Appl Biochem Biotechnol (2011) 164:655–665
DOI 10.1007/s12010-011-9165-7

Production of Astaxanthin from Cellulosic Biomass Sugars by Mutants of the Yeast *Phaffia rhodozyma*

Justin Montanti · Nhuan P. Nghiem · David B. Johnston

Received: 22 September 2010 / Accepted: 10 January 2011 /
Published online: 28 January 2011

Abstract Astaxanthin is a potential high-value coproduct in an ethanol biorefinery. Three mutant strains of the astaxanthin-producing yeast *Phaffia rhodozyma*, which were derived from the parent strain ATCC 24202 (UCD 67-210) and designated JTM166, JTM185, and SSM19, were tested for their capability of utilizing the major sugars that can be generated from cellulosic biomass, including glucose, xylose, and arabinose, for astaxanthin production. While all three strains were capable of metabolizing these sugars, individually and in mixtures, JTM185 demonstrated the greatest sugar utilization and astaxanthin production. Astaxanthin yield by this strain (milligrams astaxanthin per gram of sugar consumed) was highest for xylose, followed by arabinose and then glucose. The kinetics of sugar utilization by strain JTM185 was studied in fermenters using mixtures of glucose, xylose, and arabinose at varied concentrations. It was found that glucose was utilized preferentially, followed by xylose, and lastly, arabinose. Astaxanthin yield was significantly affected by sugar concentrations. Highest yields were observed with sugar mixtures containing the highest concentrations of xylose and arabinose. Hydrolysates produced from sugarcane bagasse and barley straw pretreated by the soaking in aqueous ammonia method and hydrolyzed with the commercial cellulase preparation, Accellerase™ 1000, were used for astaxanthin production by the mutant strain JTM185. The organism was capable of metabolizing all of the sugars present in the hydrolysates from both biomass sources and produced similar amounts of astaxanthin from both hydrolysates, although these amounts were lower when compared to yields obtained with reagent grade sugars.

Keywords *Phaffia rhodozyma* · Astaxanthin · Barley straw · Sugarcane bagasse · Cellulosic biomass

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

J. Montanti
Department of Biosystems Engineering, Clemson University, 113 Biosystems Research Complex, Clemson, SC 29631-0312, USA

N. P. Nghiem (✉) · D. B. Johnston
Eastern Regional Research Center, U.S. Department of Agriculture, Agricultural Research Service, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA
e-mail: John.Nghiem@ars.usda.gov

Introduction

Ethanol has been produced as a commodity in the US for over a century, predominantly from corn. Corn ethanol is produced typically by either the dry-grind or the wet-milling process [1]. Both of these processes generate coproducts, the marketing of which is considered critical to the economic viability of ethanol [2]. Typically, coproducts from both wet milling and dry-grind ethanol plants are marketed as low-value animal feed. Due to very fine profit margins associated with ethanol production, however, products of higher value are desirable.

Cellulosic biomass, containing cellulose and hemicellulose, are of particular interest for conversion to high-value compounds. It provides a source of hexose and pentose sugars, allowing operational flexibility in the setting of a biorefinery producing both ethanol and high-value coproducts. For example, in the conversion of starch in barley grain to ethanol, barley straw is available as a feedstock for the potential production of high-value coproducts [3]. Two process options may be considered. In the first, barley straw may be hydrolyzed and the resulting sugar stream (containing primarily glucose, xylose, and arabinose) utilized in its entirety by an organism capable of metabolizing those sugars for conversion to a high-value product. In the second case, the biomass can be fractionated to yield a glucose-rich stream and a xylose-rich stream [4]. In this case, the glucose may be converted to ethanol by the yeast *Saccharomyces cerevisiae*, which has been used universally for commercial fuel ethanol production. The xylose-rich stream may then be used for the production of a high-value coproduct by a suitable microorganism. Similarly, sugarcane bagasse (the cellulosic coproduct associated with ethanol production from sugarcane juice) may be converted to high-value coproducts as described above. In either case, the necessity of an organism capable of metabolizing all of the major biomass sugars is clear to achieve efficient utilization of the coproduct. One such organism is *Phaffia rhodozyma*, the yeast with the capability of metabolizing glucose, xylose, and arabinose that naturally produces astaxanthin [5].

