

DESCRIPTION

TITLE OF THE INVENTION: METHOD FOR KILLING SPORES

TECHNICAL FIELD

[0001]

The present invention relates to a method for killing spore forming bacteria having spores.

BACKGROUND ART

[0002]

Bacteria of Bacillus, Clostridium and the like form strong shell structures and form spores having extremely high resistance against heat, drugs, and the like. Certain types of bacteria capable of forming spores are known to produce a toxin when they enter the human body. For example, in health care setting, linens such as bed sheets and pillow covers are mainly heat disinfected, but heat disinfection cannot kill bacteria having spores, which are resistant against heat. For this reason, there have occurred damages in that a hospital-spread infection of bacteria capable of forming spores via linens sometimes results in deaths.

[0003]

In order to kill bacteria having spores, sterilization is performed with high pressure steam or strong chemical bactericides such as Na hypochlorite in a high concentration

in many cases. In food processing, severe heat treatment or a low-temperature distribution method has been employed as a hedge against spores.

[0004]

In WO 01/66471 is disclosed a method for controlling spores in a water-sealed system, which includes contacting a germinant with a water-sealed system for a time long enough for germinable bacteria to germinate and become a vegetative cell and under proper conditions, and sterilizing the germinated vegetative cell. JP-A-2009-118775 discloses a method of germinating bacteria having spores contained in liquid eggs or in egg preparations in which the bacteria is pressure treated at a prescribed temperature and a prescribed pressure, and a method in which bacteria is germinated by that method and then the germinated bacteria is killed.

SUMMARY OF THE INVENTION

[0005]

The present invention relates to a method for killing spores in which Step 2 defined below is performed after the completion of Step 1 defined below,

Step 1: a step of contacting spore forming bacteria with dipicolinic acid or a salt thereof under conditions with a pH of 6 or less; and

Step 2: a step of killing the spore forming bacteria.

[0006]

Furthermore, the present invention relates to a method for killing spores, the method having a period in which Step 1 defined below and Step 2A defined below are performed simultaneously,

Step 1: a step of contacting spore forming bacteria with dipicolinic acid or a salt thereof under conditions with a pH of 6 or less; and

Step 2A: a step of killing the spore forming bacteria physically.

[0007]

Furthermore, the present invention relates to a sporicidal assistant composition containing dipicolinic acid or a salt thereof and having a pH of 6 or less.

MODE FOR CARRYING OUT THE INVENTION

[0008]

The technology concretely disclosed in Patent Document 1 of treating bacteria having spores with a germination inducer such as L-alanine is not sufficient in bactericidal effect.

[0009]

Methods for killing spores with high concentrations of bactericides or by high pressure steam sterilization are not suited for disinfecting an extensive environment, such as hospitals, and are possibly damaging to instruments or toxic

to human bodies, and therefore such methods are limited in application and use environment. For example, the technology disclosed in Patent Document 2 has the disadvantage that the technology requires application of high pressure of 10 MPa or more for germination of bacteria having spores and also requires a special device. In addition, one is concerned about deterioration in quality due to application to foods or the like.

[0010]

In food processing, severe heat treatment or a low-temperature distribution method has been employed as a hedge against spores, but these would cause disadvantages such as deterioration in flavor or quality and power cost.

[0011]

The present invention relates to a method for killing spores by which bacteria having spores which would cause hazards in the medical or food field can be killed efficiently and effectively regardless of the species of the bacteria. Furthermore, the present invention relates to a sporicidal assistant composition to be used in order to kill spore forming bacteria.

[0012]

The present inventor has found that if dipicolinic acid (2,6-pyridinedicarboxylic acid) or a salt thereof is contacted with spore forming bacteria at pH 6 or less, the spore can be

killed effectively by use of a bactericide with a concentration lower than the usual concentration or by the use of a temperature lower than usual, and thus has reached the present invention.

[0013]

According to the present invention, there is provided a method for killing spores by which spore forming bacteria having spores can be killed. Furthermore, according to the present invention, there is provided a sporicidal assistant composition to be used to kill spore forming bacteria.

