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OPTIMIZATION OF CONDITIONS FOR THE EFFICIENT PRODUCTION OF MUTAN IN STREPTOCOCCAL CULTURES AND POST-CULTURE LIQUIDS

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The strain *Streptococcus sobrinus* CCUG 21020 was found to produce water-insoluble and adhesive mutan. The factors influencing both stages of the mutan production, i.e. streptococcal cultures and glucan synthesis in post-culture supernatants were standardized. The application of optimized process parameters for mutan production on a larger scale made it possible to obtain approximately 2.2 g of water-insoluble glucan per 1 l of culture supernate – this productivity was higher than the best reported in the literature. It was shown that some of the tested beet sugars might be successfully utilized as substitutes for pure sucrose in the process of mutan synthesis. Nuclear magnetic resonance analyses confirmed that the insoluble biopolymer synthesized by a mixture of crude glucosyltransferases was a mixed-linkage (1→3), (1→6)-α-D-glucan (the so-called mutan) with a greater proportion of 1,3 to 1,6 linkages.

Keywords: Streptococcus sobrinus – bacterial cultures – post-culture supernatants – mutan production – optimization – structural study

INTRODUCTION

The crucial role of mutans streptococci (especially *S. mutans* and *S. sobrinus*), together with dietary sucrose, in the initiation and progression of human dental caries has been well documented [13, 20]. The cariogenicity of these organisms is well correlated with their ability to convert ingested sucrose into adhesive water-insoluble glucans (referred to as mutans). This process is catalyzed by a constitutive group of extracellular and/or cell-associated enzymes termed glucosyltransferases (GTFs). The sizes and structures of mutans as well as the overall properties of the biopolymers produced depend on the relative activity of different GTFs as one GTF may modify the product of another [3, 21]. Consequently, mutans have a highly branched structure in which α-1,3 glycosidic bonds predominate. The abundance of α-1,3 linkages is associated with water insolubility while the presence of α-1,6-linked side chains contributes to the adhesive properties of these exopolysaccharides [36].

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Mutans synthesized in salivary pellicle by GTFs can promote the selective adherence of oral bacteria which colonize human teeth [27] and which play an essential role in the accumulation of dental plaque. Accordingly, strategies to reduce the disease potential of dental plaque have included the possibility of using mutan-degrading enzymes to disrupt the molecular architecture of the plaque. Some of these enzymes termed mutanases (α-1,3-glucan 3-glucanohydrolases) have shown high potential as a caries preventive agent because they degrade the water-insoluble glucans present in dental plaque [16, 35, 38]. Furthermore, the hydrolysis of mutans by these enzymes has supplied important structural information from analyses of the products of digestion [14]. Various preparations of mutanases have also been successfully used for obtaining fungal protoplasts [1, 32]. However, to induce the synthesis and secretion of mutanase, it was necessary to prepare first a mutan *in vitro* as the sole carbon source in microbial cultures, using sucrose and glucosyltransferase enzymes prepared from mutans streptococci. This type of water-insoluble glucan has not yet been made available as a commercial product. Thus, the enhancement of mutan productivity in streptococcal cultures and post-culture supernates would be useful in facilitating mutanase production on a larger scale and at a relatively low cost.

To our knowledge, no article has been published so far on optimal conditions for intensive production of mutan, and the output of this process in streptococcal cultures is still relatively low. Moreover, data concerning the productivity of insoluble glucan in bacterial cultures or post-culture supernatants have hardly been presented [6, 34]. For these reasons, the present study was conducted in order to find the best operating parameters that might contribute to the efficient formation of mutan by mutans streptococci as well as to determine the detailed structure of this biopolymer.

