

USE OF *PEDIOCOCCUS ACIDILACTICI* GKA4 IN PREPARATION OF COMPOSITION FOR PREVENTING OR TREATING RENAL DYSFUNCTION

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0001] The present disclosure relates to *Pediococcus acidilactici* and, in particular, to a use of *Pediococcus acidilactici* GKA4 in preparation of a composition for preventing or treating renal dysfunction, a culture medium thereof and a culture method thereof.

DESCRIPTION OF THE PRIOR ART

[0002] After cisplatin has been accidentally discovered in the 1960s that it can inhibit bacterial cell division, it has become one of the most indispensable drugs in the world's chemotherapeutic drugs for treating tumors. The mechanism of cisplatin in inhibition of cancer cells has not been fully understood, but the mechanism confirmed by current experiments includes the cross-binding of cisplatin and deoxyribonucleic acid (DNA), which makes DNA replicate and synthesize hindered from proceeding. Therefore, in rapidly dividing cells, such as cancer cells, cisplatin can cause damage to DNA. Despite its chemotherapeutic activity, different studies have reported toxicity of this molecule in several vital organs of the human body, including heart, liver, brain, spleen, and most notably deleterious effects in kidney tissue.

[0003] However, until now, cisplatin has remained the only drug of choice for several cancers. Renal insufficiency or nephrotoxicity caused by cisplatin

administration can be classified in several ways, among which acute kidney injury (AKI) was found to be problematic in more than 30% of patients. The common and severe pathophysiological states of AKI are shock, ischemia-reperfusion, sepsis, etc., which can be classified by rapid decline in renal function and extensive renal tubular damage, and have a very high mortality rate.

[0004] The high-concentration distribution and long-term accumulation of cisplatin in the kidney can lead to different degrees of renal damage. The pathological mechanism of cisplatin nephrotoxicity is mainly divided into two aspects: the first is to reduce the glomerular filtration rate and increase the content of creatinine (Cr) and blood urea nitrogen (BUN) in serum; and the second is ischemia and even necrosis of renal proximal tubular epithelial cells. Histopathological analysis found that cisplatin could seriously damage the renal tissue structure, resulting in large areas of necrosis, loose arrangement of cells, disappearance of brush border, obvious atrophy of glomeruli, and degeneration of renal tubular vacuoles.

[0005] In order to avoid irreparable damage to the kidney after cancer treatment, a composition for protecting the kidney and a method for protecting the kidney are needed.

SUMMARY OF THE INVENTION

[0006] To achieve the object described above, the disclosure provides a *Pediococcus acidilactici* GKA4 deposited in Bioresource Collection and Research Center of Food Industry Research and Development Institute of the Republic of China with a deposit number of BCRC 910876.

[0007] According to another object of the disclosure, a culture medium for cultivating *Pediococcus acidilactici* is provided, which comprises sucrose, a yeast extract or a combination thereof.

[0008] According to further object of the disclosure, a use of *Pediococcus acidilactici* GKA4 and/or an active substance thereof in preparation of a composition for preventing or treating a renal dysfunction is provided, wherein the *Pediococcus acidilactici* GKA4 is deposited in Bioresource Collection and Research Center of Food Industry Research and Development Institute with a deposit number of BCRC 910876.

[0009] Preferably, the composition is administered to a subject in an effective amount of 0.05 to 25 g/60 kg body weight/day.

[0010] Preferably, the renal dysfunction refers to acute kidney injury (AKI).

[0011] Preferably, the preventing or treating renal dysfunction means that a concentration of blood urea nitrogen (BUN) in serum of a subject after administration of the composition is lower than that before administration of the composition.

[0012] Preferably, the preventing or treating renal dysfunction means that a concentration of Creatinine (Cr) in serum of a subject after administration of the composition is lower than that before administration of the composition.

[0013] Preferably, the preventing or treating renal dysfunction means that a renal index of the subject after administration of the composition is lower than that before administration of the composition, wherein the renal index refers to a percentage of a subject's kidney weight to the subject's body weight.

[0014] Preferably, necrosis of renal tissue refers to necrosis of proximal renal tubules and abnormal appearance of hyaline cylinders.