Astaxanthin is of particular interest as a high-value coproduct in an ethanol biorefinery due to the ability of *P. rhodozyma* to utilize all of the major biomass sugars. Astaxanthin is the carotenoid that provides salmon and crustaceans with their characteristic coloration. The presence of astaxanthin is extremely important, as food products such as salmon would likely not be accepted by the consumer if they lacked proper coloration. As these organisms lack the ability for the de novo synthesis of astaxanthin, they must obtain it through their diet. In the marine environment, algae and plankton which naturally produce astaxanthin are consumed. In an aquaculture setting, this is not the case, necessitating the addition of astaxanthin or astaxanthin-containing materials to the feed. Additionally, astaxanthin has been found to be a powerful antioxidant, attracting the attention of the nutraceutical industry [6].

Astaxanthin is produced on the commercial scale either synthetically in a chemical process or biologically. The commercial scale biological production of astaxanthin is accomplished through cultivation of the green algae *Haematococcus pluvalis* or the red yeast *P. rhodozyma* [6]. Due to its ability to utilize all of the major sugars that can be generated from biomass (i.e., glucose, xylose, and arabinose), *P. rhodozyma* is of particular interest in biomass applications [7]. Unfortunately, astaxanthin levels achieved by wild-type strains of the organism often are too low to be economically feasible. To overcome this, mutant strains may be developed for improved production of astaxanthin.

In the present study, astaxanthin is considered as one potential high-value product that may be produced together with ethanol in a biorefinery utilizing barley straw and sugarcane

bagasse. Barley is an important ethanol feedstock that offers several key benefits over corn [3]. As such, its commercial usage is expected to expand, generating an increased amount of barley straw available for use. Sugar cane is the predominant ethanol feedstock in Brazil, similarly resulting in significant sugar cane bagasse availability.

The objectives of this project were to develop astaxanthin-overproducing mutants that retained the ability to utilize the three major biomass sugars and to investigate their capability to produce astaxanthin from selected actual biomass hydrolysates.

Materials and Methods

Microbial Strains and Maintenance

The *P. rhodozyma* parent strain, ATCC 24202 (UCD 67-210), was selected for mutagenesis because it was not protected under patents. The strain was obtained from the American Type Culture Collection (Manassas, VA). The freeze-dried culture was inoculated into 25 ml yeast malt broth (YM) media (pH 5.0) in a 250-ml shake flask. The flask was incubated at 22 °C with 250 RPM orbital shaking for 2 days. The culture was then mixed with 10 g glycerol and dispensed into cryo-vials and stored at −80 °C. A stock culture was prepared for each mutant (mutagenesis is described below) by isolating a colony from an agar plate and treating it in the same manner as above.

Chemicals and Biomass Feedstock

All chemicals were of reagent grade with the exception of yeast extract (Amberex 695 AG, Sensient Technologies, Milwaukee, WI), which was of industrial grade. The enzymes Accellerase™ 1000 and Multifect Xylanase were provided by Genencor, a Danisco division (Rochester, NY). Barley straw was provided by the Virginia Crop Improvement Association (Mt. Holly, VA). Sugarcane bagasse was provided by the USDA ARS Southern Regional Research Center (New Orleans, LA).

Mutagenesis

N-Methyl-*N*′-nitro-*N*-nitrosoguanidine was used to create mutants from the parent strain, ATCC 24202 (UCD 67-210), by the method of An et al. and Bon et al. [8, 9]. Three of the developed mutant strains were selected for use in this study based on their total carotenoid contents. They were designated as JTM 185, JTM 166, and SSM 19.

