

The effect of water activity and cosolvents on the $r_{T/H}$ ratio was also evaluated in the present study (Fig. 6b). In the presence of acetone, α -L-fucosidase showed higher transglycosylation than hydrolytic activity which corresponded to the $r_{T/H}$ value greater than 1, with a maximum recorded at 30 min ($r_{T/H}$ 1.43). In case of DMSO, the highest $r_{T/H}$ was obtained after 120 min and equaled to 1.21. Among the three cosolvents, acetonitrile presented the $r_{T/H}$ below 1, indicating on the predominant hydrolytic activity rather than transglycosylation. Overall, the addition of cosolvents, such as acetone and DMSO, to the reaction media improved kinetic equilibrium towards enzymatically catalyzed transglycosylation, as indicated by their maximal $r_{T/H}$ values when compared to the control (0.39).

Similar results were reported by Li *et al.* [40], who observed a decrease in the transglycosylation activity of a commercially available collection of thermophilic glycosidases (CLONEZYME) in the reaction media containing acetonitrile at a_w of 0.88. On the contrary, Li *et al.* [41] showed that addition of DMSO as a cosolvent at a_w of 0.94 improved the yield of daidzein transglycosylation product by 14% (from 39 to 44.47%). The reaction was catalyzed by a recombinant maltosyl transferase from *T. maritima* expressed in *E. coli*. Additionally, Baek *et al.* [42] reported higher yield of transglycosylation products in a reaction catalyzed by maltogenic amylase from a *Thermus* strain when acetone was added as a cosolvent to the reaction media. The authors observed suppressed hydrolytic activity.

Conclusions

The gathered results indicate that α -L-fucosidase from *T. maritima* can catalyze the synthesis of fucosylated oligosaccharides in water-organic cosolvent media. The best results were observed for acetone (0.51 mM/h). Moreover, the addition of acetone or DMSO to the medium shifted the reaction equilibrium favoring transglycosylation over the enzyme-assisted hydrolysis as the calculated $r_{T/H}$ values of 1.43 and 1.21, respectively, were higher compared to the value recorded for the control assay (0.39). Finally, it is possible that the fucosylated oligosaccharides synthesized in this study have biological functions, like human milk oligosaccharides, and can be used either for clinical applications or as an additive in infant formulas.

Declarations

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Conflicts of interest. The authors declare that they had no conflict of interest.

Authors contribution **R-A. M:** Methodology, formal analysis and investigation, writing-original draft preparation. **A-S. S:** Formal analysis and investigation, writing - review and editing. **G-G. M.:** Conceptualization, formal analysis and investigation, funding acquisition, resources. **G-R. F:** Methodology. **G-R. L.:** Formal analysis and investigation, writing - review and editing. **R-S. G.:** Funding acquisition, resources. **C-G.A.:** Conceptualization, formal analysis and investigation, writing-original draft preparation, funding acquisition, resources, supervision.

Compliance with Ethical Standards

Ethics approval. This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate. Not applicable

Consent for publication. Not applicable

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Table

Table 1. a_w values estimated at different solvent concentrations.

Solvent volume (μ L)	*Buffer volume (μ L)	a_w Acetone	a_w Acetonitrile	a_w DMSO
50	400	0.97	0.96	0.99
100	350	0.95	0.93	0.96
150	300	0.93	0.91	0.91
200	250	0.91	0.90	0.83
250	200	0.90	0.88	0.74

* Phosphate buffer (0.1 M pH 8)

Figures

Figure 1

Typical Chromatograms obtained by HPLC from (a) sample of the transglycosylation reaction and (b) 2'fucosyllactose standard. Transglycosylation reaction was performed at 90 °C and pH 8 by using 3.5 mM pNP-Fuc as the donor substrate, 438 mM D-lactose as the acceptor substrate and 0.0065 U/mL α -L-fucosidase.

Figure 2

Kinetics of transglycosylation reaction catalysed by α -L-fucosidase from *T. maritima*. Reaction was performed at 90 °C using 3.5 mM pNP-Fuc as donor substrate, 438 mM D-lactose as acceptor substrate and 0.0065 U/mL of α -L-fucosidase. All reacts were dissolved in 0.1 M phosphate buffer at pH 8.

Figure 3

Effect of pH (a) and temperature (b) on the initial velocity of transglycosylation activity of α -L-fucosidase from *T. maritima*. The effect of pH on the initial reaction velocity of the transglycosylation activity was performed at different pH and 60 $^{\circ}$ C by using pNP-Fuc 3.5 mM, lactose 438 mM and 0.0065 U/mL of α -L-fucosidase. Initial reaction velocity of the transglycosylation activity. The effect of temperature was evaluated under the same conditions but at pH 8 and different temperature. Error bars represent the standard deviation of the mean of triplicates.

Figure 4

Sorption isotherms for Acetone ($\square$), Acetonitrile ($\bullet$) and DMSO ($\blacklozenge$). The a_w values at different ratios were estimated by a combination of the models reported by Bell et al. [23], and García-Garibay et al. [24].

Figure 5

Stability(a) and Residual hydrolytic activity (b) of α -L-fucosidase from *T. maritima*. The time course of change in the stability of the α -L-fucosidase was determined by incubating 0.013 U/mL α -L-fucosidase from *T. maritima* at 60 °C in phosphate buffer (0.1 M, pH 8) as control (a_w 0.998) and buffer-organic solvent; acetone (a_w 0.97), acetonitrile (a_w 0.96) and DMSO (a_w 0.99) during 180 min: $\triangle$ control, $\square$ acetone, $\bullet$ acetonitrile and $\blacklozenge$ DMSO. To observe the residual hydrolytic activity reaction was performed

at 60 °C and using either acetone, acetonitrile or DMSO as a cosolvent, 3.5 mM pNP-Fuc and 0.0065 U/mL α -L-fucosidase from *T. maritima*. Control assay was performed in absence of cosolvent under the same conditions and considered as 100% of the residual hydrolytic activity. Error bars represent the standard deviation of the mean of triplicates.

Figure 6

Effect of the solvents on the (a) synthesis of fucosylated oligosaccharides and (b) rS/H ratio catalyzed by α -L-fucosidase from *T. maritima*. Reaction was performed at 60 °C during 180 min either with acetone (aw 0.97), acetonitrile (aw 0.96) and DMSO (aw 0.99) as cosolvent in the reaction medium, pNP-Fuc as the donor, 438 mM D-lactose as the acceptor and 0.0065 U/mL of α -L-fucosidase from *T. maritima*: $\square$ acetone, $\bullet$ acetonitrile and $\blacktriangle$ DMSO. Error bars represent the standard deviation of the mean of triplicate.