

Description

Prophylactic Agent for Squamous Carcinoma, Squamous Carcinoma Model Animal, and Method for Producing Said Model Animal

Technical Field

[0001]

The present invention relates to an agent for preventing squamous carcinoma caused by *Candida* infection (hereinafter may be referred to as a "prophylactic agent for squamous carcinoma caused by *Candida* infection"); to a method for producing a squamous carcinoma model animal; to a squamous carcinoma model animal produced through the method; and to a screening method employing the model animal.

Background Art

[0002]

Oral cancer and esophageal cancer are derived from stratified squamous epithelium, and particularly, esophageal cancer is intractable and has a high mortality rate. As has been known, *Candida albicans* (*C. albicans*), which is an indigenous bacterium found in the oral cavity of healthy individuals, may often cause severe oral candidiasis or esophageal candidiasis in patients with impaired immune functions, such as patients having undergone organ transplantation or major surgery, or patients with hepatic cirrhosis or diabetes. An increase in the number of bedridden elderly people problematically causes

undernutrition, which may result in impaired immune functions and increased opportunity for infection. Thus, a serious problem arises in that patients or elderly people with impaired immune functions are readily infected with *C. albicans*, which may cause deep mycosis, further leading to malignant tumor (i.e., severe pathological condition).

Therefore, demand has arisen for provision of a drug or food which suppresses the progression from candidiasis to squamous carcinoma and prevents development of squamous carcinoma.

[0003]

Meanwhile, a fermented milk containing *Lactobacillus gasseri* (*L. gasseri*) is known to exhibit the effect of eliminating *H. pylori*, and thus the effect of preventing gastritis or gastric ulcer (Patent Documents 2 and 3). However, the effect of such a fermented milk on *Candida* infection has been completely unknown.

[0004]

In general, production of a disease model is effective for screening of drugs or foods efficacious for diseases. Hitherto reported squamous carcinoma model animals include a squamous carcinoma model animal produced through intradermal or subcutaneous administration of ethanol and/or acetaldehyde to an Aldh2-deficient mammal (except for human) (Patent Document 1). However, this squamous carcinoma model animal is provided for the purpose of elucidating the pathogenic mechanism of squamous carcinoma frequently developed in human

in which the ALDH2 gene involved in alcohol metabolism is inactivated, or of developing a method for treatment of the disease. That is, the squamous carcinoma model animal has no relation to development of squamous carcinoma caused by Candida infection.

[0005]

The present inventors found that squamous carcinoma accompanied by severe infectious chronic inflammation is developed in the forestomach of a WBN/Kob rat with diabetes induced through administration of alloxan (Non-Patent Document 1). However, this alloxan-induced diabetic rat model exhibits a large individual difference in terms of the extent of proliferative forestomach lesion or the incidence thereof. Thus, a disease model exhibiting a smaller individual difference must be established for appropriate selection, through screening, of a drug or food which suppresses the progression from candidiasis to squamous carcinoma.

Citation List

Patent Document

[0006]

Patent Document 1: JP-A-2005-110601

Patent Document 2: JP-B-4509250

Patent Document 3: JP-B-3046303

Non-Patent Document

[0007]

Non-Patent Document 1: Sano T, Ozaki K, Kodama Y, Matsuura T,

Narama I., Toxicol. Pathol., vol. 37, pp. 790-798 (2009).

Summary of the Invention

Problems to be Solved by the Invention

[0008]

An object of the present invention is to provide a fermented product which is effective for preventing squamous carcinoma caused by *Candida* infection. Another object of the present invention is to provide a model animal for squamous carcinoma caused by *Candida* infection, which model animal exhibits a smaller individual difference as compared with conventional model animals, and which realizes more appropriate evaluation of drug efficacy. Yet another object of the present invention is to provide a method for producing the model animal.

Means for Solving the Problems

[0009]

In order to solve the aforementioned problems, the present inventors have conducted extensive studies, and as a result have found that when alloxan is administered to a rat for induction of diabetes, and immediately thereafter *C. albicans* is forcibly orally administered to the rat, a proliferative lesion accompanied by chronic inflammation resulting from *Candida* infection can be induced in the forestomach of the rat at an early stage, and have also found that this technique can produce a squamous carcinoma model animal exhibiting a small individual difference with respect to such a lesion. The present inventors have also found that

a fermented product produced by fermenting a milk with *Lactobacillus bulgaricus* (*L. bulgaricus*), *Streptococcus thermophilus* (*S. thermophilus*), and *L. gasseri* suppresses inflammation caused by infection with *C. albicans*, and also suppresses the progression of a proliferative lesion; i.e., the fermented product is useful for preventing squamous carcinoma caused by Candida infection.