Organism Screening

The stock cultures were used to inoculate 25 ml YM media (pH 5.0) in 250-ml shake flasks. These were maintained at 22 °C with 250 RPM orbital shaking for 72 h. They were then used to inoculate the screening media by adding 1 ml of inoculum to 25 ml media in a 250-ml shake flask. Four different media were utilized in the screening (Table 1).

Each medium was supplemented with 10% (*v*/*v*) yeast nitrogen base (prepared at 10× concentration) as a nitrogen source. The mutants were cultivated in each medium in duplicate. Incubations were carried out at pH 5.0, 22 °C, with 250 RPM orbital shaking. At the end of the screening (164 h), the cell dry weight, astaxanthin concentration, and total sugar consumption were determined. These analyses were carried out in duplicate, with the

Table 1 Screening media

Media	Carbon sources	Concentrations (g/L)
1	Glucose	20
2	Xylose	10
3	Arabinose	10
4	Glucose, xylose, arabinose	20, 10, 10

averages and standard deviations reported. The data were used to calculate the cell-specific productivity ($Y_{P/X}$, milligrams astaxanthin per gram of dry cell weight), astaxanthin yield ($Y_{P/S}$, milligrams astaxanthin per gram of sugar consumed), and cell yield ($Y_{X/S}$, grams dry cell weight per gram of sugar consumed).

Reagent Grade Sugars' Fermentation Studies

Fermentations were carried out in a 2-L benchtop bioreactor (New Brunswick Scientific, Edison, NJ). The inoculum was prepared by inoculating 25 ml of YM media in a 250-ml shake flask with 0.2 ml of the previously prepared JTM 185 stock culture. To produce a total of 200 ml inoculum, eight flasks were prepared. These flasks were cultured at 22 °C with 250 RPM orbital shaking for 72 h before being combined and used to inoculate 1.8 L of media in the fermenter, giving a total volume of 2 L. Carbon source levels were varied as described in Table 2. These media were prepared by adding the appropriate amount of each sugar to the following basal media, which were prepared with deionized water (Table 3).

The temperature was maintained at 22 °C using chilled water, and the pH was maintained at 5.5 by the automated addition of 15% (*w*/*w*) NH_4OH (7.75 M) or 5% (*w*/*w*) H_2SO_4 (0.92 M). Air was sparged into the media at a flow rate of 2 L/min and the dissolved oxygen level maintained at 20% of air saturation by the automatic adjustment of the agitation rate. Samples were taken at intervals and analyzed for cell dry weight, astaxanthin, and sugar concentrations. These analyses were carried out in duplicate, with the averages reported.

Preparation of Biomass Hydrolysate

Hydrolysates were prepared from barley straw and sugarcane bagasse using a procedure described by Kim et al. [10]. Biomass (10 g, dry basis) was pretreated by soaking in aqueous ammonia using 100 ml of 15% (*w*/*w*) NH_4OH (7.75 M) (a solid/liquid loading of 1:10). The mixture was then held in a sealed container at 65 °C for 15 h, the cap was then removed, and the ammonia was allowed to evaporate at room temperature (about 25 °C) for 3 h in a fume hood. The pretreated biomass was then washed with deionized water to

Table 2 Fermentation carbon sources

Media	Carbon sources	Concentrations (g/L)
A	Glucose, xylose, arabinose	36, 30, 30
B	Glucose, xylose, arabinose	36, 15, 15
C	Glucose, xylose, arabinose	3.6, 30, 30