[0015] Preferably, the preventing or treating renal dysfunction means that a degree of necrosis of a subject's kidney tissue after administration of the composition is lower than that before administration of the composition.

[0016] Preferably, the renal dysfunction is caused by a cancer treatment drug.

[0017] Preferably, the cancer treatment drug refers to cisplatin.

[0018] Preferably, the active substance of lactic acid bacteria is prepared by the following steps:

(a) inoculating a bacterial cell of the lactic acid bacteria in a solid-state medium for solid-state culture to form a colony;

(b) inoculating the colony cultured in step (a) into a liquid medium for liquid culture to obtain a liquid-state medium containing the bacterial cell;

(c) inoculating the liquid-state medium containing the bacterial cell of step (b) into a fermentation tank for liquid-state amplification to obtain a bacterial liquid;

(d) centrifuging the bacterial liquid of step (c) to obtain a bacterial sludge;
and

(e) freeze-drying the bacterial sludge of step (d) to serve as a bacterial powder.

[0019] Preferably, a temperature of the freeze-drying ranges from -196°C to -40°C.

[0020] Preferably, the composition comprises an additive selected from the group consisting of an excipient, a preservative, a diluent, a filler, an absorption enhancer, a sweetener or a combination thereof.

[0021] Preferably, the composition is a medicine, a feed, a beverage, a nutritional supplement, a dairy product, a food or a health food.

[0022] Preferably, the composition is in a form of powder, lozenge, suppository, microcapsule, ampoule, liquid spray or suppository.

[0023] By virtue of the above technical characteristics, the *Pediococcus acidilactici* GKA4 provided by the disclosure and the composition comprising the same can achieve the effect of preventing or treating renal dysfunction. At the same time, based on the culture medium, culture method and use of GKA4 provided by the disclosure, the purpose of manufacturing and producing GKA4 can be achieved, and the effect of preventing acute kidney injury caused by cisplatin can be achieved.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] FIG. 1 illustrates the impact of *Pediococcus acidilactici* GKA4 on blood urea nitrogen in serum.

[0025] FIG. 2 illustrates the effect of *Pediococcus acidilactici* GKA4 on creatinine in serum.

[0026] FIG. 3 illustrates that *Pediococcus acidilactici* GKA4 reduces the renal index increase caused by cisplatin.

[0027] FIG. 4 is a histological staining diagram of the renal tubular necrosis of the experimental animal after the sacrifice of the experimental animal, illustrating that *Pediococcus acidilactici* GKA4 reduces the renal multifocal tubular necrosis caused by cisplatin.

[0028] FIG. 5 illustrates that *Pediococcus acidilactici* GKA4 reduces the degree of renal necrosis caused by cisplatin.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0029] The “renal dysfunction” described herein refers to the abnormal excretory function of the kidneys. In one embodiment, the renal dysfunction means that the biochemical index of the renal function is outside the general benchmark range, indicating that the renal function is low. In a preferred embodiment, the biochemical indicators of the renal function refer to the concentration of blood urea nitrogen (BUN) in serum, the concentration of creatinine (Cr) in serum, renal index, degree of necrosis of renal tissue, neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1) and serum cysteine protein inhibitors (Cystatin C).

[0030] In one embodiment, the renal dysfunction refers to “acute kidney injury” with a high mortality rate. The diagnostic definition of “acute kidney injury” is an increase in serum creatinine of greater than or equal to 0.3 mg/dL within 48 hours, or a percentage increase in serum creatinine of greater than or equal to 50 % within 48 hours, or a decreased urine output over 6 hours of less than 0.5 mL/kg/hr.

[0031] The treatment of cancer includes surgical treatment, radiotherapy and chemotherapy. The “drug for treating cancer” mentioned herein refers to the drug used in chemotherapy. In a preferred embodiment, the chemical drug that causes toxicity to the kidney includes cisplatin or platinum ion preparations, cyclophosphamide or alkylating agents, Carmustine (BCNU) or nitrous urea, antibiotics such as Mitomycin-C. In one embodiment, the “drug for treating cancer” refers to cisplatin.