MATERIALS AND METHODS

Bacterial strain, media and growth conditions

The stock cultures of *Streptococcus sobrinus/downei* strain CCUG 21020 (formerly *S. mutans* OMZ 176) were provided by the Culture Collection, University of Göteborg, Sweden. They were stored at –20 °C in 50% glycerol and routinely transferred on Todd-Hewitt agar slants (pH 7.8). Unless otherwise stated, the bacteria were grown aerobically in glucose-content complex media, such as Todd-Hewitt broth (THB), tripticase soy broth (TSB), brain-heart infusion (BHI) (Baltimore Biological Laboratory, Cockeysville, MD, USA), and I, II, LW, TTY given by Quivey et al. [24], Fuglsang et al. [7], Su-Jin et al. [33] and Hamada and Torii [12], respectively. The media contained in 250-ml flasks, 200 ml each, were autoclaved for 30 mins at 121 °C. A precultured broth (24-h-old, 0.25 v/v%) of bacteria grown at 37 °C in the same media was used for flask inoculation. The growth of bacteria under strictly anaerobic conditions took place in a microbial anaerostat (anaeroJar, AG 025A, Oxoid, Basingstoke, England).

Medium I was chosen among the media tested for mutan production and optimized during the experiments with respect to the initial pH and carbon source. Some culturing parameters listed in Table 2 were also evaluated to improve the output of the process. If not stated otherwise, the batch cultures were run at 37 °C for 24 h under stationary conditions. For mutan production on a larger scale, the strain was grown on the selected medium I in 3-liter batches in 5-l flasks under optimized cultural conditions.

Production of mutan

Insoluble glucan was synthesized from sucrose by a mixture of extracellular glucosyltransferases present in the post-culture fluid of *S. sobrinus*, and its amount was assumed as the indicator of GTF activity.

The bacterial biomass was separated by centrifugation at 12,000 × *g* for 20 mins. Unless otherwise stated, clear supernatant fluid (pH 6.5) was allowed to react with sucrose (3%) in the presence of 0.05% sodium azide as a preservative. After the incubation at 37 °C for 24 h, the subsequently formed water-insoluble glucan was collected by centrifugation at 12,000 × *g* for 20 mins, washed thoroughly with deionized water, freeze-dried, and finally weighed. This water-insoluble glucan will be henceforth referred to as mutan. Some of the important factors specified in Table 3 influencing the efficiency of mutan production in the post-culture supernate were optimized during the experiments.

Structural studies

The carbohydrate composition of glucan was determined after hydrolysis of the preparation (2 M trifluoroacetic acid, 100 °C, 4 h) and conversion of the released sugars into alditol acetates. The reducing agent used was sodium borodeuteride [26]. Alditol acetates were analyzed in a gas chromatograph (HP-5890 series II, Hewlett Packard, Waldbronn, Germany) equipped with capillary column (HP-5, 0.2 mm × 25 m) and a mass selective detector (HP-5971).

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of water-insoluble glucan preparation were recorded with Avance (300 MHz) spectrometer (Bruker BioSpin GmbH, Rheinstetten/Karlsruhe, Germany) at 60 °C. ^{1}H and ^{13}C chemical shifts were obtained using acetone (δ_H – 2.225 ppm, δ_C – 31.450 ppm) as the internal standard. Glucan (20 mg) was dissolved in 0.6 ml of 30% NaOD in D_2O.

Miscellaneous methods

The growth of cultures was measured by recording the optical density at 560 nm (OD_{560}). Batch cultures were performed in triplicate, and analyses were carried out

at least in duplicate. The values reported here are the mean values and standard deviations were in all cases smaller than 3%. Other methodological details are provided in the tables and figures.

RESULTS AND DISCUSSION

The production of water-insoluble glucan by mutans streptococci involves two separate stages: synthesis and secretion of glucosyltransferases into the culture medium and glucan formation from sucrose by the same enzymes present in the post-culture supernatant. Therefore, to maximize the output of the whole process it is important to determine and optimize both cultural and environmental parameters influencing individual stages. These variables may be useful indicators of mutan accumulation as they sometimes cause drastic changes in mutan productivity, signaling the appropriate moment to collect the product.

Optimization of mutan production in streptococcal cultures

The starting point of these studies was the choice of an optimum medium for effective synthesis of water-insoluble glucan by GTFs of *S. sobrinus*. Bacterial cultures were run in 24 h at 37 °C in seven various culture media, mostly used in the produc-

Table 1
Effect of different media[a] on insoluble glucan production
by glucosyltransferases of *S. sobrinus* grown in stationary cultures[b]

Medium	Medium pH		Glucan yield, mg[c]
	initial	final	
I	6.7	6.4	95.3
II	6.5	5.2	0.0
LW	7.0	4.6	7.4
TTY	7.4	4.9	6.5
BHI	7.4	6.0	31.6
THB	7.8	6.3	81.9
TSB	7.3	4.6	14.3

[a] Media: I, Quivey et al. [24]; II, Fuglsang et al. [7]; LW, Su-Jin et al. [33]; TTY, Hamada and Torii [12]; BHI, Brain Heart Infusion; TSB, Tripticase Soy Broth; THB, Todd Hewitt Broth.