Table 3 Basal media

Component	Concentration (g/L)
Yeast extract	5.00
KH_2PO_4	5.71
$MgSO_4 \times 7H_2O$	5.71
NaCl	1.00
$MnSO_4 \times H_2O$	0.004
Sigma Antifoam 204	1 ml/L

remove soluble lignin and residual ammonia. The pretreated solids were separated from the wash water by centrifugation at 8,000 rpm for 30 min. The supernatant was then decanted, and the pretreated solids were recovered. This wash procedure was repeated four to five times, at which point, the supernatant appeared clear and was free of ammonia odor. The pretreated biomass (8 g, dry basis) was then placed in a Buchner funnel and vacuum-filtered overnight to remove the remaining liquid. The recovered pretreated material was then transferred to a 100-ml bottle. Eighty milliliters of potassium phosphate buffer (40 mM, pH 5.1) was added to the biomass, and the mixture was mixed by shaking vigorously. Accellerase™ 1000 and Multifect Xylanase were each added at a loading of 187.5 μL/g solids, and hydrolysis was carried out at 50 °C with 200 rpm orbital shaking for 90 h. After hydrolysis, the solutions were centrifuged and the residual solids discarded. The supernatants, containing the soluble sugars, were used for fermentation.

Hydrolysate Fermentation

The hydrolysate supernatants (50 ml) were transferred to 250-ml shake flasks for fermentation. To provide nitrogen and micronutrients, the components of the basal media used in previous fermentation experiments were added to the hydrolysates to achieve the same concentrations as used previously (Table 3). The only exception was the omission of Sigma Antifoam 204. The pH was adjusted to 5.5, and the flasks were autoclaved at 121 °C for 20 min. After cooling, the flasks were inoculated with 0.2 ml of stock culture prepared by inoculating 25 ml YM media with a single colony of JTM 185 from an agar plate. Fermentation was carried out at 22 °C with 250 rpm orbital shaking. Samples were taken every 2 days and analyzed for optical density at 600 nm (OD_{600}), sugars (high-performance liquid chromatography (HPLC)), and astaxanthin concentration (total carotenoid). The fermentations and analyses were carried out in duplicate, with the averages reported.

Analytical Techniques

Astaxanthin contents of the cell mass were determined by the procedure of An et al., with modifications as described by Nghiem et al. [5, 8]. Cell dry weight was determined by centrifuging a 5-ml sample of the culture, washing with distilled water to remove residual media, and drying the washed cells at 135 °C for 2 h. The dried pellet was then weighed. Individual sugar concentrations were determined using an Agilent 1200 Series HPLC equipped with a RID detector and a Bio-Rad Aminex HPX-87H column with a guard column operating at 65 °C. The mobile phase was 5 mM H_2SO_4 pumped at a flow rate of 0.6 ml/min.

Results and Discussion

Strain Screening

Each of the strains studied was able to metabolize glucose, xylose, and arabinose when the sugars were provided both individually and in combination. Additionally, each strain was capable of astaxanthin production from each sugar. The results of the screening experiments are summarized in Table 4.

Strain JTM 185 gave the highest levels of astaxanthin production. Additionally, JTM 185 demonstrated the highest sugar utilization and the greatest growth on the mixed sugars when compared with the other mutants tested. Hydrolysates derived from biomass normally will contain all three sugars, thus, the mixed media were considered the most important when comparing the above values. With individual sugars, JTM 166 was similar to JTM 185, but was outperformed by JTM 185 in the mixed media. The SSM 19 mutant performed rather poorly as compared to the other two mutants. Based on these results, JTM 185 was selected for further studies.

Astaxanthin Fermentation in Reagent Grade Sugar Solutions

To characterize the performance of JTM 185 on a larger scale, fermentations were carried out using mixtures of sugars in 2-L bioreactors, as described previously. Glucose, xylose, and arabinose were first supplied at 36 g/L, 30 g/L, and 30 g/L, respectively (medium A in Table 2). Next, the amount of xylose and arabinose was reduced by half to more closely resemble a glucose-rich hydrolysate obtained in a biomass fractionation scheme (medium B). Finally, only very low glucose levels (3.6 g/L) were provided to determine the performance on pentose sugars only (medium C). The resulting fermentation profiles are shown in Fig. 1a–c, with the letters corresponding to the media listed in Table 2.

