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Original Research

The synthesis of non-structural carbohydrates under the network security model

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Abstract: In order to explore the pathway of non-structural carbohydrate synthesis, an analysis of the pathway of non-structural carbohydrate synthesis under the network security model was proposed. Taking non-structural carbohydrates as the research object, the experimental materials and equipment were selected under the network security model. Through the establishment of detection methods, the preparation of freeze-dried carbohydrates, the influence of synthesis pathway-specific inhibitors on the synthesis of non-structural carbohydrates, the influence of precursors and intermediates in the pathway on the synthesis of non-structural carbohydrates, the effect of multiple factors on the synthesis of non-structural carbohydrates, the influence of polysaccharide synthesis, the treatment of reaction solution for detection, the preparation of detection sample, the detection conditions of a liquid phase, the detection conditions of LC-MS and the determination of carbohydrate biomass were studied. The results showed that the synthesis of nonstructural carbohydrates requires the participation of the glycolysis, shikimic acid and phenylpropane pathways, but not the polyketone pathway.

Key words: Network security model; Non-structural; Carbohydrate; Synthetic pathway.

Introduction

With the successful completion of the research program of compound synthesis in the 1990s, the research on carbohydrate officially kicked off and opened a new door for human beings to explore the mysteries of life. Since the completion of the sequencing of the first model Saccharomyces cerevisiae in 1996, the sequences of the other two models of Schizosaccharomyces cerevisiae and Neurospora harzianum were obtained in 2002 and 2003 respectively, and then the compound information of Rhizopus oryzae was obtained (1). According to incomplete statistics of the database, 23767 groups of compounds have been sequenced, including cellulose 13617 groups and starch 4146 groups. At present, more than 998 carbohydrates have been sequenced, including 682 monosaccharides. The main carbohydrate obtained is a monosaccharide, and cellulose has not been reported (2). With the development of non-structural carbohydrate research, the study of carbohydrate secondary synthesis has entered the chemical era from the biochemical era. Some functional carbohydrates with little or even unknown knowledge can be mined out by synthetic pathway sequencing; new carbohydrate resources and synthetic pathways can be found through element mining and analysis of synthetic pathway (3). For example, in the related research of cellulose synthesis pathway, through the methods of element group location analysis and element mining, the complete functional element cluster and key regulatory factors are obtained, and the control of the whole synthesis flow is realized (4). In the study of whether other carbohydrates also can synthesize such substances, it is only necessary to analyze the obtained element group to determine whether they can synthesize the target product (5). However, in laboratory analysis, many of the related elements encoding secondary synthesis in carbohydrates are silent and not expressed. In this regard, the silencing element cluster can be activated by certain methods, so as to obtain more new carbohydrates with biological activity, and then provide the possibility for the development and production of new products (6). The activation strategies of the secondary product cluster mainly include: in vivo activation, heterologous host expression, overexpression of transcription factors, and replacement of promoters. There are four ways to activate the cluster of elements related to the synthesis of secondary synthesis products in the host: activating the cluster of elements through the cluster-specific transcription factors, global regulatory factors, histone modification and changing the culture conditions (7). At the same time, the carbohydrate element group also makes us further realize the complexity of the synthesis of secondary synthesis products. In addition, the study of non-structural carbohydrate synthesis pathways can speed up and simplify the exploration of the secondary synthesis pathway, and a large number of synthetic element clusters of secondary synthesis products in the compound sequence have been gradually studied and identified (8). More than 50 years ago, it was found that carbohydrates can produce polysaccharides, and only after the completion of the element group sequencing of the compounds, could the pathway of non-structural carbohydrate synthesis be fully revealed. A monosaccharide is a kind of product produced by non-structural carbohydrates, which has important application value, high economic benefit and low yield. Its synthesis pathway was not determined

until the completion of element group sequencing (9). The determination of the non-structural carbohydrate synthesis pathway is also based on the network security model. Elemental analysis and synthesis based on the network security model provide more effective methods and means for the comprehensive and systematic analysis of carbohydrate properties and properties. The synthetic analysis provides us with some new ideas and methods when traditional research and methods are in trouble (10).

From the above research status, we can see that nonstructural carbohydrates can be used as a new compound resource. The current research mainly focuses on the discovery and identification of new carbohydrates or new synthetic products, but little is known about the synthesis pathway of non-structural carbohydrates (11). Nonstructural carbohydrates can produce a variety of active products, including cellulose, starch and sucrose compounds, but the synthesis pathway and related elements are not clear (12). A monosaccharide is a key step in the synthesis of sucrose compounds. It is of great theoretical and practical significance to fully understand and master the synthesis pathway and regulatory mechanism by identifying the enzymes involved in the reaction (13). Therefore, on the basis of previous studies, this paper intends to first reveal the key steps and pathways of synthesis of non-structural carbohydrates at the level of elements through the analysis of element group and transcriptome. Then it analyzed the relevant synthesis pathways more accurately through synthesis pathway inhibitors, precursor addition, separation and purification of key enzymes, expression of Crohn, functional verification, etc. It is to further explore the foundation of element transformation, synthesis reconstruction and regulation (14). Nonstructural carbohydrates are a kind of natural products with a wide range of biological activities. They have very important uses in food. In the synthesis process of non-structural carbohydrates, the differences of chain length, elongation unit, post-modification and oxidation level ultimately lead to the diversity of non-structural carbohydrates in their structure, and the diversity of their structure also leads to the diversity of their biological activities. Therefore, these compounds are always important ingredients needed by people, and also modern products One of the important sources of development (15).