[0032] “Prevention” or “preventing” as used herein refers to preventing an individual from developing a disease, disorder, or harmful condition. In one embodiment, the “prevention” or “preventing” herein refers to avoiding the occurrence of symptoms of renal dysfunction in the patient when the patient does

not have symptoms of renal dysfunction. As used herein, “treatment” or “treating” refers to the process of eliminating, terminating, or reducing a disease, disorder, or deleterious symptom in a subject. In a preferred embodiment, the “treatment” or “treating” mentioned herein refers to alleviating the symptoms of renal dysfunction of the patient when the symptoms of renal dysfunction occur; or for patients who have experienced symptoms of renal dysfunction, maintenance or improvement of renal function after the renal function returns to a certain level.

[0033] The “bacterial cell” described herein refers to the structure of *Pediococcus acidilactici* itself in various culture stages of the *Pediococcus acidilactici* used in this disclosure. In one embodiment, the bacterial cell refers to the complete or partial structure of *Pediococcus acidilactici*. In a preferred embodiment, the bacterial cell refers to the colony of the *Pediococcus acidilactici* group formed by the division and reproduction of a single bacterium. In a preferred embodiment, the culture stage refers to solid-state culture, liquid-state culture or liquid-state scale-up of inoculation in a fermenter.

[0034] The “active substance” described herein refers to the substance selected from the *Pediococcus acidilactici* bacterial cell used in this disclosure after being processed through specific experimental steps, or the mixture of the bacterial cell and the culture medium or culture solution. In one embodiment, the active substance refers to the centrifuged bacterial sludge, freeze-dried bacterial powder or solvent-extracted extract. In a preferred embodiment, the active substance refers to a solid medium containing the bacterial cell, a liquid-state medium containing the bacterial cell, or a bacterial liquid after liquid-state amplification in a fermentation tank.

[0035] Although cisplatin is an indispensable drug in the chemotherapy of specific types of cancer, it can cause toxicity to multiple organs of the patient, among which the damage to renal function is the most significant. Among renal function injuries, acute kidney injury is the most intractable due to its high mortality rate and difficulty in initial diagnosis. In this regard, in clinical cancer treatment, it is expected that lactobacillus or its active substances can significantly reduce multiple biochemical indicators of renal function damage, so as to effectively alleviate the acute renal function damage caused by cisplatin.

[0036] In order to prevent or treat renal dysfunction, the present disclosure has developed a use of *Pediococcus acidilactici* GKA4 and/or its active substance for the preparation of a composition for preventing or treating renal dysfunction. In a preferred embodiment, the above-mentioned *Pediococcus acidilactici* GKA4 is deposited in Bioresource Collection and Research Center of Food Industry Research and Development Institute with a deposit number of BCRC 910876.

[0037] Regarding the solid-state culture of strains, the bacteria of *Pediococcus acidilactici* are inoculated on the solid-state medium for solid-state culture to activate the bacteria and form colonies. In a preferred embodiment, the temperature of the solid-state culture ranges from 30 to 55°C, more preferably from 32 to 45°C. In a preferred embodiment, the time for the solid-state culture ranges from 0.5 to 3 days, more preferably from 1 to 2.5 days. In a preferred embodiment, the solid-state medium is MRS agar.

[0038] Regarding the liquid-state culture, after the colony growth on the solid-state medium is completed, a single colony is picked up and inoculated in a test tube containing MRS liquid medium, and activated by liquid-state culture. In a preferred embodiment, the temperature of MRS liquid-state culture is 30 to 55°C,

more preferably 32 to 45°C. In a preferred embodiment, the time for MRS liquid-state culture is 12 to 25 hours, more preferably 20 hours. In a preferred embodiment, the pH value of the liquid-state culture is from pH 5.0 to 7.0, more preferably from pH 5.5 to 6.5. In a preferred embodiment, the formulation of the liquid medium is shown in Table 1 below.

[0039] Table 1

Composition	Ratio (weight percent)
Carbon source	0 to 10 %
Soy milk	0.1~5 %
Nitrogen source	0.1~5 %
Ions	0.01~2 %
Water	Remainder

[0040] Regarding fermentation culture, the bacteria in the MRS liquid-state medium were inoculated in an Erlenmeyer flask containing 1L liquid-state culture solution. After the growth of the bacteria in the liquid-state medium is completed, the bacteria and the liquid-state medium are inoculated in a fermentation tank for liquid amplification to obtain a bacterial cell-containing liquid. In a preferred embodiment, the temperature for culturing in the fermentation tank is 30 to 55°C, more preferably 35 to 39°C. In a preferred embodiment, the time for culturing in the fermentation tank is 16 to 20 hours, more preferably 17 to 19 hours.

