Basic Study
Preparation and hypoglycemic effects of chromium- and zinc-rich *Acetobacter Aceti*

Huang YY *et al.* A new hypoglycemic method

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Abstract
BACKGROUND
At present, there is no ideal method to cure diabetes, and there are few reports on the treatment with probiotics.

AIM
To propose the method for preparing a new type of chromium- and zinc-rich *Acetobacter aceti* and explore its hypoglycemic effects on enhancing the application of probiotics in the treatment of diabetes.

METHODS
*Acetobacter aceti* was cultured in a liquid medium that contained chromium trichloride and zinc chloride, both at a concentration of 64 mg/mL, with the initial concentration of the bacterial solution $1 \times 10^4$ CFU/mL. After the bacterial solution had been inducted for 48 h, the induction was repeated once with the culture solution changed; the contents of chromium and zinc were detected by inductively coupled plasma mass spectrometry and the contents of NADH and glucose dehydrogenase using the NAD +
NADH kit, GCDH kit, etc. Streptozotocin was used to establish model mice to evaluate the hypoglycemic effects of the proposed chromium- and zinc-rich *Acetobacter aceti*; the effect on islet cells MIN6 was detected *in vitro*; 10 times the therapeutic dose of treatment was administered to mice to evaluate its biological safety.

RESULTS
The contents of *Acetobacter aceti* prepared by this method, chromium metal, metallic zinc, NADH coenzyme, and glucose dehydrogenase were 28.58 to 34.34 mg/kg, 5.35 to 7.52 mg/kg, 5.13 to 7.26 μM, and 446.812 to 567.138 U/g. The results exert a better hypoglycemic effect *in vivo* than metformin, which promotes the repair of tissues and cells of pancreatic islets and facilitates the growth of pancreatic islet cells MIN6 with increasing insulin secretion in the *in vitro* tests; 10 times the therapeutic dose of treatment was non-toxic to mice.

CONCLUSION
Chromium trichloride and zinc chloride can be employed to induce the preparation of chromium- and zinc-rich *Acetobacter Aceti*, which has a significantly enhanced hypoglycemic effect and can biotransform chromium and zinc, improving safety in terms of exposure to these metals.

**Key Words:** *Acetobacter aceti*; Chromium; Zinc; Enrichment; Blood sugar decrease

Huang YY, Qin XK, Dai YY, Huang L, Huang GR, Qin YC, Wei X, Huang YQ. Preparation and hypoglycemic effects of chromium- and zinc-rich *Acetobacter Aceti*. *World J Diabetes* 2022; In press

**Core Tip:** At present, there are no ideal drugs to treat diabetes, *Acetobacter* and other probiotics play a certain role in the treatment of diabetes, but its effect is not significant. How to enhance its hypoglycemic effect is the focus. In this study, metal compounds
were used to induce Acetobacter to enrich chromium and zinc, enhance its hypoglycemic effect. It is a better synergistic method and has a better application prospect.
INTRODUCTION