The main purpose of this paper is as follows:

(a) To obtain the elemental and transcriptome of non-structural carbohydrates and understand their synthesis diversity;

(b) To analyze the synthesis pathway and key steps of nonstructural carbohydrate synthesis from the level of elements and enzymes;

(c) To obtain the key elements and related enzymes for the synthesis of non-structural carbohydrates.

The research significance of this paper is: the research results can provide important information for the further development and utilization of non-structural carbohydrates; provide theoretical guidance for the regulation of food synthesis of nonstructural carbohydrates and provide a reference for the research of related synthesis pathways in other compounds (16). At the same time, it can also provide data support for the further study of the synthesis of other new compounds.

Analysis of the synthesis of non-structural carbohydrates under the network security model

In the network security model, this project synthesizes cDNA by extracting RNA of non-structural carbohydrates. To obtain an element group fragment of a nonstructural carbohydrate that removes an intron (17). Through the construction of heterologous expression recombinant plasmid in the synthesis system, the synthesis pathway of nonstructural carbohydrate was reconstructed in the synthesis heterologous system, and the intermediates and end products of each synthesis stage were obtained through the separation and identification of nonstructural carbohydrate. Then the recombinant compounds were purified by a compound expression system, and the function of each element was verified by a biochemical reaction in vitro with the purified soluble compounds and intermediates. Finally, the synthetic pathway of non-structural carbohydrates was determined (18).

Materials and Methods

Selection of experimental materials

Carbohydrate and plasmids

The carbohydrates used in this paper are shown in Table 1, and the original plasmids used are shown in

Table 1. Carbohydrates used in this research.

Organism name	Main characteristics	Remarks
E. coli XL-1	Cloning carbohydrate with recombinant plasmid	Experiment at 20 ℃
E. coli BL21(DE3)	Expression of carbohydrate by recombinant elements	nothing
E. coli BAP1	Expression of carbohydrate by recombinant elements	Experiment at 35 ℃
Saccharomyces cerevisiae BJ5464	Heterologous expression of carbohydrate in the synthesis system	Experiment at 30 ℃
Aspergillus nidulans FGSC A4	Production of carbohydrates by diorcinol	nothing
A. nidulans (Δors-BGC strain)	Heterologous expression of carbohydrate in Aspergillus nidulans	Experiment at 45 ℃

Table 2. The original plasmids used in the experiment.

Plasmid name	Main characteristics	Remarks
pYEU	Uracil screening markers, synthetic replication sites, amp-resistant element amp R	Store at 25 ℃
pYEL	Uracil screening markers, synthetic replication sites, amp-resistant element amp R	Nothing
pYET	Uracil screening markers, synthetic replication sites, amp-resistant element amp R	Store at 27 ℃
pCold I	6 x his label, cold shock carbohydrate expression, amp-resistant element amp R	Nothing
pANR	Screening markers of vitamin B6. Uracil screening markers, synthetic replication sites, amp-resistant element amp R	Nothing

Table 3. Recombinant plasmid for the experiment.

Plasmid name	Describe	Restriction site	Purpose
pIM 1	AN7909 cDNA in pYEU	Nde I-Spe I	Heterologous expression of *S.cerevisiae*
pIM2	AN7910 c DNA in pYET	Nde I-Pme I	Heterologous expression of *S.cerevisiae*
pIM3	AN7911 cDNA in pYEL	Nde I-Pme I	Heterologous expression of *S.cerevisiae*
pIM4	AN7910 c DNA in pCold I	Nde I-Eco RI	Expression of *E. coli* protein
pIM5	AN7911 c DNA in pCold I	Nde I-Eco RI	Expression of *E. coli* protein
pIM6	AN7910-H68A in pCold I	Nde I-Eco RI	Expression of *E. coli* protein
pIM7	AN7910-R16A in pCold I	Nde I-Eco RI	Expression of *E. coli* protein
pIM8	AcsA c DNA in pYEU	Nde I-Spe I	Heterologous expression of *S.cerevisiae*
pIM9	AN7909-ACP1m(S1676A) in pYEU	Nde I-Spe I	Heterologous expression of *S.cerevisiae*
pIM10	AN7909-ACP2m(S1775A) in pYEU	Nde I-Spe I	Heterologous expression of *S.cerevisiae*
pIM11	AN7909-TEm(S1907A) in pYEU	Nde I-Spe I	Heterologous expression of *S.cerevisiae*
pIM12	AN7909-TE in pYEL	Nde I-Pme I	Heterologous expression of *S.cerevisiae*
pIM13	AN7909 cDNA in pANR		*A. nidulans* overexpression

Table 2. Both carbohydrates and plasmids are preserved in our laboratory.