In each case, metabolism of all sugars occurred, and astaxanthin was produced. The organism utilized glucose preferentially, which was not unexpected. It has been frequently

Table 4 Results of strain screening

Strain	Media	X (g/L)	P (mg/L)	ΔS (g/L)	$Y_{P/X}$ (mg/g)	$Y_{P/S}$ (mg/g)	$Y_{X/S}$ (g/g)
JTM 185	Glucose	1.82±0.26	2.25±0.27	17.77±1.55	1.24±0.03	0.13±0.00	0.10±0.01
	Xylose	0.92±0.15	1.70±0.08	8.57±1.75	1.84±0.23	0.20±0.05	0.11±0.04
	Arabinose	0.83±0.00	1.19±0.15	9.4±1.13	1.43±0.18	0.13±0.00	0.09±0.01
	Mix	2.37±0.39	3.25±0.54	31.35±13.21	1.37±0.00	0.10±0.07	0.08±0.05
JTM 166	Glucose	1.69±0.04	1.67±0.06	11.25±4.57	0.99±0.06	0.15±0.07	0.06±0.06
	Xylose	1.33±0.01	1.76±0.09	9.88±0.03	1.33±0.08	0.18±0.01	0.13±0.00
	Arabinose	1.51±0.20	1.78±0.00	8.20±3.46	1.18±0.16	0.22±0.10	0.18±0.11
	Mix	1.99±0.06	2.73±0.13	28.04±0.77	1.37±0.02	0.10±0.01	0.07±0.00
SSM 19	Glucose	0.98±0.26	1.19±0.02	11.47±1.40	1.21±0.35	0.10±0.01	0.09±0.01
	Xylose	0.04±0.01	0.20±0.12	6.58±0.21	4.71±4.39	0.03±0.02	0.01±0.00
	Arabinose	0.32±0.07	0.47±0.10	5.37±4.51	1.48±0.67	0.09±0.08	0.06±0.01
	Mix	1.53±0.44	1.93±0.81	12.12±4.06	1.27±0.18	0.16±0.13	0.13±0.08

X cell dry weight, *P* astaxanthin production, *ΔS* sugar consumption, $Y_{P/X}$ cell-specific productivity (milligrams astaxanthin per gram cell dry weight), $Y_{P/S}$ astaxanthin yield (milligrams astaxanthin per gram of sugar consumed), $Y_{X/S}$ cell yield (grams cell dry weight per gram of sugar consumed)