[b] Culture conditions: medium, 200 ml; glucose concentration: I, 0.1%; THB and BHI, 0.2%, TSB, 0.25%, TTY, 1%, LW, 2%, II, 5%; temperature, 37 °C; cultivation time, 24 h; aerobic conditions.

[c] Glucan synthesis conditions: culture supernate, 200 ml, pH-value was not regulated; sucrose, 3%; NaN_3, 0.05%; temperature, 37 °C; reaction time, 24 h; static conditions.

tion of the GTFs by mutans streptococci. The post-culture supernatants, containing a mixture of streptococcal GTFs, were then allowed to react with sucrose, and the amount of synthesized glucan was taken as the indicator of the GTF activity for evaluation of the used media.

Table 1 shows that medium I was the most favorable to glucan productivity (over 95 mg of biopolymer were obtained from 200 ml of culture supernate). A slightly lower yield (about 82 mg) was obtained when bacteria were grown in THB broth. Other media gave much smaller effects, and medium II was found to be completely useless for glucan production by the tested strain of *S. sobrinus*. The bacteria acidified all the media, resulting in the most marked (over 2.4 units) pH drop in the case of TSB, TTY and LW media. The relatively low acidity of the medium (pH 4.6–6.0) probably decreases the glucan yield significantly, and more so as the pH-value of the obtained culture supernatants was not regulated in the initial experiments. It was shown that low acidity inhibited the activity of streptococcal GTFs and thereby decreased the yield of glucan production by these enzymes [37]. Also, our further study was confined to medium I, and the most suitable culture conditions for glucan production with the selected medium were provided. It is noteworthy that in this set of experiments the pH-value of culture supernates was adjusted to the initial value of 6.5.

As can be seen from the data in Table 2, the initial medium pH of 7.5, glucose concentration of 0.1%, and the use of 0.5% of the 24-h bacterial suspension as inoculum proved to be the best conditions yielding from 224 mg to 264 mg of insoluble glucan per 200 ml of culture supernate after 30 h of cultivation in stationary and aerobic cultures. In contrast, the efficiency of the biopolymer from slowly agitated (50 rpm) bacterial cultures was over 50% lower than that obtained by streptococci cultivation under stationary conditions. When the bacterial culture was run under anaerobic conditions (more favorable to facultative streptococci), glucan yield was higher by only 19% as compared to that obtained in an aerobic culture. However, maintaining strictly anaerobic conditions, especially during the production of glucan on a larger scale, is very difficult and costly. Consequently, in further studies, aerobic cultures of *S. sobrinus* resulting in a relatively high glucan productivity were applied.

Optimization of mutan production in post-culture supernates

To maximize the glucan yield in the cell-free supernatant fluid, basic production variables, i.e. pH, temperature, sucrose concentration, reaction time, and reaction conditions were standardized. The optimization was carried out with respect to its usefulness in the process of glucan production on a larger scale.

Table 3 illustrates the enzymatic formation of glucan at different values of supernatant pH. The maximum yield (about 295 mg) was reached at pH 6.0. However, pH variations ranging from 5.5 to 6.5 did not affect the range of glucan production so drastically compared with pHs 5.0 and 7.0 when the efficiency of the biopolymer

Table 2
Culturing factors affecting insoluble glucan production by glucosyltransferases of *S. sobrinus* grown in medium I[a,b]: effect of initial pH, cultivation time, inoculum quantity, kind of culture and some medium constituents