The recombinant plasmids used in the experiment are shown in Table 3. The recombinant plasmids are constructed by our laboratory.

The primers used in the experiment are shown in Table 4.

Main experimental reagents

The reagents and instruments required for the analysis experiment of the non-structural carbohydrate synthesis pathway are shown in Table 5.

Experimental method

Establishment of the test method

Weigh 10 mg of monosaccharide, sucrose, carbohydrate, starch and polysaccharide standards respectively, fix the volume of monosaccharide and carrot to 10 mL with chromatographically pure methanol, fix the volume of sucrose, starch and corn to 10 mL with ultrapure water, prepare a standard storage solution of 1 mg/mL, and store it at 4°C, in which carrot should be kept away from light. When using, dilute these standard storage solutions with 70% methanol to 0-0.5 mg/mL working solution, and prepare a mixed standard working solution for use. By adjusting the factors such as the amount of liquid, the speed of elution and the wavelength of detection, an HPLC method for the simultaneous detection of these substances was established, and the accuracy, precision and recovery of the method were analyzed.

Preparation of lyophilized carbohydrate

The nonstructural carbohydrates were inoculated on PDA plate culture medium, cultured and activated for 5 days at 28 °C; 4-6 pieces of culture were taken from a sterile perforator with a diameter of 1 cm and inoculated into a 250 mL shake flask filled with 100 mL of PDB culture medium, and cultured for 5 days at a constant temperature at 28°C and 180 rpm/min as seed solution. The seed solution was inoculated into a fresh PDB medium with a 10% inoculum amount. The amount of seed solution was 100 mL/250 mL, and cultured at 28 °C and 180 rpm/min for 5 days. After that, the fermentation broth was centrifuged at 4°C, 4000 rpm/min for 10 min, the compounds were collected, washed with sterile water for 3 times, and then re-suspended in sterile water, treated overnight at 4°C, the supernatant was removed, and the carbohydrate precipitate was collected as the resting compounds. Refrigerate at 4°C.

In addition, the rest compounds were treated overnight at -80°C, and then freeze-dried in a vacuum freeze dryer, that is, freeze-dried carbohydrates, which were stored at -70°C for standby.

The effect of pathway-specific inhibitors on the synthesis of nonstructural carbohydrates

Cerulenin and iodoacetamide are specific inhibitors of the synthetic pathway, trimethylamine and EDTA are specific inhibitors of the synthetic pathway. In order to investigate the effects of these inhibitors on the synthesis of carrot and corn, the above-mentioned compounds were put into sterile water (natural pH) containing 1.5% glucose (15 g/L) with 10% inoculum amount, and the liquid content was 100 mL/250mL. The reaction was carried out under the conditions of 28°C and 180 rpm/min. after 12 hours, the concentration of 10, 50 and 100 μmol/L of cerulenin were added respectively 15 mmol/L iodoacetamide, 12,48,96 mmol/L trimethylamine and 6,9,12 mmol/L EDTA were cultured for 120 h, during which the reaction solution was taken every 24 h to determine the accumulation of carrot, corn and carbohydrate biomass. The control group was sterile water with only resting compound and 1.5% glucose, which was transformed under the same conditions, with three replicates for each treatment.

Effects of precursors and intermediates in pathways on the synthesis of nonstructural carbohydrates

In order to further verify the pathway of carrot and corn synthesis in carbohydrates, the shikimic acid pathway and its downstream pathway, important intermediates in the phenylpropane pathway, were added to the resting reaction system, respectively, to investigate their effects on the synthesis of carrot and corn. Before the start of the conversion reaction, different concentrations of precursors were added first, and the reaction was terminated 36 hours later. The control was a reaction system containing only resting compounds and 1.5% glucose sterile water. Under the same conditions, the

Table 4. Primer sequence used in the experiment.