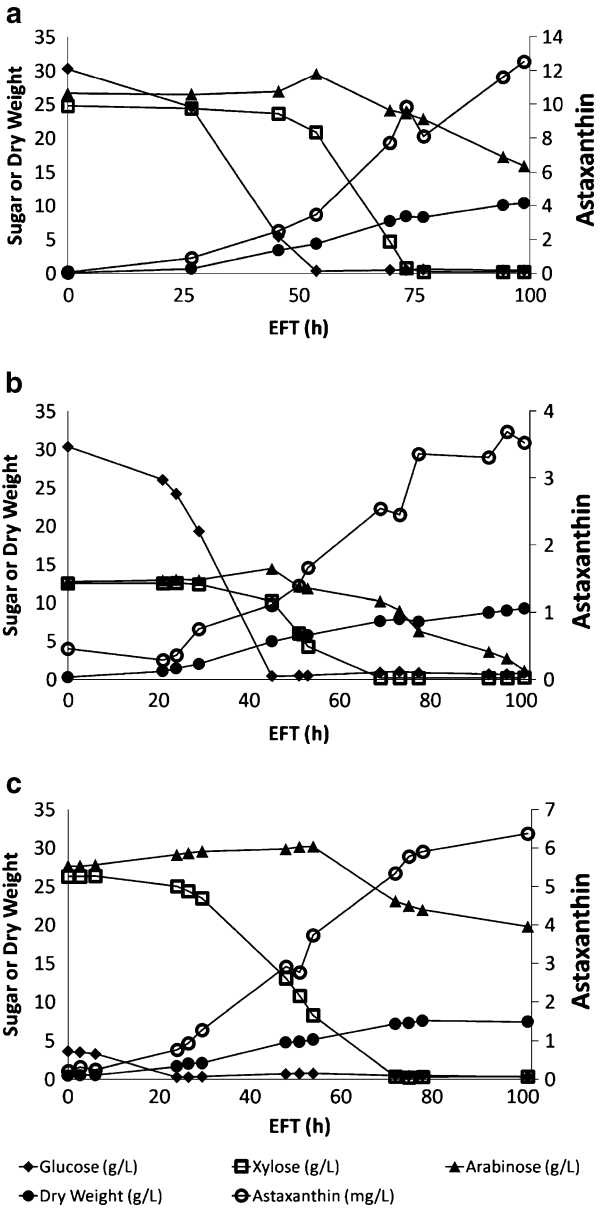

Fig. 1 **a** Fermentation of JTM 185 on mixed sugars. **b** Fermentation of JTM 185 on mixed sugars mimicking a glucose-rich hydrolysate. **c** Fermentation of JTM 185 mimicking a xylose-rich hydrolysate

reported in the literature that many organisms tend to prefer glucose over other sugars, and glucose metabolism often represses the utilization of other sugars. Xylose was utilized once glucose was nearly depleted, followed by arabinose. In each case, glucose and xylose were completely utilized during the fermentation. Arabinose, however, appeared to be utilized at lower rates than the other sugars and was only fully consumed in fermentation B. This indicates that the presence of xylose and glucose in high concentrations negatively affected the metabolism of arabinose. This is further indicated by the observation that xylose was utilized preferentially to arabinose. The final cell mass and astaxanthin concentrations and yield values for the three fermentation experiments are summarized in Table 5.

Table 5 JTM 185 fermentation at varying sugar levels

Fermentation	Max cell density (g/L)	Max carotenoid (mg/L)	$Y_{P/X}$ (mg/g)	$Y_{P/S}$ (mg/g)	$Y_{X/S}$ (g/g)	Q_P (mg/L×h)
A	10.38	12.53	1.21	0.19	0.16	0.13
B	9.27	3.53	0.38	0.07	0.17	0.04
C	7.43	6.37	0.86	0.17	0.20	0.06

$Y_{P/X}$ cell-specific productivity (milligrams astaxanthin per gram cell dry weight), $Y_{P/S}$ astaxanthin yield (milligrams astaxanthin per gram of sugar consumed), $Y_{X/S}$ cell yield (grams cell dry weight per gram of sugar consumed), Q_P volumetric productivity (milligrams astaxanthin per liter×hour)

When the largest quantities of sugars were supplied (fermentation A), the highest cell density, carotenoid levels, and volumetric productivity (Q_P, milligrams astaxanthin per liter×hour) were achieved. However, the highest cell yield was achieved when glucose was supplied only at very low levels (fermentation C). This indicates that the organism was capable of more robust growth without the presence of glucose. Additionally, reducing the initial glucose loading from 36 g/L (fermentation A) to 3.6 g/L (fermentation C) resulted in only a modest reduction in astaxanthin yields, while the reduction of xylose and arabinose from 30 g/L to 15 g/L resulted in a significant decrease in yield. These observations are promising for the inclusion of astaxanthin fermentation in a biorefinery. If a cellulosic biomass were to be hydrolyzed and the resulting sugar stream fed into ethanol fermentation, followed by recovery of the sugars remaining after ethanol removal, glucose would be available only in very small amounts relative to xylose and arabinose, as in fermentation C. Additionally, if cellulosic biomass were to be fractionated as described by Li et al., the xylose-rich stream also would contain very low levels of glucose [4].

Astaxanthin Fermentation from Biomass Hydrolysates

The hydrolysates obtained from the pretreatment and subsequent enzymatic hydrolysis of the pretreated biomass were utilized in astaxanthin production by JTM 185. The initial sugar contents of the hydrolysates are shown in Table 6. The profiles resulting from the fermentation of these hydrolysates are shown in Fig. 2a, b.