[0010]

Accordingly, the present invention encompasses the following.

[1] A prophylactic agent for squamous carcinoma caused by Candida infection, the agent comprising, as an active ingredient, a fermented product produced through fermentation of a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri*.

[2] The prophylactic agent for squamous carcinoma caused by Candida infection according to [1] above, wherein the fermented product is a fermented milk produced through fermentation of a milk with *L. bulgaricus* and *S. thermophilus*, and subsequent incorporation of *L. gasseri* into the resultant product.

[3] The prophylactic agent for squamous carcinoma caused by Candida infection according to [1] or [2] above, wherein the *L. gasseri* is *L. gasseri* OLL2716 strain (FERM BP-6999).

[4] The prophylactic agent for squamous carcinoma caused by Candida infection according to any of [1] to [3]

above, wherein a squamous epithelium is an oral and/or esophageal squamous epithelium.

[5] Use of a fermented product produced through fermentation of a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri* for producing a prophylactic agent for squamous carcinoma caused by Candida infection.

[6] A fermented product produced through fermentation of a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri* for use in preventing squamous carcinoma caused by Candida infection.

[7] A method for preventing squamous carcinoma caused by Candida infection, the method comprising administering or taking a fermented product produced through fermentation of a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri*.

[8] A method for producing a squamous carcinoma model animal, the method comprising a step of forcibly orally administering *C. albicans* to a model animal with diabetes induced through administration of a diabetogenic substance.

[9] The production method according to [8] above, wherein the animal is a rat.

[10] A squamous carcinoma model animal produced through a method as recited in [8] or [9] above.

[11] A screening method for selecting a prophylactic agent and/or therapeutic agent for squamous carcinoma, the method comprising a step of administering a test substance to a squamous carcinoma model animal as recited in [10] above, and observing a change in a squamous carcinoma lesion site.

Effects of the Invention

[0011]

The prophylactic agent of the present invention for squamous carcinoma caused by *Candida* infection can effectively suppress development of candidiasis, as well as the progression from candidiasis to squamous carcinoma. According to the present invention, there can be produced a squamous carcinoma model animal which can reproduce the progression from *Candida* infection to squamous carcinoma, with reduced individual difference in terms of the extent of lesion or the incidence thereof. The squamous carcinoma model animal realizes selection, through screening, of a substance useful for treatment of squamous carcinoma, or a prophylactic agent for squamous carcinoma caused by *Candida* infection. Also, the squamous carcinoma model animal can be employed for elucidation of the pathogenic mechanism of squamous carcinoma caused by infection with *C. albicans*, or studies on new therapeutic and prophylactic methods for squamous carcinoma.

Brief Description of the Drawings

[0012]

[Fig. 1]

Fig. 1(a) shows the forestomach of a rat of group C (i.e., a group of non-treated rats to which *C. albicans* has been administered), and Fig. 1(b) shows the forestomach of a rat of group AC (i.e., a group of alloxan-induced diabetic rats to which *C. albicans* has been administered). The extent

of squamous hyperplasia in group AC is severer than that in group C. In group AC, severe purulent inflammation accompanied by *C. albicans* infection is observed at the epithelial superficial site.

[Fig. 2]

Fig. 2 shows a lesion site of one individual with very early squamous carcinoma. The upper portion of the figure corresponds to a hyperplastic site, and irregular squamous epithelial cell cords extend downward from the bottom of the site. The portion surrounded a broken line and indicated by an arrow corresponds to a presenting portion of cancer cells infiltrating into muscularis mucosae.

[Fig. 3]

Fig. 3(a) shows a forestomach lesion site of a rat of group DC (i.e., a group of alloxan-induced diabetic rats to which *C. albicans* has been administered), and Fig. 3(b) shows a forestomach lesion site of a rat of group DC+LG21 (i.e., a group of alloxan-induced diabetic rats to which *C. albicans* and LG21 have been administered). The extent of squamous hyperplasia in group DC+LG21 is milder than that in group DC. In group DC, severe purulent inflammation accompanied by *C. albicans* infection is observed at the epithelial superficial site. In contrast, in group DC+LG21, the extent of purulent inflammation is suppressed.