Primers code	Sequences of primers (5'-3')	Remarks
pYEU-AN7909-F1	GTAGGGGGCCCTGTCGCCGCCATC	Experiment at 22 ℃
pYEU-AN7909-R1	CATTTAAATTAGTGATGGTGATGGTGATGCACGTGTTCCACCGCCCGAGTCACAGTTTC	Nothing
pYEU-AN7909-F2	GCGATTATAAGGATGATGATGATAAGACTAGTATGGCTCCAAATCACGTTCTTTTTTTC	Experiment at18 ℃
pYEU-AN7909-R2	CTTTAGGTATTCGTCGAGGGCCTG	Experiment at 25 ℃
pANR-AN7909-F	CCATTACCCCGCCACATAGACACATCTAAACAATGGCTCCAAATCACGTTCTTTTTTTC	Experiment at 25 ℃
pANR-AN7909-R	TTCTGCTAAAGGGTATCATCGAAAGGGAGTCATCCACTATTCCACCGCCCGAGTCACAG	Experiment at 18 ℃
ACP1S-F	GGGTCGATGCCCAGATGGCCATCTC	Experiment at 20 ℃
ACP1S-R	GAGATGGCCATCTGGGCATCGACCC	Nothing
ACP2S-F	GGGTATGGACGCCATGCTCAGCATC	Experiment at 25 ℃
ACP2S-R	GATGCTGAGCATGGCGTCCATACCC	Experiment at 20 ℃
TE(S)-F	CGGCGGTTGGGCAGCGGGCTCTATG	Experiment at 25 ℃
TE(S)-R	AGAGCCCGCTGCCCAACCGCCG	Nothing
pYEL-TE-F	GGGAATTCCATATGACCACGATCGACTCCAGCCGC	Experiment at 25 ℃
pYEL-TE-R	GCATGTTTAAACCTATTCCACCGCCCGAGTCAC	Experiment at 20 ℃
pYEU- AcsA-F	CTAGCGATTATAAGGATGATGATGATAAGACTAGTATGCGTTTGAAAGACACAAAGATG	Nothing
pYEU- AcsA-R	TTTAAATTAGTGATGGTGATGGTGATGCACGTGTCACCTCTCCAACTCTTGCAACAG	Nothing
pYET-AN7910-F	GGAATTCCATATGATGGCTACTGAGATTGCCGAGATC	Experiment at 18 ℃
pYET-AN7910-R	AGCTTTGTTTAAACCTATGCTACTGTGCCTTCAAC	Experiment at 18 ℃
pYEL-AN7911-F	GGAATTCCATATGACTCCGCCACTCCTCGCCCTC	Experiment at 18 ℃
pYEL-AN7911-R	AGGCATGTTTAAACTCACTGCTCCTTCTTCATGTGCAC	Experiment at 20 ℃
PcoldI-AN7910-F	GGAATTCCATATGATGGCTACTGAGATTGCCGAGATC	Experiment at 20 ℃
PcoldI-AN7910-R	CCGGAATTCCTATGCTACTGTGCCTTCAACTA	Experiment at 25 ℃
PcoldI-AN7911-F	GGAATTCCATATGATGACTCCGCCACTCCTCGCCC	Experiment at 25 ℃
PcoldI-AN7911-R	CCCAAGCTTTCACTGCTCCTTCTTCATGTGCAC	Experiment at 25 ℃
Pcold-AN7910H68A-F	CAACGTGATCGCATCCTCCTTTG	Nothing
Pcold-AN7910H68A-R	CAAAGGAGGATGCGATCACGTTG	Experiment at 20 ℃
Pcold-AN7910R16A-F	GAAACGCGAGGCCTACTACCG	Nothing
Pcold-AN7910R16A-R	CGGTAGTAGGCCTCGCGTTTC	Experiment at 20 ℃

Table 5. Statistical table of reagents required for the experiment.

Name	Company	Specifications
TIANprep Mini Plasmid Kit	Tiangen Biochemical Technology Co., Ltd	AR
RNA reverse transcription Kit	Roche	AR
TRIzol Reagent	Thermo Fisher	AR
Gel Extraction Kit	OMEGA	AR
Zymoprep, Yeast Plasmid Miniprep I	ZYMO RESEARCH	AR
Frozen-EZ Yeast Transformation II Kit	ZYMO RESEARCH	AR
Restriction endonuclease	New England Biolabs	AR
T4 DNA Ligase	New England Biolabs	AR
Q5 ® super fidelity DNA polymerase	New England Biolabs	AR
DNA marker	Beijing Bomede Gene Technology Co., Ltd	AR
Precision Plus Protein™ Pre dyed protein standard	BIO-RAD	AR
ExcelBand™ Enhanced 3-color High Range Protein Marker	Mellon	AR
SDS-PAGE gel configuration Kit	Beijing Dingguo Changsheng Biotechnology Co., Ltd	AR
BCA protein test kit	Beijing Dingguo Changsheng Biotechnology Co., Ltd	AR
Nucleic acid dye Golden View	Mellon	AR
Agarose	Biowest	AR
Ampicillin	Meilun Biotechnology Co., Ltd	AR
tryptone	Thermo Fisher Oxoid	AR
Yeast extract	Thermo Fisher Oxoid	AR
Bacto™ Casamino Acids	BD	AR
Tryptophan	Tao Su Biochemistry (China)	AR
Moscone	Tixiai (Shanghai) Chemical Industry Development Co., Ltd	AR
Common reagents	Chengdu Cologne Chemical Co., Ltd	AR

Table 6. Level and coding of experimental factors.