[0041] Regarding the preparation of bacterial powder, after the growth of the bacteria in the liquid-state culture is completed, the bacterial liquid of the liquid-state medium containing the bacteria is collected. Next, the bacterial liquid is centrifuged to remove the liquid-state medium used for fermentation to obtain the bacterial sludge. In a preferred embodiment, the centrifugation speed of the liquid-state

medium containing the bacteria is 1000 rpm to 15000 rpm, preferably 5000 rpm to 7000 rpm. The obtained bacterial sludge is mixed with the protective agent and then freeze-dried to obtain the bacterial powder, which is stored at low temperature after freeze-drying. In a preferred embodiment, the protective agent is 1% to 30% skimmed milk powder, preferably 3 to 10% skimmed milk powder. In a preferred embodiment, the storage temperature is from -30°C to 0°C, more preferably from -25°C to -15°C. The preserved bacterial powder is used as a test substance in the following animal experiments.

[0042] In the present disclosure, the composition comprising the bacteria of *Pediococcus acidilactici* and active substances thereof further comprises an additive. In a preferred embodiment, the additive can be an excipient, a preservative, a diluent, a filler, an absorption enhancer or a combination thereof. The excipient may be selected from sodium citrate, calcium carbonate, calcium phosphate, sucrose or combinations thereof. The preservative can prolong the shelf life of the pharmaceutical composition, e.g. benzyl alcohol, parabens. The diluent may be selected from water, ethanol, propylene glycol, glycerin or combinations thereof. The filler may be selected from lactose, galactose, a polyethylene glycol with high molecular weight or combinations thereof. The absorption enhancer may be selected from dimethylsulfoxide (DMSO), laurocapram, propylene glycol, glycerine, polyethylene glycol, or combinations thereof. The sweetener may be selected from Acesulfame K, aspartame, saccharin, sucralose, neotame or combinations thereof. In addition to the additives enumerated above, other suitable additives can be selected and used as required, provided that the medicinal effect of the composition is not affected.

[0043] The composition can be developed into different commodities in the field of medicine. In a preferred embodiment, the composition is a medicine, a feed, a beverage, a nutritional supplement, a dairy product, a food or a health food.

[0044] The composition can take different forms according to the needs of the recipient. In a preferred embodiment, the composition is in the form of powder, tablet, granulation, suppository, microcapsule, ampoule/ampule, liquid spray or suppository.

[0045] The composition of the present disclosure can be used in animals or humans. On the premise of not affecting the effect of the active substance of *Pediococcus acidilactici*, the composition containing the active substance of *Pediococcus acidilactici* can be made into any drug form, and administered to the animal or human in a suitable way according to the drug form. According to the form of the composition of the present disclosure, an appropriate dose of the composition is administered to the subject through an appropriate route at a predetermined time point.

[0046] In a preferred embodiment, the route of administration includes oral administration, intravenous injection, subcutaneous injection, intraperitoneal injection and the like. In a preferred embodiment, the predetermined time point includes every meal, every day, and every week.

[0047] The “effective amount” described herein refers to an amount used, which is sufficient to produce the aforementioned kidney-protecting effect.

[0048] Regarding the effective amount in in vitro cell culture experiments, the total volume of the cell culture medium used in each culture is defined as “μg/ml”. In animal experiments, the effective amount is defined as “g/60 kg body weight/day”. Through the following calculation formula, the effective amount obtained from the

in vitro cell culture experiment can be converted into the effective amount for animals, and the effective amount for animals can be converted into the effective amount for humans according to the metabolic rate.

I. In general (Reagan-Shaw et al., 2008), 1 “ $\mu\text{g/ml}$ ” unit (based on the effective amount obtained from in vitro cell culture experiments) can be equivalent to 1 “ mg/kg body weight/day” unit (based on the effective amount obtained from mouse model experiments). Based on the fact that the metabolic rate of mice is 12.3 times that of humans, the effective dosage for humans can be further obtained.