[0014]

Spore forming bacteria refers to any bacteria having resistance against certain heat treatment or drying due to the formation of spores in the absence of nutrition. Spores indicate a strong shell structure formed by bacteria and they are distinguished from bacteria itself. The "spore forming bacteria," which is to be subjected to spore killing of the present invention, is a common spore forming bacteria, which is present in medical sites, foods, and beverage products. Examples thereof include Bacillus bacteria, such as Bacillus cereus and Bacillus subtilis, Clostridium bacteria, such as Clostridium difficile, Amphibacillus bacteria, Sporosarcina bacteria, Geobacillus bacteria, Aeribacillus bacteria, and Alicyclobacillus bacteria. Since the "spore forming bacteria" amenable to the present invention has heat resistance because the spore forming bacteria is bacteria having spores. The heat

resistance as referred to herein means that 10^7 CFU/mL or more of bacteria can survive when bacteria having spores in an initial number of bacteria of 10^7 to 10^9 CFU/mL are heated at 80°C for 30 minutes or more as shown in Examples of the present invention.

[0015]

"Killing spores" in the present invention mainly intends to germinate bacteria having spores, thereby killing the bacteria. Germination is the process by which spore forming bacteria having spores germinates and the germination refers to the phenomenon that the bacteria is thereby grown to bacteria cell having normal proliferative or metabolic ability. Herein, killing spores includes killing bacteria having spores completely and sterilizing. In addition, killing spores also includes reducing the total number of bacteria existing in a population. Killing spores is not particularly limited and represents a state, expressed by a sporicidal effect index shown in Examples, of 0.21 or more, more preferably 0.3 or more, more preferably 0.5 or more. The sporicidal effect as referred to in the present invention indicates the difference expressed by a sporicidal effect index from control in bactericidal effect.

[0016]

The reason why the method for killing spores of the present invention has a superior sporicidal effect is considered as follows although it is not limited. In Step 1,

germination is promoted by applying dipicolinic acid (henceforth also referred to as DPA) or a salt thereof to spore forming bacteria having spores under conditions with a pH of 6 or less, so that sterilization in Step 2 is performed easily. It is presumed that dipicolinic acid under conditions with a pH of 6 or less easily enters into bacteria because of its high affinity with the bacteria and thus germination is promoted effectively.

[0017]

Dipicolinic acid or a salt thereof is expected to have a sporicidal effect or a bactericidal effect regardless of the type of the spore forming bacteria because the action of dipicolinic acid or a salt thereof in spore forming bacteria is not an action via a selected receptor specific with the species.

[0018]

Germination can be confirmed, for example, by checking the presence of darkening of spores with a phase contrast microscope. That is, a larger proportion of darkened spores means that germination has been induced.

[0019] <Step 1>

[0020]

Step 1 of the present invention is a step of bringing spore forming bacteria into contact with dipicolinic acid or a salt thereof under conditions with a pH of 6 or less. In Step 1,

the spore forming bacteria is desirably bacteria having spores. The spore forming bacteria may contain bacteria containing no spores but being capable of containing bacteria.

[0021]

Dipicolinic acid or a salt thereof is provided in a liquid or solid state. From the viewpoints of convenience and further improvement in sporicidal effect, dipicolinic acid or a salt thereof is preferably liquid. From the viewpoints of convenience, dipicolinic acid or a salt thereof is preferably provided in the form of a sporicidal assistant composition described below.

[0022]

The method of contacting dipicolinic acid or a salt thereof with spore forming bacteria in Step 1 of the present invention may be, in the case where the spore forming bacteria is present in a liquid, a method in which a high concentration solution of dipicolinic acid or a salt thereof or a powder of dipicolinic acid or a salt thereof is mixed with a liquid in which spore forming bacteria is present, and then contacting them while always stirring the mixed liquid, a method of stirring after mixing, or a method of resting after mixing. When spore forming bacteria is present on a solid, a method of applying a liquid sporicidal assistant composition described below, such as a solution of dipicolinic acid or a salt thereof, onto the solid is available. The method of applying a solution

of dipicolinic acid or a salt thereof onto a solid may be a method of spraying or a method using a tool such as a brush and sponge. In addition, a method in which a solid sporicidal assistant composition such as a powder is placed on a solid and then water or the like is sprayed is also available. "Onto a solid" is not particularly limited herein and refers to a hard surface or the like.