Code		A	B	C
Factor		Conversion time	Glucose concentration	Compound concentration
Unit		H	%	%
	-1	5	1	0.2
Level	0	10	1.5	0.3
	1	15	2	0.4

reaction lasted for 36 hours, and each treatment was repeated three times.

In order to eliminate the effect of carbohydrate growth on the yield of carrot and corn, freeze-dried carbohydrate was used as the material for biotransformation. During the operation, before adding the freeze-dried carbohydrate, take the pre-prepared precursor and inhibitor and add them into the distilled water containing 1.5% glucose (15 g/L), with the liquid volume of 100 mL/250mL, then add the freeze-dried carbohydrate with the inoculation volume of 0.38%, and start the reaction. The reaction conditions were: 28 ℃, 180 rpm/min, conversion for 12 hours. After that, the yield of the target product was detected by the conversion solution. The control group contained only freeze-dried carbohydrates and 1.5% glucose water, which were transformed under the same conditions. Three repeats are set for each process.

The effect of multiple factors on polysaccharide synthesis

Take a proper amount of freeze-dried carbohydrates and put them into Tris-HCl buffer with a certain concentration of glucose at 100 mM, pH 7.5. The biotransformation reaction is carried out at 28℃ and 180 rpm/min. During the reaction, the corn accumulation was detected by using the transformation solution. The experiment is arranged according to the Box-Behnken experiment design of three factors and three levels. The levels and codes of each factor are shown in Table 6. According to the preliminary experiment, carbohydrates can synthesize corn and carrot in this simple system, but because the synthesis speed of the two substances is different, this part focuses on corn yield.

Treatment of reaction solution for detection and preparation of test sample

For the reaction system of resting compounds and freeze-dried carbohydrates, after the reaction, the carbohydrates are removed by centrifugation, vacuum concentrated to one-fifth of the original volume at 50℃, and then 95% ethanol of three times the volume is added; after the alcohol is precipitated overnight, the insoluble substances are removed by filtration, the liquid phase is evaporated to dryness, and then 1 ml methanol is added for dissolution, an ultrasonic wave was used

for 3-4 min, and 1.5 mL is collected for separation The heart tube was refrigerated at 4℃ for liquid analysis. During the determination, the organic filter membrane with a pore diameter of 0.45 μm was used to filter, and the filtrate was taken to detect the yield of carrot and corn by HPLC-MS.

Liquid phase-detection conditions

Column: Xterra MS C18 (4.6×250 mm, 5 μm) (waters, USA)
Mobile phase: a phase is double distilled water; phase B is acetonitrile;
The gradient elution procedure was: 10% B for 0-10min, 10-20min, 10% - 20% B, 20-30min, 20% - 70% B, 30-40min, 70% - 10% B, 40-50min, 10% B;
Flow rate: 1.0mL/min;
Column temperature: 30 °C;
Injection volume: 20 μL;
Detection wavelength: 257 nm, 226 nm, 227 nm, 229 nm, 263 nm.

Detection conditions of the liquid mass spectrometer

The mass spectrometric analysis of the product after the enzymatic reaction was carried out with the American Thermo Fisher liquid mass spectrometer (LTQ-XL). The detection conditions were ESI ion source, capillary temperature 320℃, gas flow rate of colliding gas 35 L/h. Positive setting: voltage 4 kV, current 100 μA, capillary voltage 40 V; corresponding negative setting: 4.5 kV, 100 μA, -20V, respectively. The injection volume was 10 μL and the flow rate was 0.5 mL/min.

Determination of carbohydrate biomass

After separating the converted resting compounds from the conversion solution, place them on the filter paper that has been dried to constant weight, and then dry them to constant weight in the oven at 55 ℃, and calculate the carbohydrate biomass (mg/L) in the reaction solution per unit volume.

Results and Discussion

Types of non-structural carbohydrates synthesized in the reaction system

It can be seen in Figure 1 that in the resting compound transformation system, the yield of carbohydrate gradually increased from 24 h to 72 h, and then decreased; the yield of polysaccharide was the highest at 24 h, then decreased continuously, and then increased slightly at 144h. This may be because, in the process of transformation, polysaccharides are converted into carbohydrates, and the reduction of carbohydrates in the later stage may be due to its hydrolysis to polysaccharides.

In the transformation system of freeze-dried cells, the results of the effects of various factors on the carbohydrate production in the reaction system are shown in Table 7.