II. According to the following test results, the effective amount based on the mouse test is 250 mg/kg body weight/day, and the effective dosage for humans is about 2420 mg/60 kg body weight/day through the above conversion formula.

[0049] In a preferred embodiment, the effective amount of *Pediococcus acidilactici* or active substance contained in the composition is 1 to 5g/60kg body weight/day, preferably 2 to 3g/60kg body weight /day.

[0050] The composition for protecting the kidneys of the present disclosure is described below through specific examples.

[0051] Example 1: Strain source

[0052] The strains used to prepare the test substances in the examples of the present disclosure were purchased from Bioresource Collection and Research Center of Food Industry Research and Development Institute. The strain used in the disclosure was the strain GKA4 of *Pediococcus acidilactici*, which was deposited in Bioresource Collection and Research Center of Food Industry Research and Development Institute as BCRC910876. However, the strains described in the disclosure are not limited to those obtained from such sources, and

those with ordinary skill in the technical field can also obtain the strains of *Pediococcus acidilactici* from other microbial strain preservation units.

[0053] Example 2: Strain cultivation

[0054] The bacteria of *Pediococcus acidilactici* were inoculated on MRS agar that served as a solid-state medium, and solid-state culture was carried out at 37°C for 2 days to activate the bacteria for colony formation. After the growth of the colony on the MRS agar was completed, a single colony was picked and inoculated into a test tube containing MRS liquid-state medium, cultured and activated in liquid state at 37°C for 16 hours, and then inoculated into a 1 L liquid-state medium with a pH value of 6.0 in an Erlenmeyer flask. The formulation of the liquid-state medium is shown in Table 1 above. After the growth of the bacteria in the liquid-state medium was completed, the bacteria and the liquid-state medium were inoculated in the fermentation tank and cultured at 37 °C for 18 hours for liquid amplification to obtain the bacterial liquid.

[0055] Example 3: Preparation of bacterial powder

[0056] After the growth of the bacteria in the liquid-state culture was completed, the bacterial liquid of the liquid-state medium containing the bacteria was collected. The bacterial liquid was centrifuged at 6000 rpm to remove the liquid-state medium and obtain the bacterial sludge. The bacteria sludge was mixed with 5% skimmed milk powder that served as the protective agent and then freeze-dried to obtain bacteria powder, which was stored at about -20 °C and used as a test substance in the following animal experiments.

[0057] Example 4: Physiological test of mice fed with *Pediococcus acidilactici*
GKA4

[0058] Test animals

[0059] Thirty-two male ICR mice weighing 18 to 22 grams were purchased from BioLASCO Taiwan Co.,LTD.,Taiwan. The temperature of the animal room was controlled at 23 °C to 25 °C, the humidity was controlled at 55% to 65%, and the light and dark were 12 hours each. During the experiment, the mice had free access to feed and distilled water.

[0060] Experimental design

[0061] Thirty-two mice were divided into four groups including normal saline group, cisplatin group, amifostine group and GKA4, with 8 mice in each group. The normal saline group, the cisplatin group and the amifostine group were given normal saline and the GKA4 group was given GKA4 by oral tube feeding once a day for 10 days. On the seventh day of administration, the cisplatin group, the amifostine group and the GKA4 group were intraperitoneally injected with cisplatin 20 mg/kg B.W. (Body Weight) to induce acute kidney injury, and the amifostine group was intraperitoneally injected with amifostine 30 minutes before the intraperitoneal injection of cisplatin. The groups and administered test samples are shown in Table 2 below.

[0062] Table 2

Group	Number of rats	Experimental reagent (intraperitoneal injection)
Normal saline group	8	5 mL normal saline (0.9% NaCl) by tube feeding for 10 consecutive days
Cisplatin group	8	5 mL normal saline (0.9% NaCl) by tube feeding for 10 consecutive days and cisplatin (20 mg/kg BW) by intraperitoneal injection on the 7th day
Amifostine group	8	5 mL normal saline (0.9% NaCl) by tube feeding for 10 consecutive days and 200 mg/kg of amifostine by intraperitoneal

		injection on the 7th day, followed by cisplatin (20 mg/kg BW) by intraperitoneal injection 30 minutes later
GKA4 group	8	250 mg/kg of GKA4 by tube feeding for 10 consecutive days and cisplatin (20 mg/kg BW) by intraperitoneal injection on the 7th day