Diabetes mellitus is a disease of chronic glucose metabolism disorders[1,2]. In 2020, the world’s population with diabetes is about 495 million and by 2045 this is expected to increase to 700 million[3,4]. The pain and burden of diabetes and its complications are major health and socio-economic concerns for people all over the world. Diabetic patients are often challenged by serious complications, such as diabetic nephropathy, diabetic retinopathy, cardiovascular system disease, etc, which pose a serious threat to human life and health[5-7]. With patients with type 2 diabetes mellitus (T2DM) accounting for 90% to 95% of all diabetic patients, T2DM is an important focus of much research into the epidemiology of diabetes. Scholars have undertaken much research into the causes and methods of prevention of diabetes for many years, however, there is no effective cure for diabetes due to its unclear causes, complicated pathogenesis, so its underlying mechanism has yet to be fully elucidated[8,9]. The current comprehensive prevention and treatment measures for diabetes mainly include diet control, exercises, and drug therapy[10-12]; the drugs used for the treatment of diabetes are mainly chemical agents, with few biological drugs available. The health drinks prepared by the metabolites of kombucha, a symbiotic of yeast, lactic acid bacteria, and acetic acid bacteria and black tea can lower blood sugar, blood pressure, blood lipids, etc, and exert certain auxiliary effects on patients with hypertension, hyperlipidemia and hyperglycemia, with the main active ingredients thereof for lowering blood sugar being tea polyphenols and D-glucaric acid-1, 4-lactone[13]. However, the effect of bacterial metabolites on reducing blood sugar is not fully demonstrated in the aforementioned healthy drinks for the two components, There are not bacterial metabolites: Acetobacter aceti contains dihydronicotinamide-adenine dinucleotide, glucuronic acid dehydrogenase, etc, which decompose glucose through glycolysis while kombucha fails to improve the effect of reducing blood sugar by fully utilizing the effects of these metabolic enzymes, therefore, Acetobacter aceti can only be used as a healthy drink for adjuvant therapy. However, how to make good use of the ability of Acetobacter aceti to metabolize glucose, increase the contents of dihydronicotinamide-adenine dinucleotide,
glucuronic acid dehydrogenase, and other metabolic enzymes and exert their hypoglycemic effects are the key problems that need to be solved before using Acetobacter aceti to prepare a new hypoglycemic drug. To address the biological drug shortage in the treatment of T2DM and improve the effect of Acetobacter aceti on lowering blood sugar, the present study provides a method for preparing and applying chromium- and zinc-rich Acetobacter aceti and there was a significant increase in the amount of dihydronicotinamide-adenine dinucleotide, glucuronic acid dehydrogenase, chromium and zinc inside the cells, and other microelements in the specimens we prepared. Chromium- and zinc-rich Acetobacter aceti can have a certain repair effect on pancreatic islet cells, promotion of insulin secretion, and a good hypoglycemic effect. Therefore, it can be used as a candidate drug for the treatment of T2DM.

**MATERIALS AND METHODS**

**Materials**

Glucose (obtained from Guangdong Guanghua Technology Co., Ltd, batch number: 20200403); yeast extract (purchased from Beijing Aoboxing Bio-tech Co., Ltd, batch number: 20200422); calcium carbonate (obtained from Shanghai Titan Scientific Co., Ltd, batch number: P1260108); agar (purchased from Beijing Solarbio Technology Co., Ltd, batch number: 310C022); anhydrous alcohol (obtained from Shanghai MacLean Biochemical Technology Co., Ltd, batch number: C11974944); chromium chloride (purchased from Shanghai MacLean Biochemical Technology Co., Ltd, batch number: C10717130); zinc chloride (obtained from Shanghai MacLean Biochemical Technology Co., Ltd, batch number: C10730413); 50 mL centrifuge tubes and EP tubes (purchased from Jiangsu Lexinkang Medical Equipment Co., Ltd); STZ: streptozotocin (obtained from Shanghai McLean Biochemical Technology Co., Ltd, batch number: C20PA038100B); citric acid (purchased from Shanghai McLean Biochemical Technology Co., Ltd, batch number: C10723907); sodium citrate (obtained from Shanghai McLean Biochemical Technology Co., Ltd, batch number: C10712912); universal pH indicator paper (purchased from Hangzhou Test Three Technology Co., Ltd); metformin
hydrochloride tablets (obtained from Beijing Jingfeng Pharmaceutical Group Co., Ltd, batch number 2004032); (ACCU-CHEK) glucose test strips (purchased from Roche Diabetes Care GmbH, batch number: 26020933, etc); SPF C57BL/6 mice aged six to eight weeks (purchased from Changsha Tianqin Biological Co., Ltd); Acetobacter aceti, number: GIM1.67 (purchased from Guangdong Microorganism Conservation Centre); MIN6 cells (purchased from China Centre for Type Culture Collection) were used. The Animal Experiment Ethics Number was 20200620.