The f-value of the model is 157.39, p-value < 0.0001, and the effect of transformation time (A) and its square value on carbohydrate production is extremely significant; the effect of glucose concentration (B) and bacte-

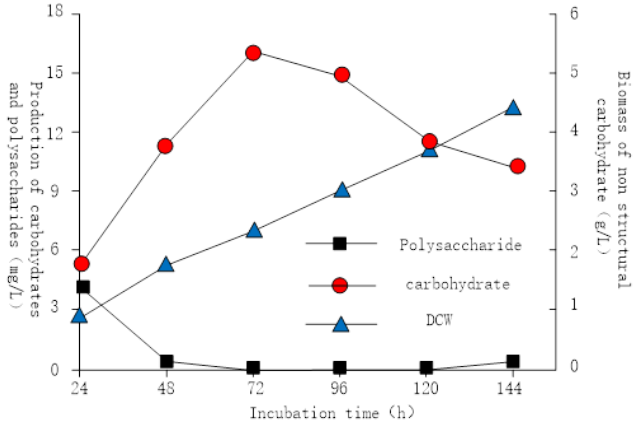

Figure 1. Yield and biomass curve of carbohydrate and polysaccharide in resting compounds.

Table 7. the effects of various factors on the carbohydrate production in the reaction system.

Running order	Bioconversion time/h	Glucose concentration g/L	Cell dosage g/L	Carbohydrate production (μg/L)	
				Experimental value/mean±SD	Predicted value
1	5	10	3	53.95±0.17	54.01
2	15	10	3	91.69±0.22	91.88
3	5	20	3	45.41±0.31	45.81
4	15	20	3	84.83±0.20	83.77
5	5	15	2	59.53±0 .28	60.15
6	15	15	2	87.84±0.17	86.91
7	10	15	3	101.48±0.08	102.05
8	15	15	4	102.34±0.09	102.02
9	10	120	2	95.97±0.25	95.59
10	10	10	2	88.44±0.16	88.03
11	10	20	4	102.43±0.05	103.94
12	10	15	4	102.61±0.16	101.09
13	10	15	3	101.61±0.09	101.83
14	10	15	3	97.86±0.16	99.32
15	5	15	4	60.42±0.27	58.92
16	10	15	3	100.19±0.11	100.69
17	10	15	3	102.28±0.06	102.22

rial concentration (C), and the square effect of B and C and the interaction of AC on carbohydrate production are all significant. The multivariate correlation coefficient R^2=0.9951 and the mismatch term is not significant (P=0.2698), indicating that the model has a high degree of fitting with the actual data, and the error of using it in the actual prediction is small; the correction determination coefficient AdjR2=0.9888, indicating that 98.88% of the variability of the experimental data can be explained by this regression model. The optimal conditions for the system to synthesize carbohydrates were as follows: the conversion time was 12.65 h, the concentration of glucose was 14.67 g/L, the concentration of bacteria was 3.87 g/L, and the theoretical value of PDG production was 110.66 μg/L. The actual result is 110.28μg/L.

Analysis of nonstructural carbohydrate synthesis pathway by polyketide pathway inhibitors

Previous studies have shown that the synthesis of non-structural compounds and structural carbohydrates have similar mechanisms, and the main catalytic enzyme, polyketide synthetase, have similar structures and functions with carbohydrate synthetase. The inhibition mechanism of non-structural carbohydrates as specific inhibitors is that β-ketoacyl-ACP synthetase cannot condense the precursors such as acetyl-CoA and malonyl CoA to form non-structural carbohydrates by binding with acyl transporters on these two enzymes. As can be seen in Figure 2 (A), with the increase of carbohydrate concentration, there is no significant effect on the synthesis of carbohydrates in the test system compared with the control, indicating that the inhibitor does not affect the cell growth of bacteria. At the same time, compared with CK, as shown in Figure 2 (B) and (C), the changing trend of non-structural carbohydrate production in this system is the same as CK. The results showed that the inhibition of the polyketide pathway had no significant effect on the synthesis of non-structural carbohydrates.

Iodoacetamide is also a specific inhibitor of the polyketone pathway. The mechanism of action is that iodoacetamide can form a covalent bond with cysteine residues in the polyketone pathway, so as to alkylate the phosphopan acyl mercaptoacetamide which transports acyl groups on ACP, thus losing the transport function, thus inhibiting the active sites of KS and acyl-coenzyme A in the polyketone pathway. The effect of different concentrations of iodoacetamide on the growth of bacteria and the synthesis of non-structural carbohydrates is shown in Figure 3. It can be seen from Figure 3 (A) that with the increase of iodoacetamide concentration from 5 to 15 mmol/L, carbohydrate biomass continued to decrease, 76% and 84.3% lower than CK at 72h and 120h, respectively. From the appearance, the non-structural carbohydrates are semi-active, and the transformation liquid is very viscous. Therefore, iodoacetamide has a strong toxic effect on carbohydrates. From the perspective of the output of non-structural carbohydrates, as shown in Figure 3 (B) and (C), iodoacetamide can inhibit the production of carbohydrates at a lower concentration of 5 mmol/L, and there is no significant effect on the continuous increase of the concentration. This may be due to the drastic reduction of carbohydrate content