[0063] Amifostine administered to mice in this experiment is a protective agent. Since the kidney is the main organ that removes cisplatin drugs, the renal tubular epithelial cells will be exposed to highly concentrated cisplatin, causing kidney damage. Therefore, at the initial stage of cisplatin injection, the reabsorption capacity of the patient's proximal renal tubules for sodium and water will be significantly reduced, which will reduce the glomerular filtration efficiency and cause abnormalities in the distal renal tubules. Amifostine is a known protective agent approved by the Food and Drug Administration (FDA) for alleviating nephrotoxicity caused by high-dose cisplatin administration in lung cancer patients.

[0064] Test record and analysis

[0065] On day 11 (end of the test), the animals were sacrificed with CO₂, and the appearance as well as tissues and organs in the chest and abdomen were observed and recorded. The blood was collected, and the test kit purchased from Randox Laboratories Ltd. was used for analysis of biochemical indicators in serum. The body weight of the mice was measured, and the kidneys were isolated from the mice to measure the weight of kidney. The kidney index was calculated according to the following formula 1.

Formula 1: Kidney index = [(mouse kidney weight/mouse body weight)×100]

[0066] Kidneys were fixed in 10% buffered formalin and embedded in paraffin to prepare sections of approximately 5 μm , and the sections were mounted on slides to be stained with hematoxylin and eosin. The sliced tissue on the slide was observed under an optical microscope at a magnification of 400X, and the pathological changes of the sliced tissue were recorded. Depending on the severity of tissue lesions in the sections, had a veterinarian assess the kidney necrosis score. Scores range from 0 to 5, wherein 1 represents very mild (<1%), 2 represents mild (1%-25%), 3 represents moderate (26%-50%), 4 represents moderate/severe (51%-75%), and 5 represents serious/very serious (76%-100%). The higher the score, the more severe the degree of kidney necrosis.

[0067] Inflammation test (NO assay)

[0068] Griess reagent (0.1% (w/v) sulfonamide and 0.1% (w/v) naphthylethylenediamine dihydrochloride were taken and freshly prepared and used in a ratio of 1:1 by volume) was used to determine the content of nitrite (NO^{2-}) in the culture solution. Since NO is quickly oxidized and transformed into nitrite anion (NO^{2-}) and nitrate anion (NO^{3-}) after LPS-induced NO is generated, the amount of NO produced can be measured indirectly by measuring nitrite anion. 100 ml of Griess reagent was added to 100 ml of the culture solution. The well plate was placed on a shaker and shook at 100 rpm for 5 minutes. The nitrite anion reacted with Griess reagent to exhibit blue-purple color, and the absorbance value at 550 nm can be determined by an ELISA reader. The nitrite anions contained in the culture solution can be known by comparing with the standard curve made with NaNO_2 , which indirectly represents the amount of NO produced.

[0069] The data are represented by Mean $\pm$ standard deviation (SD) of the experimental results. The statistical analysis method of body weight adopts

Student's t-test to test the difference between the test substance group and the control group, and $p < 0.05$ indicates a significant difference.

[0070] Test results

[0071] (1) Content of blood urea nitrogen (BUN)

[0072] With reference to FIG. 1 and as shown in Table 3, by comparing the blood urea nitrogen concentration in the serum of each group, the blood urea nitrogen concentration in the mouse serum of the GKA4 group administrated with GKA4 and induced by cisplatin was significantly lower than that of the cisplatin group, which was similarly induced by cisplatin but not fed with GKA4 ($p < 0.05$). The blood urea nitrogen concentration in the mice serum of GKA4 group was similar to that of the amifostine group. This result shows that GKA4 can reduce the concentration of serum urea nitrogen as an indicator of renal function, slow down the acute kidney injury caused by cisplatin, and achieve the effect of restoring renal function. Compared with the currently used protective agent (amifostine group), GKA4 has a considerable effect on restoring renal function.