**Preparation of chromium- and zinc-rich Acetobacter aceti**

First, *Acetobacter aceti* was revived and cultured in a liquid enriched medium, with the concentration of the bacterial solution OD<sub>600</sub> = 0.9 to 1.5, which is about 3 × 10<sup>8</sup> to 5 × 10<sup>8</sup> CFU/mL; second, chromium- and zinc-rich *Acetobacter aceti* was cultured in a liquid medium (the concentrations of chromium trichloride and zinc chloride were both 64 mg/mL) with the initial concentration of the bacterial solution set to 1 × 10<sup>9</sup> CFU/mL and shaken for 48 h at 250 rpm; third, the bacterial solution was collected, centrifuged to remove the supernatant and washed by phosphate buffer saline (PBS), with the precipitate being chromium- and zinc-rich *Acetobacter aceti*.

**Detect coenzymes and metals in chromium- and zinc-rich Acetobacter aceti**

**Collection of chromium- and zinc-rich Acetobacter aceti:** 100 mL cell suspensions of chromium- and zinc-rich *Acetobacter aceti* were removed and distributed across a sterile 96-microwell plate with the culture solution of chromium- and zinc-rich *Acetobacter aceti* used as a blank control. The absorption of the suspension and the chromium- and zinc-rich *Acetobacter aceti* culture solution were measured at 600 nm using the microplate reader and calculated as OD<sub>1</sub> and OD<sub>2</sub>, respectively with the final OD value of the chromic-and zinc-rich *Acetobacter aceti* suspension taken as the difference OD<sub>1</sub>-OD<sub>2</sub>. The chromium- and zinc-rich *Acetobacter aceti* suspension was centrifuged at 8000 rpm for ten minutes to remove the supernatant with the obtained precipitate being the
Acetobacter aceti induced and cultured by chromium and zinc, which was then washed once with 1 mL sterile PBS and centrifuged at 13000 rpm for 1 min to remove the supernatant. Thereafter, the precipitate was weighed and treated with sterile water, with the concentration of Acetobacter aceti being 0.1 mg/mL.

Detection of chromium and zinc in chromium- and zinc-rich Acetobacter aceti: The samples that had been collected were sent to Shanghai WEIPU Chemical Technology Service Co., Ltd, and the contents of chromium and zinc therein were detected by the inductively coupled plasma mass spectrometry method.

Detection of NAD+/NADH in chromium- and zinc-rich Acetobacter aceti: The detection of NAD+/NADH in the chromium- and zinc-rich Acetobacter aceti that had been collected was conducted using the NAD+/NADH assay kit with WST-8 (Beyotime Biotechnology).

Detection of glucose dehydrogenase in chromium- and zinc-rich Acetobacter aceti: Chromium- and zinc-rich Acetobacter aceti was collected using Solarbio’s “Glucose dehydrogenase microplate assay kit” according to the instruction manual.

Evaluation of the hypoglycemic effect of chromium- and zinc-rich Acetobacter aceti

Construction of a diabetes mouse model: Preparation of citrate buffer: 2.10 g of citric acid was treated with 100 mL of double distilled water to make a citric acid mother liquor: solution A; 2.94 g of trisodium citrate was treated with 100 mL of double-distilled water to make a sodium citrate mother liquor: solution B; solution A and solution B were mixed in a ratio of 1:1.32 (or 1:1) and the pH value thereof was measured with a pH meter and adjusted from 4.2 to 4.5, which was the 0.1 mol/L sodium citrate-hydrochloric acid buffer solution required to prepare STZ.

Selected animals: 70 SPF grade C57BL/6J female mice aged six weeks and weighing 20 ± 2 g, were allowed to eat and drink without restrictions during five days of
adjustable feeding. Six mice were randomly selected as the normal control group, and the rest were used for model construction.