[Fig. 4]

Fig. 4(a) shows an immunostaining image of Ki-67 (i.e., a cell proliferation marker) in group DC, and Fig. 4(b) shows

an immunostaining image of Ki-67 in group DC+LG21. Ki-67 is positive in the nucleus of proliferated squamous epithelial cells, and the positive rate thereof in group DC+LG21 is lower than that in group DC.

Modes for Carrying Out the Invention

[0013]

The present invention will next be described in detail, but the invention is not limited to the below-described embodiments.

(Prophylactic agent for squamous carcinoma caused by Candida infection)

The prophylactic agent of the present invention for squamous carcinoma caused by Candida infection contains, as an active ingredient, a fermented product produced through fermentation of a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri*.

The fermented product may be produced by fermenting a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri* added thereto. Alternatively, the fermented product may be produced by fermenting a milk with *L. bulgaricus* and *S. thermophilus* added thereto, followed by addition of *L. gasseri* and further fermentation.

No particular limitation is imposed on the milk serving as a fermentation raw material, and an animal milk (e.g., a mammal-derived milk such as cow milk, sheep milk, goat milk, or buffalo milk) may be generally employed. The milk serving as a raw material does not necessarily contain only raw milk

or sterilized milk, and may contain a reconstituted milk raw material (e.g., butter, cream, skim milk, concentrated whole milk, concentrated skim milk, whole milk powder, skim milk powder, or condensed milk) and optionally an additional food or food additive, so long as fermentation is not impeded.

[0014]

The *L. bulgaricus* and *S. thermophilus* employed in the present invention is preferably a starter containing, as a base, a mixed starter of *L. bulgaricus* and *S. thermophilus*, which are generally used for fermented milk production and are standardized as yogurt starters by the Codex Alimentarius. Examples of the *L. bulgaricus* and *S. thermophilus* employed include *L. delbrueckii* subsp. *bulgaricus* JCM1002^T and *S. thermophilus* ATCC19258. Optionally, there may be added a lactic acid bacterium such as a bacterium belonging to the genus *Streptococcus*, the genus *Leuconostoc*, or the genus *Pediococcus*, a bifidobacterium, yeast, etc.

[0015]

No particular limitation is imposed on the *L. gasseri* employed, and the *L. gasseri* may be, for example, *L. gasseri* OLL2716 strain. As described in Patent Document 3, *L. gasseri* OLL2716 strain was internationally deposited as FERM BP-6999 under the Budapest Treaty in International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology (Tsukuba Center, Central 6, 1-1-1, Higashi, Tsukuba, Ibaraki, Japan; original deposition

date: May 24, 1999). This strain was transferred from domestic deposition (original deposition) (Fermentation Research Institute Deposition Number: P-17399, deposition date: May 24, 1999) to international deposition under the Budapest Treaty on January 14, 2000.

[0016]

As described in the Examples hereinbelow, the fermented product of the present invention suppresses inflammation caused by infection with *C. albicans*, and significantly suppresses malignant transformation of squamous epithelial cells. Therefore, the fermented product of the present invention can be employed as a prophylactic agent for squamous carcinoma caused by Candida infection.

The fermented product may be employed in a human or a non-human animal, and may be employed for a therapeutic or non-therapeutic purpose. As used herein, the term "non-therapeutic" refers to a concept not including medical practice; i.e., a concept not including a surgical, therapeutic, or diagnostic method for human (more specifically, a concept not including a surgical, therapeutic, or diagnostic method for human carried out by a physician or a person directed by a physician).

[0017]

As used herein, the expression "prevention of squamous carcinoma caused by Candida infection" refers to suppression of malignant transformation of a tissue consisting of squamous epithelial cells infected with Candida (preferably

C. albicans), for prevention of development of squamous carcinoma; i.e., suppression of the progression from candidiasis to squamous carcinoma, and also refers to suppression (prevention) or treatment of a premalignant lesion before malignant transformation of squamous epithelial cells; i.e., inflammation (candidiasis) caused by *Candida* infection. Examples of the tissue consisting of squamous epithelial cells include oral cavity, esophagus, pharynx, larynx, anus, and skin. The present invention is suitably applied to oral cavity, esophagus, pharynx, or larynx, which correspond to the upper gastrointestinal tract.

As used herein, the term "squamous carcinoma" encompasses morphologically distinguishable typical squamous carcinoma, and squamous hyperplastic lesions.