Serial No.	Factor varied	Final pH	Glucan yield, mg[c]
1.	Initial pH of the medium[d]		
	6.5	6.2	70.5
	7.0	6.7	108.2
	7.5	6.8	226.0
	8.0	7.4	152.3
	8.5	8.2	50.6
2.	Glucose concentration (%)[e]		
	0.05	7.0	193.1
	0.10	6.6	224.3
	0.25	5.9	169.6
	0.50	4.8	131.2
	1.00	4.6	100.7
3.	Cultivation time (h)[f]		
	6	7.4	69.9
	18	6.9	170.1
	24	6.6	203.9
	30	6.2	240.2
	48	5.9	216.4
4.	Inoculum quantity (v/v%)[g]		
	0.10	6.5	179.9
	0.25	6.3	241.6
	0.50	6.1	264.9
	1.00	6.0	233.4
	5.00	5.9	130.3
5.	Kind of culture:		
	stationary[h]	6.2	256.8
	aerobic[i]	6.2	252.9
	anaerobic[j]	6.3	300.8
	agitated (50 rpm)[k]	5.9	117.6

[a] Composition of the medium was the same as that of the original one, except for the factor or its concentration that varied as indicated.

[b] Culture conditions: [d–k]medium, 200 ml; temperature, 37 °C; cultures were inoculated with 24-h suspension of bacteria (OD_{560}, 1.5) grown in medium I; [d–f]inoculum quantity, 0.25 v/v%; [d–i,k]aerobic conditions; [d–j]stationary cultures; [d]glucose, 0.1%; cultivation time, 24 h; [e]cultivation time, 24 h; medium pH, 7.5; [f–k]glucose, 0.1%; medium pH, 7.5; [g–k]cultivation time, 30 h; [h–k]inoculum quantity, 0.5 v/v%.

[c] Glucan synthesis conditions: [d–k]culture supernate (200 ml, pH-value was adjusted to that of 6.5); sucrose, 3%; NaN_3, 0.05%; temperature 37 °C; reaction time, 24 h; static conditions.

Table 3
Optimization of parameters influencing the enzymatic production of insoluble glucan in post-culture supernate[a]

Serial No.	Factor varied	Glucan yield, mg[b]
1.	pH of culture supernate[c]	
	5.0	163.4
	5.5	224.0
	6.0	294.4
	6.5	249.8
	7.0	162.6
2.	Temperature (°C)[d]	
	30	127.6
	37	296.0
	40	233.2
	45	178.8
3.	Reaction time (h)[e]	
	12	165.1
	24	293.5
	36	364.2
	48	357.3
4.	Sucrose concentration (%)[f]	
	1	291.8
	3	363.5
	5	397.9
	10	419.3
	15	457.5
	20	448.3
5.	Reaction conditions:	
	static[g]	452.9
	agitation (50 rpm)[h]	158.1

[a] The supernatant fluid, containing a mixture of bacterial glucosyltransferases, was obtained after 30 h cultivation of *S. sobrinus* at 37 °C on the optimized medium I under stationary and aerobic conditions: pH, 7.5; glucose, 0.1%; inoculum quantity, 0.5 v/v%.

[b] Glucan synthesis conditions: [c–h]culture supernate, 200 ml, temperature, 37 °C; NaN_3, 0.05%; [c–g]glucan was formed in static conditions; [c]sucrose, 3%, reaction time, 24 h; [d]sucrose, 3%, reaction time, 24 h, pH, 6.0; [e]sucrose, 3%, pH, 6.0; [f]reaction time, 36 h, pH, 6.0; [g,h]sucrose, 15%, reaction time, 36 h, pH, 6.0.

reached only 55% of its maximum. Also, the temperature of 37 °C and reaction time of 36 h were adopted as the best parameters to obtain 296–364 mg of polymer.

Sucrose concentrations ranging from 1% to 5% were mostly reported for the production of glucan [9, 11, 17, 24, 33]. During the initial experiments sucrose concentration in the post-culture supernate was 3%. To ensure suitable conditions for glu-

can production by GTFs of *S. sobrinus*, we evaluated its efficiency at sucrose concentrations between 1% and 20%. The data in Table 3 clearly suggests that a 5-fold increase in sucrose amounts from 3% to 15% raised the glucan yield by about 26%. Accordingly, in further studies the sucrose of 15% inducing a high glucan productivity (about 458 mg/200 ml of culture supernate) would be an adequate substrate concentration for the formation of water-insoluble glucan. However, when running the enzymatic reaction under slow agitation (50 rpm), an approximately 3-fold decrease in glucan yield was observed in comparison with that obtained under mostly popularly applied static conditions.