In both cases, all of the sugars were consumed and astaxanthin was produced. The sugar utilization pattern was determined to be the same as observed in the reagent grade sugar fermentations (Fig. 1a–c), i.e., glucose was utilized preferentially, followed by xylose, and finally, arabinose. The cell mass, sugar consumption, and astaxanthin production data from these fermentations are summarized in Table 7.

Table 6 Biomass hydrolysate composition

Sugar	Concentration (g/L)	
	Barley straw	Sugarcane bagasse
Glucose	23.77	20.29
Xylose	12.68	11.33
Arabinose	1.63	1.42

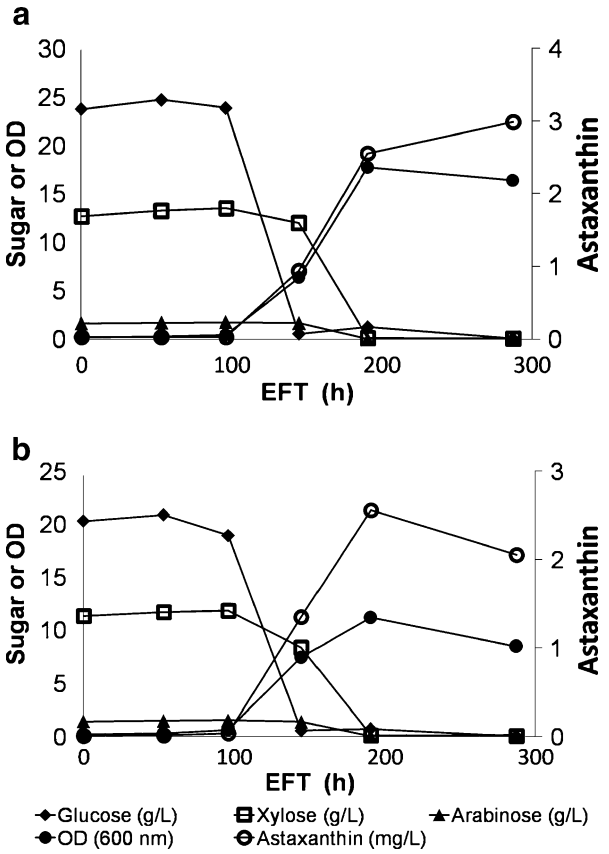

Fig. 2 **a** Astaxanthin production from barley straw hydrolysate. **b** Astaxanthin production from sugarcane bagasse hydrolysate

A greater cell-specific productivity ($Y_{P/X}$) was observed for sugarcane bagasse compared to barley straw. However, this is likely due to the reduced cell concentration rather than increased astaxanthin production. Astaxanthin yields were the same in both cases, and the cell yield was greater when JTM 185 was grown on barley straw. Compared to the reagent grade sugars, however, astaxanthin yields were lower in the biomass hydrolysates (0.14 mg astaxanthin per gram of sugar consumed in reagent grade sugar solutions as compared to 0.08 mg astaxanthin per gram of sugar consumed in hydrolysates). Cell-specific productivity was reduced when the hydrolysates were used as compared to reagent grade sugar solutions. Cell yield, however, was higher. These

Table 7 JTM 185 fermentation of biomass hydrolysates

Fermentation	Max cell density (g/L)	Max carotenoid (mg/L)	S_{init} (g/L)	ΔS (g/L)	$Y_{P/X}$ (mg/g)	$Y_{P/S}$ (mg/g)	$Y_{X/S}$ (g/g)
Barley straw	12.78±0.02	2.99±0.70	38.08±0.02	37.84±0.04	0.23±0.72	0.08±0.74	0.34±0.06
Sugarcane bagasse	7.16±0.09	2.56±0.01	33.05±0.15	32.73±0.17	0.36±0.10	0.08±0.18	0.22±0.26