Models of mice administered by STZ: 24 mL of 0.1 mol/L sodium citrate buffer was treated with 120 mg of STZ away from the light (equivalent to a concentration of 5 mg/mL) and placed in an ice environment. The mice were made to fast for 10 h in advance, and then treated with STZ at a dose of 0.15 mL/10 g of mouse weight (equivalent to 75 mg/kg). STZ was administered by intraperitoneal injection for three consecutive days. Before each intraperitoneal injection of STZ, the STZ liquid should be pipetted with a 1 mL syringe to mix the precipitate before it was extracted. The STZ concentration should be maintained at the same level. After each intraperitoneal injection, mice should be deprived of food and water for 90 min. On the seventh day after the last administration (the mice were made to fast for ten hours before blood collection), blood was collected from their caudal veins, and the fasting blood glucose (FBG) levels of the mice were measured with a Roche glucometer. When FBG ≥ 16.7 mmol/L, pathological models of mice with diabetes were confirmed as having been successfully established. Grouping and administration: the diabetic mice were numbered according to their blood glucose levels from high to low, and the model mice were divided into a model group (PBS), positive control group (metformin), metal chromium plus zinc group (the concentrations of chromium and zinc were calculated as 1 × 10⁻⁷ mg/mL and 2 × 10⁻⁸ mg/mL according to the highest content of chromium- and zinc-rich Acetobacter aceti), Acetobacter aceti group (OD = 1), and chromium- and zinc-rich Acetobacter aceti group (OD = 1). There were six mice in each group, and each was given 0.5 mL of treatment by gavage.

Evaluation of hypoglycemic activity in vivo: After the models were successfully established, the mice were given intragastric administration of treatment on the second day, once a day, for 15 consecutive days. The normal control group and the model group were given the same amount of PBS water, with the positive drug control group given diformin tablets (ground into powder, prepared into a suspension with reverse
osmosis water and administered intragastrically at 0.320 g/kg.d), the metal chromium plus zinc group was given administration of treatment prepared by chromium trichloride and zinc chloride. Through general observation, during the 15 d period of administration, the activity and spirit of the mice, their eating and drinking, urine and feces, and the dryness and wetness of the bedding were observed every day during the 15 d of administration. Glucose monitoring: from the beginning of treatment, fasting blood glucose was measured every three days. After 15 d of administration, the mice were fasted for 10 h, with their blood collected from the eyeballs. Thereafter, the mice were sacrificed, with the tissue taken from their pancreas islets, fixed with formaldehyde and sliced for hematoxylin and eosin (HE) staining. The apoptosis-related Bax genes were detected by immunohistochemistry; after the tissue taken from pancreas islets was made into electron microscope section, the ultrastructural damage thereof was observed to detect the repair effect of chromium- and zinc-rich Acetobacter aceti on tissues and cells of pancreatic islets. The weights of mice were recorded every three days, with the effects of chromium- and zinc-rich Acetobacter aceti on weight recovery of diabetic mice detected.

_Evaluation of chromium- and zinc-rich Acetobacter aceti on pancreatic islet cells MIN6_  
**Effects of chromium- and zinc-rich Acetobacter aceti on growth of pancreatic islet cells MIN6:** Chromium- and zinc-rich Acetobacter aceti was collected (OD = 10), sonicated and stored at -20 °C for later use. Pancreatic islet cells MIN6 were revived, with the cell concentration adjusted to $1 \times 10^5$ CFU/mL, cultured in 5 mmol/L and 25 mmol/L high-glucose 1640 medium, respectively, and laid on a 96-well plate, 90 mL/well. The cells that had been cultured for 12 h were diluted 10-fold and dosed with 10 mL collected chromium- and zinc-rich Acetobacter aceti (OD = 10), the dose of which was equivalent to $1 \times 10^7$ CFU/mL of bacteria. Positive drug control group (diformin tablets) and negative drug control group (PBS) were set. Some 24 h after dosing, cell growth was detected with the CCK-8 kit (Beyotime Biotechnology).
Effects of chromium- and zinc-rich *Acetobacter acetii* on promoting insulin secretion of pancreatic islet cells MIN6: Chromium- and zinc-rich *Acetobacter acetii* was collected (OD = 10), sonicated and stored at -20 °C for later use. Pancreatic islet cells MIN6 were revived, with the cell concentration adjusted to $1 \times 10^5$ CFU/mL, cultured in 5 mmol/L and 25 mmol/L high-glucose 1640 medium, respectively, and laid on a 6-well plate, 1.98 mL/well. The cells that had been cultured for 12 h were dosed with 20 mL collected chromium- and zinc-rich *Acetobacter acetii* (OD = 10), the dose of which was equivalent to $1 \times 10^7$ CFU/mL of bacteria. Positive drug control group (diformin tablets) and negative drug control group (PBS) were set. Some 24 h after dosing, the cell supernatant was collected, with the insulin content detected by the enzyme-immunized “mouse insulin Elisa kit”.

