ELSEVIER

Carbohydrate Research 337 (2002) 1641–1645

CARBOHYDRATE RESEARCH

www.elsevier.com/locate/carres

An alternate carbon source for enhancing production of polysaccharides by *Silene vulgaris* callus

Elena A. Günter, Yury S. Ovodov*

Institute of Physiology, Komi Science Centre, The Urals Branch of the Russian Academy of Sciences, 50, Pervomaiskaya strasse, 167982 Syktyvkar, Russia

Received 25 September 2001; received in revised form 12 February 2002; accepted 15 March 2002

Abstract

Pectin termed silenan and acidic arabinogalactan were isolated as cell-wall polysaccharides of *Silene vulgaris* callus in the presence of various carbon sources as components of the media. The maximum yields, productivity per litre of medium and production per day of acidic arabinogalactan, were achieved using glucose or galactose as the carbon source. Sucrose was found to increase the production of the polysaccharides. Yields, productivity and rate of production of arabinogalactan per day were decreased in the presence of arabinose. Yields of silenan, productivity and rate of production per day were closely related irrespective of the sugar used as the carbon source in the media (sucrose, glucose or galactose) and yields of silenan from the callus growing on arabinose were comparable. A concentration of sucrose in the 20–50 g/L range enhanced the biosynthesis of silenan and at 50 g/L the silenan contained the linear backbone and the ramified regions of the macromolecule.

Keywords: *Silene vulgaris* (Moench) Garcke; Callus; Arabinogalactan; Pectin; Silenan; Carbons

1. Introduction

Preliminary studies of *Silene vulgaris* callus and intact plant indicated the synthesis of polysaccharides possessing immunomodulating activity.[1,2] Pectic polysaccharide, silenan, from the *S. vulgaris* intact plant contains a linear α-(1→4)-D-galacturonan backbone with 2-substituted α-rhamnopyranose residues and ramified regions. The silenan side chains are composed of blocks containing terminal α-(1→5)-linked arabinofuranose and β-(1→4)-linked galactopyranose residues.[3] Sucrose is the most important substrate for catabolism of carbohydrates in higher plants.[4] Several reports[5,6] have indicated a stimulatory effect by elevated levels of carbon source on natural product yields. The aim of the present study was to investigate an influence of various carbons on the production and compositions of the *S. vulgaris* callus polysaccharides.

2. Experimental

General methods.—The callus of *S. vulgaris* was obtained as described earlier.[2,7] Callus cultures were maintained on the modified Murashige and Skoog solid medium[8] with an addition of saccharides (30 g/L). Sucrose (0.09 mol/L), glucose (0.17 mol/L), galactose (0.17 mol/L) or arabinose (0.20 mol/L) were used as a carbon source. The callus cells were subcultured for 21 days at 26 °C in the darkness in the medium containing sucrose at the following initial concentrations, g/L (mol/L): 10 (0.03), 20 (0.06), 30 (0.09), 40 (0.12), 50 (0.15).

Total galacturonic acid and protein contents were estimated colorimetrically with 3,5-dimethylphenol[9] and using the Lowry method,[10] respectively. Spectrophotometric measurements were run on a Ultraspec 3000 instrument (UK).

Isolation of polysaccharides.—The callus was treated with boiling MeOH and $CHCl_3$. The residual material (1 g) was extracted with water (1.0 L) at 50 °C for 2 h, and then concentrated and the residual material was centrifuged. A crude polysaccharide fraction was pre-

* Corresponding author. Tel. +7-8212-241001; fax: +7-8212-241001

E-mail address: ovoys@physiol.komisc.ru (Y.S. Ovodov).

0008-6215/02/$ - see front matter
PII: S0008-6215(02)00079-4

cipitated with 2 vols of 96% EtOH. The precipitate was dissolved in distilled water followed by dialysis against distilled water and lyophilisation. The residual material was treated with dilute HCl (up to pH 4) at 50 °C for 3 h, then the mixture was filtered, and the plant material was extracted with 0.7% aq ammonium oxalate (1.3 L) at 68 °C for 2 h. The solution was treated as described above to afford the purified silenan. The yields were calculated with respect of the dry weight of the callus treated with MeOH and $CHCl_3$. Productivity per litre of medium and production per day of acidic arabinogalactan and silenan were detected.