[0073] Table 3

Group	Normal saline group	Cisplatin group	Amifostine group	GKA4
BUN concentration (mg/dL)	24.00±0.44	138.65±4.07	48.20±2.29	64.18±2.10

[0074] (2) Creatinine content

[0075] With reference to FIG. 2 and as shown in Table 4, by comparing the creatinine concentration in the serum of each group, the creatinine concentration in the mouse serum of the GKA4 group administrated with GKA4 and induced by cisplatin was significantly lower than that of the cisplatin group, which was similarly

induced by cisplatin but not fed with GKA4 ($p<0.05$). The creatinine concentration in the mice serum of GKA4 group was similar to that of the amifostine group. This result shows that GKA4 can reduce the concentration of serum creatinine as an indicator of renal function, slow down the acute kidney injury caused by cisplatin, and achieve the effect of restoring renal function. Compared with the currently used protective agent (amifostine group), GKA4 has a considerable effect on restoring renal function.

[0076] Table 4

Group	Normal saline group	Cisplatin group	Amifostine group	GKA4
Creatinine concentration (mg/dL)	0.90±0.02	3.31±0.11	1.45±0.04	1.53±0.03

[0077] With reference to FIG. 3 and as shown in Table 5, the body weight and kidney weight of the animals were recorded at the end of the experiment. The renal index can be calculated according to Formula 1. By comparing the renal index of each group, the mouse renal index of the GKA4 group administrated with GKA4 and induced by cisplatin was significantly lower than that of the cisplatin group, which was similarly induced by cisplatin but not fed with GKA4 ($p<0.05$). The mouse renal index of the GKA4 group was similar to that of the amifostine group, and approached that of the normal saline group. This result shows that GKA4 can reduce the renal index as an indicator of renal function, slow down the acute kidney injury caused by cisplatin, and achieve the effect of restoring renal function.

[0078] Table 5

Group	Normal saline group	Cisplatin group	Amifostine group	GKA4
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Initial weight (g)	34.17 ± 0.74	34.12 ± 0.15	34.05 ± 0.28	34.00 ± 0.24
Final body weight (g)	39.68 ± 0.58	32.55 ± 0.19	36.05 ± 0.20	36.27 ± 0.31
Weight difference (g)	5.51	-1.57	2	2.27
Kidney weight (g)	0.53 ± 0.03	0.77 ± 0.03	0.55 ± 0.03	0.58 ± 0.03
Renal index	1.35 ± 0.08	2.37 ± 0.09###	1.51 ± 0.07***	1.60 ± 0.06***

means comparison with normal saline group; * means comparison with cisplatin group.

[0079] With reference to FIG. 4, in the histopathological examination, there is obvious multifocal renal tubular necrosis due to the renal proximal tubule accumulation in the renal tissue of the cisplatin group, while the histopathological changes of the kidney of the GKA4 group have a protective effect.

[0080] With reference to FIG. 5 and as shown in Table 6, the renal necrosis index was scored by assessing the necrosis of proximal tubules and the abnormal presence of hyaline cylinders. If the renal proximal tubules of the mice accumulate multifocal tubular necrosis, and the arrangement of the cells is not complete, and even vacuoles and transparent cylinders appear, it shows that the kidneys are inflamed. By comparing the score of renal tissue necrosis of each group, the score of renal tissue necrosis of mice of the GKA4 group administrated with GKA4, which induced by cisplatin was significantly lower than that of the cisplatin group, which was similarly induced by cisplatin but not fed with GKA4 ($p < 0.05$) and close to that of the amifostine group. This result shows that GKA4 can reduce the score of renal tissue necrosis as an indicator of renal function, slow down the acute kidney injury caused by cisplatin, and achieve the effect of restoring renal function.

[0081] Table 6

Group	Normal saline group	Cisplatin group	Amifostine group	GKA4
Necrosis score of proximal renal tubules	0±0	5±0	1.17±0.41	0.83±0.75
Abnormal score of hyaline cylinders	0±0	5±0	0.83±0.41	0.33±0.52

[0082] In summary, it can be seen that by pre-administering a composition having bacteria or active substances of GKA4 to an object, the acute injury caused by cisplatin to the kidney can be effectively reduced, thereby achieving the effect of protecting the kidney and maintaining renal function.

[0083] Example 5: preparation of composition

[0084] The present disclosure provides a composition, which includes GKA4 and exerts the effect of protecting the kidney.