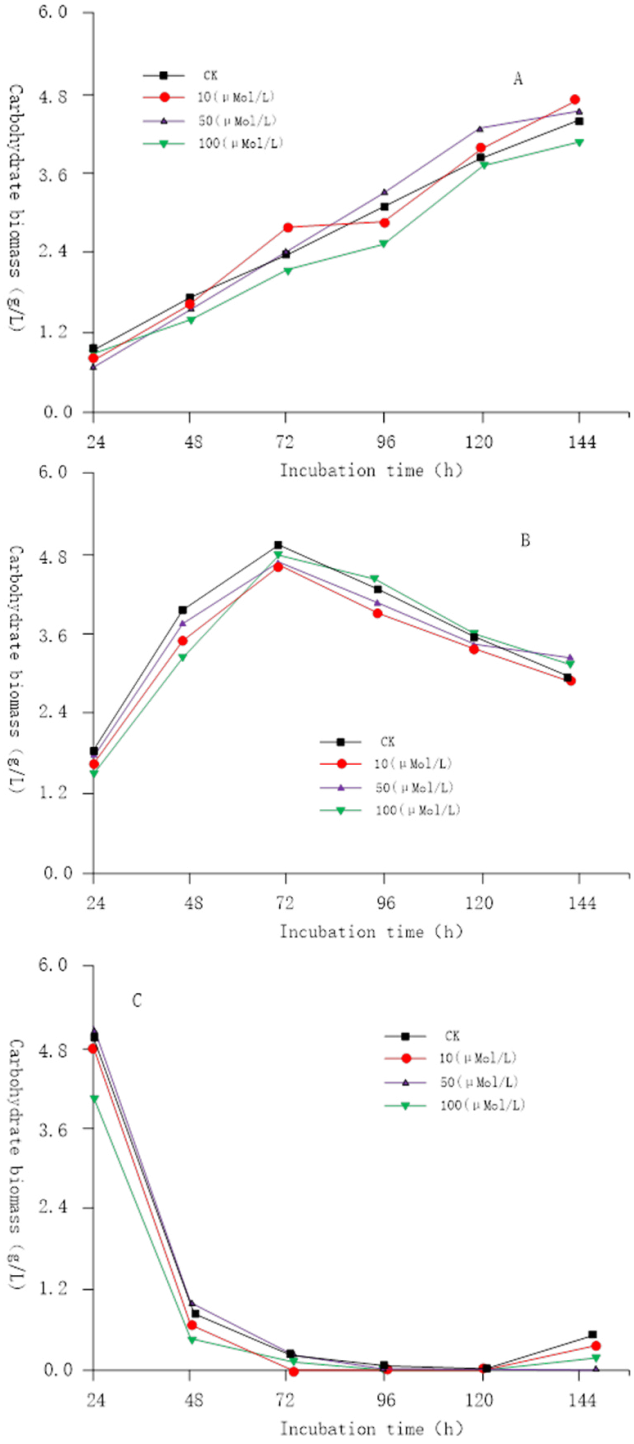

Figure 2. Analysis of nonstructural carbohydrate synthesis pathway by polyketide pathway inhibitors. For explanations of sections A, B and C, refer to the text.

and semi activity. It has been reported that iodoacetamide can not only inhibit the activity of compounds but also inhibit the activity of 3-phosphate-glyceraldehyde dehydrogenase and malate dehydrogenase in TCA. In view of the above research results that the light cyanobacterin does not affect the synthesis of non-structural carbohydrates, it is speculated that iodoacetamide may destroy the synthesis of carbohydrates by inhibiting the synthesis pathway of bacteria, thus inhibiting the corresponding synthesis pathway, hindering the generation of non-structural carbohydrates synthesis precursors, and ultimately affecting the synthesis of carbohydrates Products. It is suggested that the production of phosphoenolpyruvate from glucose is the necessary pathway