Complete acidic hydrolysis.—Polysaccharides (2 mg of each) were hydrolysed with 2 M TFA (0.5 mL) at 100 °C for 3–4 h in sealed tubes. The acid was removed by the repeated co-evaporation with MeOH. The neutral sugars were quantified by GLC as the corresponding alditol acetates using *myo*-inositol as the internal standard.[11] The molar ratios were calculated from the peak areas. The data obtained are expressed as mean values from two separate experiments.

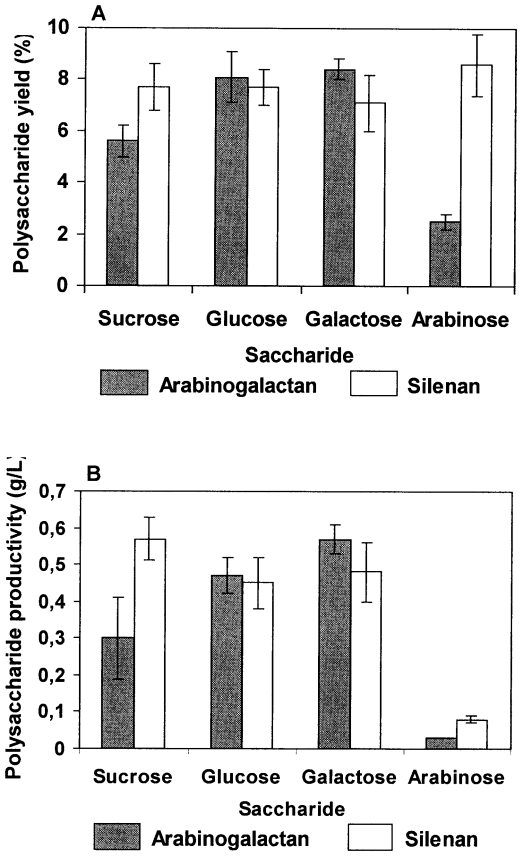

Fig. 1. The effect of saccharides on the yields (A) and productivity (B) of polysaccharides in *S. vulgaris* callus. The data are mean values from three separate experiments. Bars indicate SD.

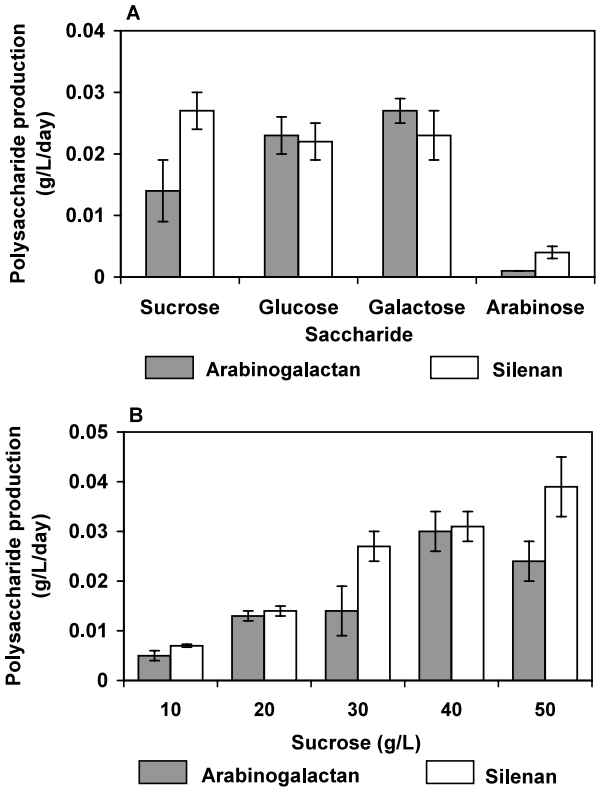

Fig. 2. The effect of saccharides (A) and sucrose concentrations (B) on the polysaccharide production per litre in a day by *S. vulgaris* callus. The data are mean values from three separate experiments. Bars indicate SD.