The optimization of both cultural and environmental factors affecting respective stages of glucan production resulted in an almost 5-fold increase in product output compared with the initial, non-optimized process (see Tables 1 and 3). Thus, the optimization of operating conditions plays a significant role in the economy of glucan production process. Based on the results presented in Tables 1–3, Quivey medium (marked as I) with initial pH 7.5, containing 0.1% glucose, inoculated with 0.5% of the 24-h suspension of *S. sobrinus* and incubated in 30 h at 37 °C under stationary and aerobic conditions, was proven to be the best for glucan production. The optimal

Table 4
Influence of beet sugar as a substitute for sucrose on the efficiency of insoluble glucan production in streptococcal culture supernate[a]

Sugar source	Glucan yield, mg[b]
Sucrose (control)[c]	424.7
Beet sugar[d] coming from the following Polish sugar factories:	
Strzyżów	525.2
Wróblin	513.9
Świdnica	468.1
Glinojeck	458.6
Krasnystaw	404.0
Cerekiew	328.5
Małoszyn	326.1
Chybie	321.5
Rejowiec	300.9
Opole Lubelskie	272.0
Kościan	222.1
Lublin	193.7

[a] The supernatant fluid, containing a mixture of bacterial glucosyltransferases, was obtained after 30 h cultivation of *S. sobrinus* at 37 °C on the optimized medium I under stationary and aerobic conditions. Other details are given in Table 3.

[b] Glucan synthesis conditions: culture supernate, 200 ml; NaN_3, 0.05%; sugar/sucrose, 15%; temperature, 37 °C; reaction time, 36 h; pH 6.0; static conditions.

[c] Analytical grade reagent purchased locally.

[d] Granulated sugar as a substitute for pure sucrose.

parameters for efficient synthesis of glucan in the culture supernate were as follows: pH, 6.0; temperature, 37 °C; sucrose concentration, 15%; and the enzymatic reaction was run in 36 h under static conditions. These conditions were utilized in our further studies.

An attempt was also made to replace pure sucrose used for the production of insoluble glucan by much cheaper sugar beet. Of the twelve granulated sugars coming from various sugar factories, four – produced in Strzyżów, Wróblin, Świdnica and Glinojeck, respectively – were found to form, by means of streptococcal GTFs, much larger amounts of insoluble glucan than those obtained with pure sucrose used as a control (Table 4). Accordingly, these sugars may be successfully utilized as a substitute for pure sucrose in the process of glucan synthesis. Other sugars tested decreased (in some cases even over twice) the efficiency of biopolymer accumulation in culture supernatant.

The differential effects of particular sugars on the output of insoluble glucan production are probably caused by some ions present in non-purified sugar, which may activate or inhibit the activity of bacterial GTFs. It was found that divalent metal ions (Cu, Fe, Zn, Sn, Ag) and the fluoride ion (F) were effective caries inhibitors when used alone or co-crystallized with sucrose [5, 25]. However, the combinations of these cariostatic elements were more effective in caries prevention than single ions. Among different possible mechanisms for the effects of these ions in reducing the cariogenic potential of sugar, an inhibitory action of Cu^{2+} and Fe^{2+} on the activity of two GTF enzymes (B, D) from *S. mutans* was reported [25]. It was also shown that the fluoride ions may also decrease the synthesis of extracellular polysaccharides in *S. mutans* [2].

On the other hand, Mukasa et al. [22] have shown that the formation of water-insoluble glucans by extracellular GTF from *S. mutans* 6715 was greatly stimulated by mono- or divalent cations such as Na^+, K^+, NH_4^+, Ca^{2+}, Ba^{2+}. The authors have found that high concentrations of these cations stimulated GTF activity up to 2.7-fold and also shifted the product from water-soluble to insoluble.