Therefore, "prevention of squamous carcinoma caused by *Candida* infection" as used herein encompasses, for example, prevention of oral cancer, pharyngeal cancer, laryngeal cancer, or esophageal cancer, and suppression (prevention) or treatment of oral mycosis such as acute oral candidiasis, acute pseudomembranous candidiasis, oral thrush, acute atrophic candidiasis, chronic oral candidiasis, chronic atrophic stomatitis, chronic hypertrophic candidiasis, chronic mucocutaneous candidiasis, or median rhomboid glossitis, esophageal candidiasis, etc.

[0018]

The prophylactic agent of the present invention for squamous carcinoma caused by *Candida* infection may be a drug

or food for human or animal, which exhibits the effect of suppressing malignant transformation of squamous epithelial cells infected with Candida, thereby suppressing the progression from candidiasis to squamous carcinoma, when the drug or food is administered to an animal (including human) or the animal is caused to take the drug or food.

Alternatively, the prophylactic agent may be a material or preparation which is incorporated into such a drug or food. The food employed in the present invention encompasses a functional food, a patient food, and a food for specified health use, which are provided for the purpose of preventing squamous carcinoma caused by Candida infection, or preventing or treating candidiasis, and on which the purpose is optionally displayed.

[0019]

The dosage form of the aforementioned drug containing the fermented product of the present invention may be determined in consideration of, for example, the purpose of prevention or amelioration, or the route of administration. Examples of the dosage form include tablet, coated tablet, pill, capsule, granule, powder, liquid, suspension, emulsion, syrup, injection, suppository, infusion, decoction, and tincture. Such a drug product may be produced through a customary method by optionally mixing a base component with a known adjuvant which is generally used in a drug production technique, such as a filler, an extender, an excipient, a binder, a humectant, a disintegrant, a surfactant, a

lubricant, a colorant, a flavoring agent, a solubilizer, a suspending agent, or a coating agent. Such a drug product may contain, for example, a colorant, a preservative, a flavor, a flavoring agent, a sweetening agent, or another drug.

[0020]

The aforementioned food containing the fermented product of the present invention may be provided in any form, for example, in the form of milk, refreshing beverage, fermented milk, yogurt, cheese, bread, biscuit, cracker, pizza crust, infant formula, liquid diet, patient food, infant milk powder or the like, milk powder for lactating woman or the like, or nutritional food. The food may be provided in a generally used state of food or beverage, such as solid (powder, granule, etc.), paste, liquid, or suspension.

[0021]

For preparation of the food, the fermented product of the present invention may be employed as is. Alternatively, the food may be produced through a method which is generally used for common food or beverage compositions; for example, mixing of the fermented product with an additional food or food ingredient.

No particular limitation is imposed on the additional food ingredient, and the food ingredient may be, for example, water, a protein, a sugar, a lipid, a vitamin, a mineral, an organic acid, an organic base, a fruit juice, or a flavor.

Examples of the protein include animal and vegetable proteins such as whole milk powder, skim milk powder, partially skimmed milk powder, casein, whey powder, whey protein, concentrated whey protein, separated whey protein, α -casein, β -casein, κ -casein, β -lactoglobulin, α -lactalbumin, lactoferrin, soybean protein, egg protein, and meat protein; hydrolysates thereof; and milk-derived ingredients such as butter, whey mineral, cream, whey, non-protein nitrogen, sialic acid, phospholipid, and lactose. Examples of the sugar include saccharides, modified starch (e.g., dextrin, soluble starch, British starch, oxidized starch, starch ester, and starch ether), and dietary fiber. Examples of the lipid include animal oils and fats, such as lard, fish oil, and fractionated oil, hydrogenated oil, and transesterified oil thereof; and vegetable oils and fats, such as palm oil, safflower oil, corn oil, rapeseed oil, coconut oil, and fractionated oil, hydrogenated oil, and transesterified oil thereof. Examples of the vitamin include vitamin A, carotenes, vitamin B group, vitamin C, vitamin D group, vitamin E, vitamin K group, vitamin P, vitamin Q, niacin, nicotinic acid, pantothenic acid, biotin, inositol, choline, and folic acid. Examples of the mineral include calcium, potassium, magnesium, sodium, copper, iron, manganese, zinc, selenium, and whey mineral. Examples of the organic acid include malic acid, citric acid, lactic acid, and tartaric acid. These ingredients may be employed in combination of two or more species. There may be employed a synthetic

product and/or a food containing a large amount of such an ingredient.