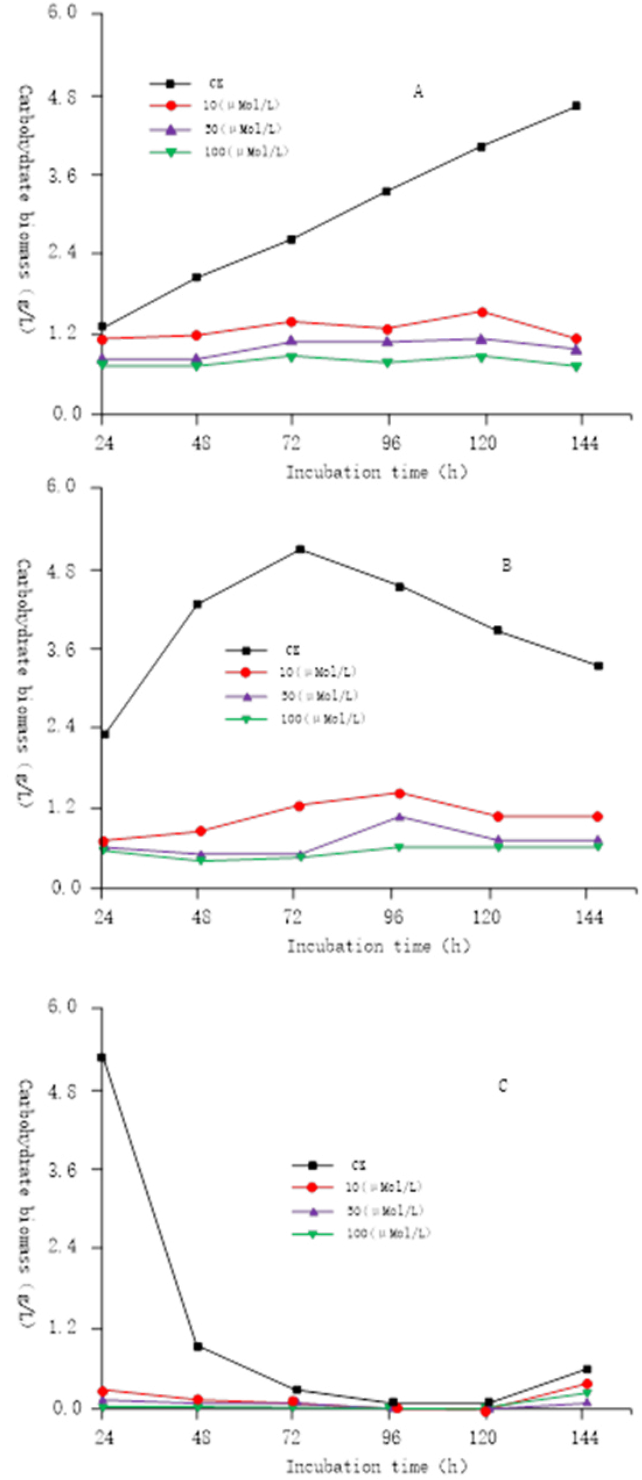

Figure 3. Analysis of the synthesis of nonstructural carbohydrates by iodoacetamide, a polyketide pathway inhibitor**.** For explanations of sections A, B and C, refer to the text.

for the synthesis of non-structural carbohydrates.

The main synthetic pathways of carbohydrate products are polyketone pathway, mevalonate pathway, shikimic acid pathway, polypeptide pathway, sugar derived pathway and mixed pathway, among which polyketone pathway, mevalonate pathway and mangrove acid pathway are the main pathways involved in the synthesis of aromatic compounds. In molecular structure, polysaccharides and their glycosides belong to the class of xylans. The lignans are aromatic compounds containing a benzene ring. The way to synthesize these two compounds is either polyketone way or shikimic acid way. Up to now, there is no report on the specific pathway of the synthesis of these two compounds. In this study, by adding synthesis pathway-specific inhibitors to control a part of the synthesis pathway, and then further observe the synthesis of its final metabolites, so as to speculate the main synthesis pathway related to the synthesis of the target synthesis products. According to the results of previous studies and our preliminary experiments, we found that under different culture systems and transformation conditions, in addition to the synthesis of non-structural carbohydrates, p. corynensis XP-8 can also synthesize monosaccharides and polysaccharides, but the synthesis of carbohydrates requires specific medium and conditions. Therefore, the accumulation of carbohydrate and polysaccharide was analyzed in this paper. shikimic acid pathway has been previously investigated (19-24).

In this paper, based on the network security model, by adding synthesis pathway-specific inhibitors and related precursors to the reaction system, the effects of these synthesis pathways on the synthesis of non-structural carbohydrates are investigated, and the synthesis pathway involved is inferred. Firstly, a method system for the simultaneous determination of carbohydrates by high-performance liquid chromatography was established. The established method and conditions can make the carbohydrates to be measured show a good linear relationship, precision and high recovery within their respective concentration range. In the reaction system of resting compounds and freeze-dried carbohydrates, polyketide pathway inhibitors, light cynomycin and iodoethylene were added. It was found that there was no significant effect on the formation of the compounds, while iodoacetamide significantly inhibited the synthesis of the resting compounds, and iodoacetamide had no significant effect on the synthesis of carbohydrates in the freeze-dried carbohydrate reaction excluding the synthesis of the compounds. It is suggested that glycolysis, another pathway of iodoacetamide inhibition, plays an important role in carbohydrate synthesis. Based on the above analysis, the following conclusions can be drawn: the synthesis of non-structural carbohydrates requires the participation of glycolysis pathway, shikimic acid pathway and phenylpropane pathway, but has nothing to do with polyketone pathway.

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