In order to scale up the glucan production, *S. sobrinus* strain was grown on the selected medium I in 3-liter batches in 5-l flasks under optimized culture conditions. Also, the enzymatic synthesis of insoluble glucan in post-culture supernatant was carried out under previously established conditions. The application of the best operating parameters made it possible to obtain a high glucan productivity (in average 2.2 g of water-insoluble biopolymer per 1 l of culture supernate) within a relatively short time. This yield is eleven times higher than that obtained by Ebisu et al. [6] for the best insoluble glucan-producing strain *S. mutans* OMZ 176 (now known as *S. sobrinus* OMZ 176 [31]). Also, basing on twenty-seven of the *S. mutans* strains isolated from dental plaque or carious lesions of patients, Trautner et al. [34], obtained from 2.02 g to 3.94 g of insoluble glucan (for the strains of type c and of type d, respectively) from the incubation medium of 4000 ml. In this paper, however, the glucans were produced in a one-stage process, in which sucrose was added directly to the culture medium. Thus, the final products were contaminated with bacterial cells. The above-mentioned authors have reported that the water-insoluble fractions of the type

c strains were composed of only 50 per cent extracellular polysaccharide and considerable quantities of bacteria; those of the type d strains consisted of 88 per cent glucan. Having taken these facts into account, we calculated that glucan yields reached by these authors were from 2.5 times to 9 times lower as compared with those obtained in our present study.

Structural study of water-insoluble glucan

The preparation of water-insoluble glucan synthesized under optimized conditions was subjected to the structural study. The sugar analysis revealed glucose to be the only component of insoluble material. To determine the α-glucosidic linkages in tested glucan it was subjected to nuclear magnetic resonance (NMR) analyses. The determination of anomeric configuration was based on ^{1}H and ^{13}C NMR spectra. In ^{1}H NMR spectrum signals (broad singlets) for anomeric atoms have been found in the 5000–5500 ppm region characteristic of α-anomers of glucose (Fig. 1). The results from ^{13}C NMR analysis of insoluble glucan are presented in Figure 2. Signal assignments were made according to Colson et al. [4], Gorin [10], Fukui et al. [8] and Shimamura [30]. As shown in Figure 2, six signals arising from α-1,3-linked glucose and six signals from α-1,6-linked glucose were present. The approximate amounts of α-1,3-(60.3%) and α-1,6-glucosidic linkages (21.9%) and those of branched glucose

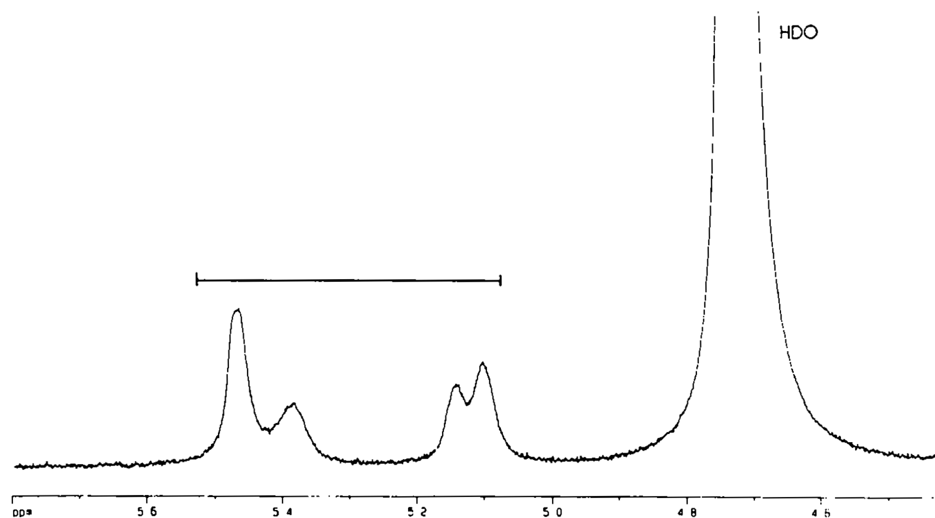

Fig. 1. ^{1}H NMR spectrum of water-insoluble glucan synthesized in culture supernate by glucosyltransferases obtained after cultivation of *S. sobrinus* on medium I. Glucan (20 mg) was dissolved in 0.6 ml of 30% NaOD/D_2O. Acetone was used as internal standard (δ_H = 3.350 ppm). Recording temperature was 60 °C