[0022]

The amount of the fermented product of the present invention contained in the aforementioned drug or food may be optionally determined in consideration of the intended use or application thereof. The amount of the fermented product, as reduced to dry matter, is generally 0.001 to 100 wt.%, preferably 0.01 to 100 wt.%, more preferably 0.1 to 100 wt.%, with respect to the total amount of the drug or food. The total number of cells of *L. bulgaricus* and *S. thermophilus* (lactic acid bacteria) contained in the fermented product is preferably 10,000,000 (1×10^7)/mL or more. The number of cells of *L. gasseri* is preferably 1,000,000 (1×10^6)/mL or more, more preferably 10,000,000 (1×10^7)/mL or more.

[0023]

The amount of the aforementioned drug or food to be administered or taken—which may vary with, for example, the biological species, age, or body weight of a subject, or the number of cycles of administration or intake—may be appropriately determined at the discretion of those skilled in the art. For example, the dose of the fermented product of the present invention (as reduced to dry matter) may be 0.01 to 10,000 mg/kg body weight/day, and is preferably 0.05 to 6,000 mg/kg body weight/day, more preferably 0.3 to 5,000 mg/kg body weight/day. The administration or intake of the drug or food may be carried out singly or in a repeated

manner. Preferably, the administration or intake is carried out in a repeated manner.

No particular limitation is imposed on the subject for administration or intake of the drug or food, so long as the subject is a human in need thereof. Examples of the subject include patients with oral candidiasis, esophageal candidiasis, etc., and people who are likely to develop such a disease.

[0024]

(Squamous carcinoma model animal)

The "animal" employed for producing the squamous carcinoma model animal of the present invention refers to, for example, mouse or rat. No particular limitation is imposed on the sex, age (week old), body weight, etc. of the animal, so long as the animal can be applied to intended screening. The oral cavity, tongue, or esophagus of human is covered with stratified squamous epithelium, and only a portion of the oral cavity, tongue, or esophagus of human, monkey, or dog is cornified. Meanwhile, it has been known that the entire region (from the oral cavity to the forestomach) of mouse or rat is completely cornified (Folia Pharmacologica Japonica, 131, 373-377, 2008). A characteristic feature of the present invention resides in that a lesion is formed in the forestomach of mouse or rat. Examples of the "animal" include WBN/Kob rat and F344 rat.

[0025]

A characteristic feature of the squamous carcinoma

model animal production method of the present invention resides in that *C. albicans* is forcibly orally administered to a diabetic animal. The diabetic animal employed may be, for example, a diabetes-induced animal produced through administration of a drug (e.g., alloxan or streptozotocin) to a rat (normal rat) or a mouse (normal mouse), or an animal with hereditary diabetes. However, animals with diabetes developed through administration of a diabetes-inducing substance are preferably employed, since the period of diabetes development or the degree of severity can be equalized. Preferably, there is employed a rat with diabetes developed through administration of alloxan (i.e., a diabetogenic substance), since alloxan is not a genotoxic carcinogen. Whether or not diabetes is developed in an animal can be determined through a customary method; for example, measurement of the blood glucose level or urine glucose level of the animal.

[0026]

A characteristic feature of the present invention resides in that *C. albicans* is forcibly orally administered after induction of diabetes. Preferably, a *C. albicans* sample is suspended in saline, and the suspension is orally administered. In the present invention, no particular limitation is imposed on the number of cells of forcibly orally administered *C. albicans*, the frequency of administration, or the period of administration, so long as *C. albicans* is fixed on squamous epithelial cells of an

animal employed. The number of cells of *C. albicans*, the frequency of administration, or the period of administration—which may vary with, for example, the biological species, age, or body weight of the animal, the route of administration, or the number of cycles of administration—may be appropriately determined at the discretion of those skilled in the art. When, for example, *C. albicans* is administered to a 10-week-old WBN/Kob rat, cells of *C. albicans* (1×10^6 /mL or more, preferably 1×10^7 /mL or more) are forcibly orally administered at a frequency of once to four times a week.

[0027]

According to the squamous carcinoma model animal production method of the present invention, generally, squamous hyperplasia occurs at the forestomach of a rat about three months (preferably about six months) after initiation of administration of *C. albicans*. As used herein, the term "squamous carcinoma" encompasses morphologically distinguishable typical squamous carcinoma, and squamous hyperplastic lesions.

After development of a lesion in the model animal of the present invention, a portion of the lesion site can be collected and employed for histopathological determination of a premalignant lesion. For example, when papillary proliferation of squamous epithelium is found through hematoxylin-eosin staining (HE staining) of the collected tissue, hyperplasia at the lesion site can be demonstrated.

In addition, the presence of cancer cells can be determined by demonstrating structural atypia or nuclear atypia through finding of a hyperplastic to continuously invasively proliferated lesion.