[0023]

Because of convenience consideration, the method of contacting dipicolinic acid or a salt thereof with spore forming bacteria is preferably a method of contacting the bacteria with a liquid composition containing dipicolinic acid or a salt thereof, more preferably with a solution of dipicolinic acid or a salt thereof, and more preferably with an aqueous solution of dipicolinic acid or a salt thereof.

[0024]

In Step 1, when contacting spore forming bacteria with dipicolinic acid or a salt thereof in a solution, the pH of the solution at 24°C is preferably, from the viewpoint of further improvement in sporicidal effect, 5 or less, more preferably 4 or less, more preferably 3 or less, more preferably 2.5 or less, more preferably 1.8 or less. From the viewpoint of safety, the pH of the solution at 24°C is preferably 1 or more, and more preferably 1.2 or more. Totally considering the aforementioned points of view, the pH of the solution at 24°C

is preferably 1 to 6, more preferably 1 to 5, more preferably 1 to 4, more preferably 1 to 3, more preferably 1 to 2.5, and more preferably 1.2 to 1.8. pH can be measured using a tabletop pH meter, Model 9611 (manufactured by HORIBA, Ltd.) at 24°C.

[0025]

pH adjustment may be performed when a solution of dipicolinic acid or a salt thereof is prepared in advance. Alternatively, pH adjustment can be performed after adding a solution of dipicolinic acid or a salt thereof to a solution containing spore forming bacteria when contacting with the spore forming bacteria. Examples of a pH adjuster include conventional acids and bases, for example, inorganic acids such as hydrochloric acid and sulfuric acid, organic acids such as lactic acids, citric acid, and salts thereof, inorganic bases such as sodium hydroxide and potassium hydroxide, and organic bases such as triisopropanolamine. As to the pH adjuster, from the viewpoint of versatility, inorganic acids such as hydrochloric acid and sulfuric acid are preferred as an acid and inorganic bases such as sodium hydroxide and potassium hydroxide are preferred as a base.

[0026]

In the case of contacting spore forming bacteria with dipicolinic acid or a salt thereof in a solution, the content of dipicolinic acid in the solution is preferably, from the viewpoint of further improvement in sporicidal effect, 0.05 mM

or more, more preferably 0.5 mM or more, more preferably 3 mM or more, more preferably 4 mM or more, more preferably 6 mM or more, and more preferably 8 mM or more, and from the viewpoints of the stability of the solution of dipicolinic acid or a salt thereof and economical efficiency, the content is preferably 30 mM or less, more preferably 25 mM or less, more preferably 20 mM or less, more preferably 15 mM or less, more preferably 12 mM or less, more preferably 10 mM or less. In the case of contacting spore forming bacteria with dipicolinic acid in a solution, totally considering the aforementioned points of view, the content of dipicolinic acid in the solution is preferably 0.05 to 30 mM, more preferably 0.5 to 30 mM, more preferably 3 to 30 mM, more preferably 3 to 25 mM, more preferably 3 to 20 mM, more preferably 3 to 15 mM, more preferably 4 to 15 mM, more preferably 6 to 12, more preferably 8 to 10 mM. The content of dipicolinic acid as referred to herein is a value in terms of dipicolinic acid when using a salt of dipicolinic acid.

[0027]

In contact, the relationship between the initial number of bacteria and the concentration of dipicolinic acid is not particularly limited. For example, from the viewpoint of further improvement in sporicidal effect, the concentration of dipicolinic acid relative to 10^8 CFU/mL, the initial number of bacteria, is preferably 0.1 mM or more, more preferably 1 mM or more, more preferably 10 mM or more, more preferably 100 mM

or more, and from the viewpoint of economical efficiency, the concentration of dipicolinic acid relative to 1 CFU/mL, the initial number of bacteria, is preferably 1 M or less, more preferably 100 mM or less, more preferably 30 mM or less, more preferably 10 mM or less, more preferably 1 mM or less.