3. Results and discussion

The influence of different sugars, such as sucrose, glucose, galactose and arabinose, on the cell-wall polysaccharide biosynthesis by *S. vulgaris* callus is demonstrated in Figs. 1 and 2. Analysis of 21 day-old callus indicated that the maximum yields (8.1–8.4% of the dry weight of the callus treated with methanol and chloroform) (Fig. 1(A)), productivity (0.47–0.57 g/L) (Fig. 1(B)) and production per day (0.023–0.027 g/L/day) (Fig. 2(A)) of acidic arabinogalactan were achieved using glucose or galactose as the carbon source. Although a slight decrease of arabinogalactan production was observed in the case of using sucrose, the latter was found to increase polysaccharide production.

Sucrose and glucose were shown to sustain high growth rates in cell cultures and carbon conversion efficiency is also high. These hexoses participate in glycolytic and pentose phosphate pathways.[5] Cells of *S. vulgaris* callus are galactose-adapted and rapid use of galactose is likely due to a high activity of UDP-galactose-4-epimerase (EC 5.1.3.2), a key enzyme in the utilisation of galactose.[5] Information concerning the metabolic utilisation of carbon substrates other than sucrose, glucose and fructose is extremely limited. It is

known that the pentoses, arabinose, xylose and ribose failed to support growth of plant cells.[5] Yields, productivity and rate of production of arabinogalactan per day were decreased in the presence of arabinose (Figs. 1 and 2(A)). It is likely that arabinogalactan was consumed for the formation of arabinogalactan regions of silenan while new biosynthesis of arabinogalactan appeared to be limited due to the cells failed to utilise arabinose.

Yields of silenan (7.1–8.6%), productivity (0.45–0.57 g/L) of the callus in the biosynthesis of silenan and rate of production per day were closely related for all carbon sources (sucrose, glucose or galactose) (Figs. 1 and 2(A)). Yields of silenan from the callus grown on arabinose were comparable with those for the callus grown on sucrose, glucose or galactose. However, more than a fivefold decrease of the silenan productivity by the callus per litre of medium and production per day was observed with arabinose (Figs. 1(B) and 2(A)). Thus, glucose, galactose and sucrose were shown to be effective in sustaining biosynthesis of polysaccharides by *S. vulgaris* callus.

Figs. 2(B) and 3 illustrate the effects of sucrose concentration on polysaccharide production by *S. vulgaris* callus. Media contained the following initial sucrose concentrations, g/L (mol/L): 10 (0.03), 20 (0.06), 30 (0.09), 40 (0.12), 50 (0.15). A concentration of sucrose in the 20–50 g/L range was shown to be enough for the biosynthesis of arabinogalactan. Yields of arabinogalactan appeared to be constant (5.4–6.0%) at sucrose level of 20–50 g/L while this yield decreased to 3.9% for 10 g/L sucrose (Fig. 3(A)). The maximum productivity (0.51–0.64 g/L) and production per day (0.024–0.030 g/L/day) of arabinogalactan were observed at 40–50 g/L of sucrose (Figs. 2(B) and 3(B)). These parameters decreased significantly when sucrose concentration dropped to 10 g/L.