[0028]

A model animal with squamous carcinoma produced through the method of the present invention can be employed for selection, through screening, of a therapeutic drug for squamous carcinoma. A substance useful for treatment of squamous carcinoma can be selected through screening by orally or parenterally administering a test substance to the model animal, and observing the therapeutic effect of the test substance (e.g., reduction of a cancer lesion site, or suppression of cancer progression). Also, a prophylactic drug for squamous carcinoma can be selected through screening by administering a test substance to the model animal during production thereof, and then forcibly orally administering *C. albicans* to the model animal.

Also, the model animal of the present invention is useful for elucidation of the pathogenic mechanism of squamous carcinoma caused by infection with *C. albicans*, or studies on therapeutic and prophylactic methods for squamous carcinoma.

Examples

[0029]

The present invention will next be described in more detail by way of Examples, which should not be construed as

limiting the invention thereto. Unless otherwise specified, the symbol "%" shown in the Examples refers to wt.%.

[0030]

[Example 1] Establishment of disease model

Attempts were made to induce early inflammation and proliferative lesion through experimental administration of *C. albicans* immediately after induction of diabetes with alloxan.

[0031]

Alloxan (product of Sigma) (dose: 40 mg/kg) was administered to 10-week-old female WBN/Kob rats through tail vein single injection, and a diabetic condition was observed over two weeks. Alloxan was again administered to animals showing no onset of diabetes, so as to develop diabetes therein. Table 1 shows groups of rats employed; specifically, a group of rats to which *C. albicans* (6×10^7 cells/mL) was forcibly orally administered after induction of diabetes (group AC, 15 rats), a group of rats to which *C. albicans* was not forcibly orally administered after induction of diabetes (group A, 14 rats), and a group of rats to which only *C. albicans* was forcibly orally administered without administration of alloxan (group C, 10 rats). Two weeks after administration of alloxan, a test substance diluted with saline (*C. albicans*: 6×10^7 cells/mL) was forcibly orally administered to each rat of group AC (dose: 1 mL) thrice a week. Thereafter, forcible oral administration was carried out once a week for eight weeks (total: 14 times for

10 weeks). Twenty-five weeks after administration of *C. albicans*, each rat of all the groups was subjected to autopsy, and the upper gastrointestinal tract was histopathologically observed. There were employed viable cells of *C. albicans* which had been collected, through anatomy (February, 2008), from the stomach of an F344 male rat (1896) with proliferative lesion induced by alloxan, and which had been cultured and stored in Department of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University.

[0032]

[Table 1]

Groups	Development of diabetes through alloxan administration	Forcible oral administration of <i>C. albicans</i>
Group AC	Developed	Done
Group A	Developed	Not done
Group C	Not developed	Done

[0033]

Table 2 shows pathological findings of the rats of groups AC, A, and C. In group C (Fig. 1), forestomach squamous hyperplasia, *C. albicans* infection, and inflammation were not observed. In group A, four of the 14 rats (28.6%) showed mild to moderate squamous cell hyperplasia at the forestomach; seven of the 14 rats (50%) showed accumulation of neutrophils; and nine of the 14 rats (64.3%) showed chronic inflammation caused by lymphocytic and plasmacytic infiltration from the lamina propria to submucosa. In group

A, the extent of lesion was mild to moderate, and the incidence thereof was low. In contrast, in group AC, 14 of the 15 rats showed moderate to severe squamous cell hyperplasia at the forestomach; *C. albicans* infection and purulent inflammation were observed at the mucosal superficial site; and chronic inflammation caused by lymphocytic and plasmacytic infiltration was observed at the lamina propria and submucosa (Fig. 1). One of the rats of group AC developed very early squamous carcinoma (Fig. 2).

These data indicated that infection of an alloxan-induced diabetic rat with *C. albicans* induces a proliferative lesion accompanied by chronic inflammation in the forestomach of the rat at an early stage. Also, it was found that this disease model exhibits a very small individual difference and is useful for evaluation of drug efficacy.