S_{init} initial sugar content, ΔS sugar consumption, $Y_{P/X}$ cell-specific productivity (milligrams astaxanthin per gram cell dry weight, $Y_{P/S}$ astaxanthin yield (milligrams astaxanthin per gram of sugar consumed, $Y_{X/S}$ cell yield (grams cell dry weight per gram of sugar consumed)

observations may be attributed to enzymes used in the preparation of the hydrolysates. Accellerase™ 1000 is a very crude preparation that contains nutrients remaining from its production, likely including nitrogen sources. The increase in N levels would make for a richer media, resulting in the observed increase in cell yield. Additionally, the decreased C/N ratio would likely have a negative effect on the synthesis of astaxanthin, which is a secondary metabolite [5].

Conclusion

Each of the *P. rhodozyma* mutant studied demonstrated the capability to produce astaxanthin, utilizing the major sugars derived from biomass both individually and in combination. Of the tested strains, JTM 185 demonstrated the highest levels of sugar utilization and astaxanthin production.

In fermentations of reagent grade sugars, it was further observed that JTM 185 was capable of astaxanthin production and metabolism of all sugars provided. It was repeatedly observed that glucose was utilized preferentially, followed by xylose and then arabinose. Additionally, it appeared that high concentrations of xylose repressed arabinose utilization. When glucose was supplied in very low levels relative to xylose and arabinose, the highest cell yield (based on total sugar consumption) was observed, with only a very modest decrease in astaxanthin yield as compared to when glucose was supplied at much higher levels. This is a promising result, as these characteristics would make the organism very useful in a biorefinery setting, in which glucose was utilized in ethanol production and xylose and arabinose were available for the production of high-value coproducts.

It was determined that the organism was able to utilize biomass hydrolysates produced from barley straw and sugarcane bagasse for growth and astaxanthin production. When these hydrolysates were used, however, lower cell-specific productivity (as compared to reagent grade sugar solutions) was observed. Cell yields, however, were increased in the fermentation of the hydrolysates.

Acknowledgements The authors would like to thank Mr. Gerard Senske and Ms. Jennifer Thomas of the USDA ARS ERRC for their invaluable assistance and expertise during the course of this study. The assistance of Dr. Sarah Lingle at the USDA ARS SRRC in obtaining the sugarcane bagasse is also greatly appreciated.

References

1. Rausch, K. D., & Belyea, R. L. (2006). *Applied Biochemistry and Biotechnology, 128*, 47–86.
2. Bothast, R. J., & Schlicher, M. A. (2005). *Applied Microbiology and Biotechnology, 67*, 19–25. doi:10.1007/s00253-004-1819-8.
3. Nghiem, N. P., Hicks, K. B., Johnston, D. B., Senske, G., Kurantz, M., Li, M., et al. (2010). *Biotechnology for Biofuels, 3*, 8.
4. Li, X., Kim, T. H., & Nghiem, N. P. (2010). *Bioresearch Technology, 101*, 5910–5916. doi:10.1016/j.biortech.2010.03.015.
5. Nghiem, N. P., Montanti, J. M., & Johnston, D. B. (2009). *Applied Biochemistry and Biotechnology, 154*, 227–237.
6. Higuera-Ciapara, I., Felix-Valenzuela, I., & Goycoolea, F. M. (2006). *Critical Reviews in Food Science and Nutrition, 46*, 185–196. doi:10.1080/10408690590957188.

7. Palágyi, Z., Ferenczy, L., & Vágvölgyi, C. (2001). *World Journal of Microbiology & Biotechnology, 17*, 95–97. doi:10.1023/A:1016689512718.
8. An, G. H., Schuman, D. B., & Johnson, E. A. (1989). *Applied and Environmental Microbiology, 55*(1), 116–124.
9. Bon, J. A., Leathers, T. D., & Jayaswal, R. K. (1997). *Biotechnological Letters, 19*(2), 109–112.
10. Kim, T. H., Taylor, F., & Hicks, K. B. (2008). *Bioresearch Technology, 99*, 5694–5702. doi:10.1016/j.biortech.2007.10.055.