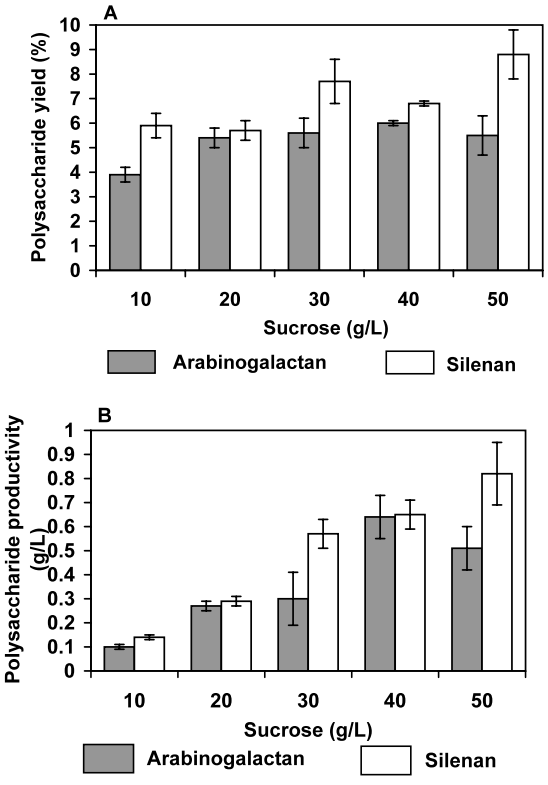

Fig. 3. The effect of sucrose concentrations on the yields (A) and productivity (B) of polysaccharides in *S. vulgaris* callus. The data are mean values from three separate experiments. Bars indicate SD.

Increasing the sucrose concentration from 10 to 50 g/L was accompanied by enhancing the biosynthesis of silenan, achieving the highest values at 30–50 g/L of sucrose (Figs. 2(B) and 3). The maximum yield (8.8%), productivity (0.82 g/L) and rate production per day (0.039 g/L/day) of silenan were obtained at 50 g/L of sucrose. Thus, sucrose concentration as 50 g/L was found to be sufficient for producing maximum amounts of pectin.

The compositions of the cell-wall polysaccharides of *S. vulgaris* callus cultured in the presence of various sugars are given in Table 1. D-Galactose and L-arabinose were shown to be the main neutral sugar constituents of arabinogalactan. Negligible amounts of rhamnose, xylose, mannose and glucose were also detected. The galactose/arabinose ratio was close to ca. 3.7:5.7. In addition, 11–14% of galacturonic acid residues were detected using sucrose, glucose or arabinose as a carbon source. The galacturonic acid contents were increased up to 23% in the presence of galactose. Silenan was essentially composed of the residues of galacturonic acid (73–86%). D-Galactose and L-arabinose were the main neutral sugar constituents. The galactose/arabinose ratio was close to ca. 1.7:2.9.

Arabinogalactan of *S. vulgaris* callus cultured in the presence of various sucrose concentrations was shown to have a similar sugar composition (Table 2). The galactose/arabinose ratio was close to ca. 3.7:4.6. This polysaccharide contained 11–15% of galacturonic acid. The galactose/arabinose ratio in silenan was close to ca. 1.1:1.8. The contents of galactose and arabinose in silenan increased at sucrose 40–50 g/L while the relative amounts of other neutral sugars were unchanged (Table 2). Silenan obtained at 10–40 g/L of sucrose was shown to contain 71–81% of galacturonic acid residues while the contents of galacturonic acid were found to decrease by 63% for the concentration of sucrose 50 g/L (Table 2). Therefore, the latter concentration of sucrose appeared to be sufficient for the biosynthesis of genuine pectin containing the linear backbone and the hairy regions of the macromolecule.

Thus, the alternate carbon-source strategy may be successfully used for regulation of biosynthesis and

Table 1
The effect of saccharides (30 g/L) on the polysaccharide fractions of *S. vulgaris* callus

Contents [a]	Sucrose		Glucose		Galactose		Arabinose	
	I [d]	II [e]	I [d]	II [e]	I [d]	II [e]	I [d]	II [e]
Rhamnose [b]	2.8	0.6	2.9	1.2	3.1	1.2	2.3	1.4
Arabinose [b]	8.2	0.9	9.7	1.8	11.8	1.8	5.3	2.2
Xylose [b]	2.0	0.5	4.3	0.8	3.6	1.0	3.2	0.8
Mannose [b]	1.5	0.7	1.7	1.2	1.8	0.7	2.3	1.1
Glucose [b]	1.8	1.5	3.4	1.4	3.8	0.9	2.8	1.2
Galactose [b]	43.6	1.6	35.7	3.2	43.1	3.2	30.0	6.4
Galacturonic acid [c]	11.1	73.1	14.4	77.4	22.5	79.0	11.8	86.1
Protein [c]	10.2	13.9	15.7	14.3	14.5	13.0	13.4	4.0

[a] The data obtained are mean values from two experiments.
[b] Expressed as mol%.
[c] Weight percentage.
[d] Acidic arabinogalactan fraction.
[e] Silenan fraction.