[0034]

[Table 2]

	AC	A	C		
Effective no, of rats	15	14	10		
Squamous cell carcinoma	1 (6.7%)	0	0		
Squamous cell hyperplasia	15(100%)	4 (28.6%)	10(100%)	**	**
hyp-	0		0		
hyp±	0		3 (30,0%)		
hyp+	3 (20,0%)	1 (7.1%)	7 (70,0%)		
hyp++	8 (53,3%)	3 (21.4%)	0		
hyp+++	4 (26,7%)		0		
hyp++++	0		0		
Accumulation of neutrophils	13 (86.7%)	7 (50%)	0	**	**
-	2 (13.3%)	7 (50%)	10 (100%)		
+	1 (6.7%)	3 (21.4%)	0		
++	0	4 (28.6%)	0		
+++	12 (80%)	0	0		
Lymphocytic and plasmacytic infiltration in submucosa	13 (86.7%)	9 (64.3%)	0	*	**
-	2 (13,3%)	5 (35.7%)	10 (100%)		
+	3 (20%)	7 (50%)	0		
++	4 (26.7%)	1 (7.1%)	0		
+++	6 (40%)	1 (7.1%)	0		
C. albicans infection	13 (86,7%)	4 (28.6%)	0	**	**
-	2 (13,3%)	1 (7.1%)	10 (100%)		
+	13 (86,7%)	3 (21.4%)	0		
hyp:hyperplasia					
^b *Significantly different from AC		$P < 0.01$			
^b Significantly different from AC		$P < 0.05$			

[0035]

[Example 2]

The data obtained in Example 1 indicated that

administration of *C. albicans* to an alloxan-induced diabetic rat induces a proliferative and inflammatory lesion in the forestomach of the rat, and malignant transformation of the lesion.

It was examined whether or not the fermented product of the present invention suppresses a proliferative and inflammatory lesion in the forestomach induced by *C. albicans* infection, the fermented product being prepared through fermentation of a milk with a starter containing, as a base, a mixed starter of *L. bulgaricus* and *S. thermophilus*, which are standardized as yogurt starters by the Codex Alimentarius, and subsequent fermentation with *L. gasseri* OLL2716 added thereto.

The fermented product of the present invention was prepared as follows. Firstly, each of *L. gasseri* OLL2716 strain, *L. delbrueckii* subsp. *bulgaricus* JCM1002^T, and *S. thermophilus* ATCC19258 (1% each) was inoculated into a 10% skim milk powder medium, and culturing was carried out at 37°C for 15 hours, to thereby prepare a bulk starter, respectively. Thereafter, *L. delbrueckii* subsp. *bulgaricus* JCM1002^T starter (1%), *S. thermophilus* ATCC19258 starter (1%), and *L. gasseri* OLL2716 strain starter (5%) were inoculated into a yogurt mix (SNF: 9.5%, FAT: 3.0%) which had been heat-treated at 95°C for five minutes, followed by fermentation at 43°C for four hours. Immediately after fermentation and cooling, the numbers of viable cells of *L. gasseri* OLL2716 strain, *L. delbrueckii* subsp. *bulgaricus*

JCM1002^T, and *S. thermophilus* ATCC19258 were found to be 9.0×10^7 CFU/mL, 6.4×10^7 CFU/mL, and 11.0×10^8 CFU/mL, respectively. Hereinafter, the fermented product of the present invention may be referred to as "LG21."

[0036]

Alloxan (product of Sigma) was administered to WBN/Kob rats for induction of diabetes, and then the rats were divided into groups shown in Table 3 for testing. Specifically, the rats were divided into group DC+LG21 (i.e., a group of rats with oral administration of *C. albicans* and the fermented product of the present invention (LG21)), and group DC (i.e., a group of rats with oral administration of only *C. albicans*). For group DC, three weeks after administration of alloxan, a test substance diluted with saline (*C. albicans*: 6×10^7 cells/mL) was forcibly orally administered to each rat (dose: 1 mL) thrice a week for initial two weeks. Thereafter, forcible oral administration of *C. albicans* was carried out once a week until 27 weeks after administration of alloxan. For group DC+LG21, from 2 to 27 weeks after administration of alloxan, the fermented product of the present invention (LG21) was forcibly orally administered to each rat (dose: 5 mL/kg body weight) once a day, and three weeks after administration of alloxan, a test substance diluted with saline (*C. albicans*: 6×10^7 cells/mL) was forcibly orally administered to each rat (dose: 1 mL) thrice a week for initial two weeks. Thereafter, forcible oral administration of *C. albicans* was carried out once a

week until 27 weeks after administration of alloxan. Twenty-seven weeks after administration of alloxan, the rats (35-week-old) of both groups DC+LG21 and DC were subjected to autopsy, and the rats were compared with one another in terms of proliferative forestomach lesion, *C. albicans* infection, and inflammatory lesion.