**Detection of chromium- and zinc-rich Acetobacter acetii for processing glucose**

Chromium- and zinc-rich *Acetobacter acetii* and *Acetobacter acetii* were collected, with the initial concentration adjusted to $1 \times 10^4$ CFU/mL. The glucose concentration of the liquid medium was detected with a blood glucometer. The capacity of chromium- and zinc-rich *Acetobacter acetii* that had been cultured at 30 °C for 12 h, 24 h, 36 h, and 48 h for processing glucose in the medium was detected.

**Safety evaluation of chromium- and zinc-rich Acetobacter acetii**

Chromium- and zinc-rich *Acetobacter acetii* was collected and adjusted to 10 times the therapeutic dose (OD = 10). 1 mL was administered to the mice each time, three times a day for seven consecutive days, with the body weights and the pathological changes of the organs detected.

**RESULTS**

Detect coenzymes and metals in chromium- and zinc-rich *Acetobacter acetii*
The content of chromium metal in the _Acetobacter aceti_ prepared by the aforementioned method was 28.58-34.34 mg/kg and that of the metal zinc was 5.35-7.52 mg/kg, both of which were significantly higher than those in the non-induced _Acetobacter aceti_ (chromium metal: 1.05-2.29 mg/kg; metal zinc: 0.18-0.26 mg/kg) (Figure 1A). The content of NADH was 5.13 to 7.26 mM, which was significantly higher than that in the non-cultured _Acetobacter aceti_: 0.86 to 1.02 mM (Figure 1B). The content of glucose dehydrogenase was 446.812-567.138 U/g, which was significantly higher than that of non-cultivated _Acetobacter aceti_: 54.126-93.651 U/g (Figure 1C).

**Evaluation of the therapeutic effects of chromium- and zinc-rich _Acetobacter aceti_ on mice with diabetes**

At FBG ≥ 16.7 mmol/L, after administration for seven days, the mice treated with chromium- and zinc-rich _Acetobacter aceti_ were found to be in a good mental state with bright eyes and normal activities on-going, and take food, drink water, urinate and defecate in a normal manner with their beddings dry. The fasting blood glucose was detected every three days after the initial administration of treatment, with that of the diabetic mice in the chromium- and zinc-rich _Acetobacter aceti_ (OD = 1) found to be significantly lower than that in the positive drug control group (diformin tablets) and the metal chromium plus zinc group (Figure 2).

After 15 d of treatment with chromium- and zinc-rich _Acetobacter aceti_, the tissue taken from pancreas islets was treated with HE staining, with less apoptosis of cells and structural atrophy and vacuole barely found in the chromium- and zinc-rich _Acetobacter aceti_ group (OD = 1) under the microscope; Through the detection of the apoptosis-related Bax genes by immunohistochemistry, the apoptosis of islet cells was found to be significantly reduced; after the tissue taken from pancreas islets was made into electron microscope cross-sections, the ultrastructural damage thereto was found to be alleviated with a small expansion range of endoplasmic reticulum, slightly swollen mitochondria, and the amount of autophagic vacuolization significantly reduced.
However, the recovery of islet tissues and cells in this group was significantly better than that in the positive drug control group (diformin tablets), as shown in Figure 3.

The body weights of the mice after treatment with chromium- and zinc-rich *Acetobacter aceti* were recorded every three days. The weights of diabetic mice in the chromium- and zinc-rich *Acetobacter aceti* (OD = 1) were found to recover well, with no significant difference from those of the positive drug control group (metformin), as displayed in Figure 4.

**Evaluation of chromium- and zinc-rich Acetobacter aceti on pancreatic islet cells MIN6**

Through the detection of cytotoxicity of the prepared chromium- and zinc-rich *Acetobacter aceti* by the CCK-8 kit (Beyotime Biotechnology), the chromium- and zinc-rich *Acetobacter aceti* group was found to promote the growth of MIN6 cells significantly better than the positive control group and the negative control group, which was evident in the high glucose 1640 medium (Figure 5). This result implied that chromium- and zinc-rich *Acetobacter aceti* could promote the growth of pancreatic islet cells MIN6.