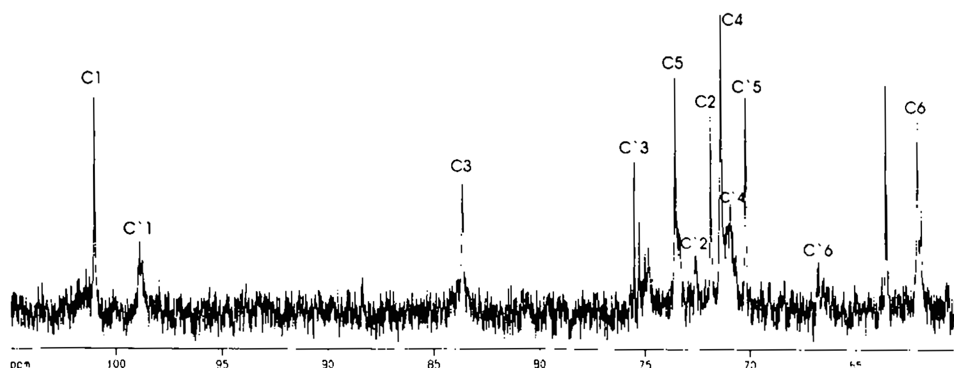

Fig. 2. ^{13}C NMR spectrum of water-insoluble glucan synthesized in culture supernate by glucosyltransferases obtained after cultivation of *S. sobrinus* on medium I. Glucan (20 mg) was dissolved in 0.6 ml of 30% NaOD/D_2O. Acetone was used as internal standard (δ_C = 49,640 ppm). Recording temperature was 60 °C. Peaks were assigned for each carbon in glucose moieties in α-1,3 and α-1,6 glucosidic linkages, and designated C1 through C6 and C'1 through C'6, respectively

(17.8%) were calculated from the integration of carbon signal areas of C3, C6 and C'6 [30]. The ^{13}C NMR spectrum shown in Figure 2 resembled those from articles concerned with glucans synthesized by *S. mutans* [8, 10, 28], and decisively proved the mixed (α-1,3 and α-1,6) nature of the analyzed water insoluble glucan preparation. Also, a greater proportion (by 2.7 times) of 1,3 to 1,6 linkages in the analyzed glucan confirms that the insoluble material has a structure typical of mutan.

Although there have been many studies on the linkage analysis of insoluble glucans most of these studies have been made on polymers synthesized by the isolated and highly purified GTFs [8, 9, 19, 23, 29]. The structural information for glucans formed by crude GTF preparations is scarce and too general [11]. On the other hand, the data from *in vitro* studies indicates that insoluble glucans (mutans) formed by separated and highly purified GTF preparations are not appropriate substrates for the induction of enzymes hydrolyzing these biopolymers since they are structurally distinct products from glucans naturally occurring in dental plaque [17], their synthesis is mostly dependent on exogenous primer dextran [19], and they are more readily degradable during enzymatic hydrolysis [18]. Having taken these facts into account, in the production of insoluble glucans we utilized crude enzymatic supernatants containing GTF complexes which, as shown by Guggenheim [11], Ebisu et al. [6] and Inoue et al. [15], lead to obtaining natural products more resistant to mutanase digestion and synthesized in the absence of primer molecule.

In conclusion, the data presented here show that the *S. sobrinus* CCUG 21020 strain, constitutes a culture of high extracellular GTF activity that gives reasonable yields of insoluble mixed-linkage (1→3), (1→6)-α-D-glucan (mutan) with a greater proportion of 1,3 to 1,6 linkages. The optimization of the whole process allowed for the effective mutan production. Its productivity was almost five times higher than

that obtained under initial, non-standardized conditions. The scale-up of mutan production gave a yield of 2.2 g glucan per 1 l of culture supernate, which is a higher productivity than the best reported so far. Moreover, some of beet sugars may be effectively used as a substitute for pure sucrose in post-culture supernatants to reduce the costs of mutan production considerably. In further studies mutan will be used as a natural substrate inducing the synthesis and secretion of specific mutanases degrading this polymer. These enzymes are currently being tested in our laboratory for their potential as caries preventive agent.

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