[0037]

[Table 3]

Groups	Presence or absence of diabetes induced through alloxan administration	Forcible oral administration of <i>C. albicans</i>	Continuous administration of LG21
Group DC+LG21	Presence	Done	Done
Group DC	Presence	Done	Not done

[0038]

Table 4 shows pathological findings of the rats of groups DC+LG21 and DC. In both groups, the extent of forestomach squamous hyperplasia and inflammation was severe at the lesser curvature and mild at the greater curvature. In group DC+LG21, hyperplasia at the greater curvature was significantly mild as compared with the case of group DC (Fig. 3). Also, the positive rate of Ki-67 (i.e., a cell proliferation marker) was significantly low in group DC+LG21 (Fig. 4). Furthermore, in group DC+LG21, the extent of *C. albicans* infection and mucosal purulent inflammation was slightly mild at the greater curvature. As is clear from these data, the fermented product of the present invention significantly suppresses malignant transformation of squamous epithelial cells.

[0039]

[Table 4]

Greater Curvature

Table 4. Histopathologic findings in forestomach of diabetic rats

	LG21+DC	DC	
Effective no. of rats	10	12	
Squamous cell hyperplasia	9 (90%)	12 (100%)	
hyp-	1 (10%)	0	} *
hyp+	7 (70%)	4 (33.3%)	
hyp++	2 (20%)	6 (50.0%)	
hyp+++	0	2 (16.7%)	
hyp++++	0	0	
Accumulation of neutrophils	7 (70%)	9 (75%)	
-	3 (30 %)	3 (25.0%)	
+	5 (50 %)	3 (25.0%)	
++	2 (20 %)	4 (33.3%)	
+++	0	2 (16.7%)	
Lymphocytic and plasmacytic infiltration in submucosa	7 (70%)	9 (75%)	
-	3 (30%)	3 (25.0%)	
+	3 (30%)	5 (41.7%)	
++	3 (30%)	2 (16.7%)	
+++	1 (10%)	2 (16.7%)	
<i>C. albicans</i> infection	7 (70%)	9 (75%)	
-	3 (30%)	3 (25.0%)	
+	5 (50%)	4 (33.3%)	
++	2 (20%)	5 (41.6%)	
+++	0	0	
Ki-67 positive index (%)	37.69 ± 4.58	43.89 ± 7.40	*

hyp: hyperplasia

^bSignificantly different from LG21+DC

P < 0.05

Industrial Applicability

[0040]

According to the present invention, there can be

provided a prophylactic agent for squamous carcinoma caused by Candida infection. Also, there can be provided a model animal for squamous carcinoma caused by Candida infection, which model animal exhibits a smaller individual difference as compared with conventional model animals, and which realizes more appropriate evaluation of drug efficacy; a method for producing the model animal; and a screening method employing the model animal.

Claims

1. A prophylactic agent for squamous carcinoma caused by Candida infection, the agent comprising, as an active ingredient, a fermented product produced through fermentation of a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri*.

2. The prophylactic agent for squamous carcinoma caused by Candida infection according to claim 1, wherein the fermented product is a fermented milk produced through fermentation of a milk with *L. bulgaricus* and *S. thermophilus*, and subsequent incorporation of *L. gasseri* into the resultant product.

3. The prophylactic agent for squamous carcinoma caused by Candida infection according to claim 1 or 2, wherein the *L. gasseri* is *L. gasseri* OLL2716 strain (FERM BP-6999).

4. The prophylactic agent for squamous carcinoma caused by Candida infection according to any one of claims 1 to 3, wherein a squamous epithelium is an oral and/or esophageal squamous epithelium.

5. Use of a fermented product produced through fermentation of a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri* for producing a prophylactic agent for squamous carcinoma caused by Candida infection.

6. A fermented product produced through fermentation of a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri* for use in preventing squamous carcinoma caused by Candida

infection.

7. A method for preventing squamous carcinoma caused by Candida infection, the method comprising administering or taking a fermented product produced through fermentation of a milk with *L. bulgaricus*, *S. thermophilus*, and *L. gasseri*.

8. A method for producing a squamous carcinoma model animal, the method comprising a step of forcibly orally administering *C. albicans* to a model animal with diabetes induced through administration of a diabetogenic substance.

9. The production method according to claim 8, wherein the animal is a rat.

10. A squamous carcinoma model animal produced through a method as recited in claim 8 or 9.

11. A screening method for selecting a prophylactic agent and/or therapeutic agent for squamous carcinoma, the method comprising a step of administering a test substance to a squamous carcinoma model animal as recited in claim 10, and observing a change in a squamous carcinoma lesion site.