The prepared chromium- and zinc-rich *Acetobacter aceti* (OD = 10) was sonicated, added to the medium of pancreatic islet cells MIN6 and cultured for 12 h, after which the insulin content was detected by the enzyme-immunized "mouse insulin Elisa kit". The insulin content of the supernatant in cells in the chromium- and zinc-rich *Acetobacter aceti* (OD = 10) group was significantly higher than that in the positive control group and the negative control group, which was evident in the 25 mmol/L high glucose 1640 medium (Figure 6). This result implied that chromium- and zinc-rich *Acetobacter aceti* could promote insulin secretion of islet MIN6 cells.

**Detection of the capacity of chromium- and zinc-rich Acetobacter aceti for processing glucose**

A blood glucometer was used to detect the decomposition of glucose in the medium by chromium- and zinc-rich *Acetobacter aceti*. The glucose content in the medium that had been cultured at 30 °C for 12 h, 24 h, 36 h, and 48 h was significantly decreased in a ratio
that was higher than that in the *Acetobacter aceti* group (Figure 7). This result implied that chromium- and zinc-rich *Acetobacter aceti* had better effects in terms of decomposing glucose.

**Safety evaluation of chromium- and zinc-rich Acetobacter aceti**
Ten times the dose of chromium- and zinc-rich *Acetobacter aceti* was administered to mice by gavage with no change detected in the weights and no pathological damage found in the liver, spleen, kidneys, and stomach (Figure 8). This result implied that *Acetobacter aceti* was biosafe.

**DISCUSSION**
At present, the incidence of diabetes is high with no cure therefor. The conventional therapy is long-term use of hypoglycemic drugs and symptomatic treatment. However, in the long-term treatment process, the drug is prone to resistance among non-compliant patients who fail to adhere to treatment regimens. Therefore, to help patients control blood sugar over a long time requires finding high risk factors for diabetes and blocks them from the source. However, with diabetic patients prone to be deficient in B vitamins and many micronutrients such as chromium, zinc, and selenium[14-16], it is of significance to explore how to supplement micronutrients for the prevention and treatment of diabetes.

Studies have found that plasma chromium levels are negatively correlated with the risk of type 2 diabetes and prediabetes. Chromium deficiency may lead to impaired glucose tolerance and insulin resistance, and elevated blood sugar. Besides, chromium levels in the human body will decrease with age with chromium deficiency becoming severe in old people[17-19]. According to evidence-based medicine meta-analysis studies, chromium was found to increase insulin sensitivity mainly by activating insulin receptor kinase activity, inhibiting phosphatase activity, and increasing phosphorylation of insulin receptors[20,21]. Low molecular weight chromium binding substance was found to be combined with insulin receptors and activate tyrosine kinase
activity on insulin P receptors, thus enhancing insulin signal transduction. However, at an insulin concentration of 100 nmol/L, the tyrosine kinase activity in insulin receptor was significantly enhanced if insulin was dosed with chromium[22,23]. In addition, it was found that chromium could also promote the translocation of GLUT4 to the cell membrane by activating protein kinase B and adenosine monophosphate to activate protein kinase signaling pathways and by activating the activity of p38 mitogen-activated protein kinase[24,25]. Despite much related research, the specific mechanism of lowering blood sugar through chromium supplementation remains unclear. Chromium was listed as an essential microelement for the human body in 1989; the body absorbs Cr³⁺ mainly through food, including meat, whole grain, millet, pepper, etc. The daily intake for adults recommended by the United States Food and Drug Administration’s Center for Food Safety and Applied Nutrition Food Safety Recommendations Committee was 25-35 μg in 2001.