[0085] If the GKA4 of the disclosure is applied to food applications, the following composition 1 can be used as an illustrative example.

[0086] Composition 1: The freeze-dried powder of GKA4 as the active substance of *Pediococcus acidilactici* (20 wt%) was fully mixed with benzyl alcohol (8 wt%) as the preservative and glycerine (7 wt%) as the diluent, dissolved in pure water (65 wt%), and stored at 4 °C for later use. The aforementioned wt% refers to the ratio of each component to the total weight of the composition.

[0087] If GKA4 of the disclosure is applied to medical purposes in liquid dosage form, the following composition 2 is used as an illustrative example.

[0088] Composition 2: The freeze-dried powder of GKA4 as the active substance of *Pediococcus acidilactici* (20 wt%) was fully mixed with benzyl alcohol (8 wt%) as the preservative, glycerine (7 wt%) as the diluent and sucrose (10 wt%) as the diluent, dissolved in pure water (55 wt%), and stored at 4 °C for later use. The aforementioned wt% refers to the ratio of each component to the total weight of the composition.

CLAIMS

What is claimed is:

1. A *Pediococcus acidilactici* GKA4 deposited in Bioresource Collection and Research Center of Food Industry Research and Development Institute of the Republic of China with a deposit number of BCRC 910876.
2. A use of *Pediococcus acidilactici* GKA4 and/or an active substance thereof in preparation of a composition for preventing or treating renal dysfunction, wherein the *Pediococcus acidilactici* GKA4 is deposited in Bioresource Collection and Research Center of Food Industry Research and Development Institute with a deposit number of BCRC 910876.
3. The use of claim 2, wherein the composition is administered to a subject in an effective amount of 0.05 to 25 g/60 kg body weight/day.
4. The use of claim 3, wherein the renal dysfunction refers to acute kidney injury (AKI).
5. The use of claim 2, wherein the preventing or treating the renal dysfunction means that a concentration of blood urea nitrogen (BUN) in serum of a subject after administration of the composition is lower than that before administration of the composition.
6. The use of claim 2, wherein the preventing or treating the renal dysfunction means that a concentration of Creatinine (Cr) in serum of a subject after administration of the composition is lower than that before administration of the composition.
7. The use of claim 2, wherein the preventing or treating the renal dysfunction means that a renal index of the subject after administration of the composition is

lower than that before administration of the composition, wherein the renal index refers to a percentage of a subject's kidney weight to the subject's body weight.

8. The use of claim 2, wherein necrosis of renal tissue refers to necrosis of proximal renal tubules and abnormal appearance of hyaline cylinders.

9. The use of claim 2, wherein the preventing or treating the renal dysfunction means that a degree of necrosis of a subject's kidney tissue after administration of the composition is lower than that before administration of the composition.

10. The use of claim 2, wherein the renal dysfunction is caused by a cancer treatment drug.

11. The use of claim 10, wherein the cancer treatment drug refers to cisplatin.

12. The use of claim 2, wherein the active substance of lactic acid bacteria is prepared by the following steps:

(a) inoculating a bacteria of the lactic acid bacteria in a solid-state medium for solid-state culture to form a colony;

(b) inoculating the colony cultured in step (a) into a liquid medium for liquid culture to obtain a liquid-state medium containing the bacterial cell;

(c) inoculating the liquid-state medium containing the bacterial cell of step (b) into a fermentation tank for liquid-state amplification to obtain a bacterial liquid;

(d) centrifuging the bacterial liquid of step (c) to obtain a bacterial sludge; and

(e) freeze-drying the bacterial sludge of step (d) to obtain a bacterial powder.

13. The use of claim 12, wherein a temperature of the freeze-drying ranges from -196°C to -40°C.

14. The use of claim 2, wherein the composition comprises an additive selected from the group consisting of an excipient, a preservative, a diluent, a filler, an absorption enhancer, a sweetener or a combination thereof.
15. The use of claim 2, wherein the composition is a medicine, a feed, a beverage, a nutritional supplement, a dairy product, a food or a health food.
16. The use of claim 3, wherein the composition is in a form of powder, tablet, suppository, microcapsule, ampoule, liquid spray or suppository.