Table 2
The effect of the initial sucrose concentrations on the polysaccharide fractions produced by *S. vulgaris* callus

Contents [a]	Sucrose concentration (g/L)							
	10		20		40		50	
	I [d]	II [e]	I [d]	II [e]	I [d]	II [e]	I [d]	II [e]
Rhamnose [b]	2.8	1.2	2.6	1.6	3.0	1.4	2.8	1.7
Arabinose [b]	8.1	1.8	7.8	2.8	12.5	3.8	12.1	3.9
Xylose [b]	2.2	0.8	2.6	1.1	3.4	0.6	2.8	0.7
Mannose [b]	1.3	1.0	0.9	1.2	2.4	0.9	3.6	1.3
Glucose [b]	0.9	1.7	2.7	1.6	4.3	1.0	3.4	1.8
Galactose [b]	34.3	3.2	35.8	2.9	47.5	4.9	39.8	5.3
Galacturonic acid [c]	15.1	78.6	13.8	80.6	10.7	70.7	11.5	63.2
Protein [c]	24.1	9.5	22.4	13.3	15.1	17.2	16.8	20.6

[a] The data obtained are mean values from two experiments.
[b] Expressed as mol%.
[c] Weight percentage.
[d] Acidic arabinogalactan fraction.
[e] Silenan fraction.

enhancing production of silenan and acidic arabinogalactan by *S. vulgaris* callus.

Acknowledgements

This work was supported by the grant nos. 00-04-48063 and 02-04-06936 of the Russian Foundation for Basic Research and by the grant no. 8.1.13 of the Scientific Council: "Chemistry and Technology of the Renewing Plant Material Processing" (Ministry of Industry and Science of the Russian Federation).

References

1. Popov S. V.; Popova G. Yu.; Ovodova R. G.; Bushneva O. A.; Ovodov Yu. S. *Int. J. Immunopharmacol.* **1999**, *21*, 614–622.
2. Misharina E. A.; Ovodova R. G.; Bushneva O. A.; Ovodov Yu. S. *Rast. Resursy (Russia)* **1999**, *35*, 88–95.
3. Ovodova R. G.; Bushneva O. A.; Shashkov A. S.; Ovodov Yu. S. *Russ. J. Bioorg. Chem.* **2000**, *26*, 616–622.
4. Sagishima K.; Kubota K.; Ashihara H. *Ann. Bot.* **1989**, *64*, 185–193.
5. Stepan-Sarkissian, G.; Fowler, M. W. The Metabolism and Utilization of Carbohydrates by Suspension Cultures of Plant Cells. In *Carbohydrate Metabolism in Plant*

Cells; Morgan, M. J., Ed.; Plenum Press: New York and London, 1986; pp 151–181.
6. Ryu D. Y. *Biotechnol. Bioeng.* **1993**, *42*, 793–799.
7. Günter, E. A.; Ovodov, Yu. S. RU Patent 2171841, 1999.
8. Murashige T.; Skoog S. A. *Physiol. Plant* **1962**, *15*, 473–479.
9. Usov A. I.; Bilan M. I.; Klochkova N. G. *Bot. Marina* **1995**, *38*, 43–51.
10. Lowry O. H.; Rosebrough N. J.; Farr A. L.; Randall R. J. *J. Biol. Chem.* **1951**, *193*, 265–275.
11. York W. S.; Darvill A. G.; McNeil M.; Stevenson T. T.; Albersheim P. *Methods Enzymol.* **1985**, *118*, 3–40.