Zinc atoms are an important part of the insulin molecule and involved in maintaining the stability and biological effects of insulin[26-28]. Zinc that plays a key role in the synthesis, storage, and secretion of insulin in pancreatic β cells can increase the activity of the insulin signaling pathway. However, zinc deficiency can lead to a decrease in insulin secretion[29,30]. In addition, with the formation of hexamers that contain zinc required in the synthesis of insulin, zinc deficiency will lead to restricted insulin synthesis and reduced insulin sensitivity, thus increasing the risk of diabetes[31,32]. Studies found that zinc lowers blood sugar mainly through antioxidant responses, inhibition of inflammatory factors, and anti-apoptosis effects[30,33]. Intracellular zinc was found to be regulated by zinc transporters, with the uptake, storage, and distribution thereof regulated by metallothioneins[34,35]. Studies have found that zinc can exert effects of inhibiting inflammatory response and anti-apoptosis effect at a concentration higher than 100 μmol/L[36], therefore, increasing the intake of zinc can increase the level of metallothionein, which helps mediate anti-apoptosis, etc[31,37]. Some scholars suggested that the effects of a high zinc-intake on lowering blood sugar may be associated with its ability to reduce variation in zinc transporters[38]. Therefore, given
the important role of zinc in glycemic control, a deficiency thereof can lead to glucose metabolism disorders, while high intake thereof may reduce the risk of glucose metabolism disorders and diabetes.

Above all, chromium and zinc supplementation can stabilize blood sugar in an indisputable manner. The key is how to supplement chromium and zinc through a proper, safe, and scientific approach. In the present study, chromium trichloride, zinc chloride, and Acetobacter aceti were co-cultured to induce the production of chromium- and zinc-rich Acetobacter aceti following simple protocols with high yield. Chromium- and zinc-rich Acetobacter aceti prepared using this method not only helped Acetobacter aceti exert the effect of decomposing glucose, but also properly supplemented with chromium and zinc, enhancing the hypoglycemic effect. Meanwhile, with chromium and zinc being transformed by bacteria, biological safety was also ensured, therefore, it could be regarded as a better new hypoglycemic biological drug. The hypoglycemic mechanism of chromium- and zinc-rich Acetobacter aceti was preliminarily explored: it mainly increased the contents of dihydronicotinamide-adenine dinucleotide and glucuronide dehydrogenase in Acetobacter aceti and enhanced the ability to degrade glucose. In addition, its hypoglycemic mechanism was not much different from those of chromium and zinc, which are metal microelements. However, for ethanol that serves as the source of nutrition for the growth of Acetobacter aceti, this cannot be supplied by human body in the long-term as it barely survives in the body, accordingly being detected in feces, therefore, it cannot exert long-term hypoglycemic effects, which will be further addressed in future research.

**CONCLUSION**

Chromium trichloride and zinc chloride can be employed to induce the preparation of chromium- and zinc-rich Acetobacter Aceti, which has a significantly enhanced hypoglycemic effect and can biotransform chromium and zinc, improving safety in terms of exposure to these metals.
ARTICLE HIGHLIGHTS

Research background
At present, there are no ideal drugs to treat diabetes, Acetobacter and other probiotics play a certain role in the treatment of diabetes, but its effect is not significant. How to enhance its hypoglycemic effect is the focus. In this study, metal compounds were used to induce Acetobacter to enrich chromium and zinc, enhance its hypoglycemic effect. It is a better synergistic method and has a better application.

Research motivation
It provides a theoretical basis for the application of new chromium and zinc rich Acetobacter aceti.

Research objectives
To prepare a new type of chromium- and zinc-rich Acetobacter aceti and explore its hypoglycemic effects on enhancing the application of probiotics in the treatment of diabetes.

Research methods
Acetobacter aceti was cultured in a liquid medium that contained chromium trichloride and zinc chloride.

Research results
A new type of chromium and zinc rich Acetobacter aceti was successfully prepared.

Research conclusions
Chromium- and zinc-rich Acetobacter Aceti has a significantly enhanced hypoglycemic effect and can biotransform chromium and zinc, improving safety in terms of exposure to these metals.
Research perspectives

It has very good application prospects.
<p>| 3 | Hang Liu, Min Zhou, Xin Ju, Hang Shu, Cuiying Hu, Liangzhi Li. &quot;Inactivation Mechanism of 1-Ethyl-3-Methylimidazolium-Based Ionic Liquid on β-Glucosidase Produced by Paenibacillus sp. LLZ1 and Enhanced Activity Using a Surfactant&quot;, Applied Biochemistry and Biotechnology, 2019 | 22 words — &lt; 1% |
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