[0028]

The contact temperature of spore forming bacteria with dipicolinic acid or a salt thereof in Step 1 before the commencement of Step 2 of the present invention is preferably, from the viewpoint of further improvement in sporicidal effect and the viewpoint of economical efficiency, 15°C or more, more preferably 20°C or more, and from the viewpoint of economical efficiency, the contact temperature is preferably less than 50°C, more preferably 49°C or less, more preferably 45°C or less, more preferably 40°C or less, more preferably 30°C or less. From the same points of view, the contact temperature of spore forming bacteria with a solution of dipicolinic acid or a salt thereof in Step 1 is preferably 15°C to less than 50°C, more preferably 15°C to 49°C, more preferably 15°C or 40°C, more preferably 15 to 30°C.

[0029]

The contact time for which spore forming bacteria is contacted with dipicolinic acid or a salt thereof at pH 6 or less in Step 1 before the commencement of Step 2 of the present invention is preferably, from the viewpoint of further

improvement in sporicidal effect, 30 seconds or more, more preferably 1 minute or more, more preferably 3 minutes or more, more preferably 5 minutes or more, more preferably 8 minutes or more, more preferably 10 minutes or more, more preferably 15 minutes or more, more preferably 20 minutes or more, more preferably 25 minutes or more, and from the viewpoint of economical efficiency, the contact time is preferably 60 minutes or less, more preferably 50 minutes or less, more preferably 40 minutes or less, more preferably 35 minutes or less. Totally considering the aforementioned points of view, the contact time for which a solution of dipicolinic acid or a salt thereof with spore forming bacteria in Step 1 before the commencement of Step 2 is preferably 30 seconds to 60 minutes, more preferably 1 minute to 60 minutes, more preferably 3 minutes to 60 minutes, more preferably 5 minutes to 60 minutes, more preferably 5 minutes to 50 minutes, more preferably 5 minutes to 40 minutes, more preferably 8 minutes to 40 minutes, more preferably 8 minutes to 35 minutes, more preferably 10 minutes to 35 minutes, more preferably 15 minutes to 35 minutes, more preferably 20 minutes to 35 minutes, more preferably 25 minutes to 35 minutes.

[0030]

The sum total of the contact time of spore forming bacteria and dipicolinic acid or a salt thereof in Step 1 of the present invention is preferably, from the viewpoint of

further improvement in sporicidal effect, 5 minutes or more, more preferably 10 minutes or more, more preferably 15 minutes or more, more preferably 20 minutes or more, more preferably 30 minutes or more, more preferably 45 minutes or more, and from the viewpoint of economical efficiency, the sum total is preferably 90 minutes or less, more preferably 80 minutes or less, more preferably 70 minutes or less.

When Step 2 is finished before Step 1, the time from the finish of Step 2 to the finish of Step 1 is not included in the sum total of the contact time.

[0031]

Although it is fundamental to perform Step 2 after Step 1, Step 2 may be performed after Step 1 is finished and Step 1 and Step 2 may be performed at the same time before Step 1 is finished. Step 1 and Step 2 may be commenced at the same time or Step 1 may be commenced after Step 2 is commenced.

[0032]

Step 1 can be finished by removing dipicolinic acid or a salt thereof. The method of removing dipicolinic acid or a salt thereof when finishing Step 1 may, in the case where the spore forming bacteria and dipicolinic acid or a salt thereof are present in a liquid, be to discard a supernatant by centrifugal separation, thereby removing dipicolinic acid or a salt thereof.

Step 1 may be finished by adjusting pH to more than 6.

As means for pH adjustment to be used when finishing Step 1, a pH adjuster can be used. Examples of the pH adjuster include those given above, and from the viewpoint of versatility, an inorganic acid or an inorganic base is preferred, and hydrochloric acid or sulfuric acid, or sodium hydroxide or potassium hydroxide is more preferred.

[0033] <Step 2>

Step 2 of the present invention is a step of killing spore forming bacteria. The spore forming bacteria in Step 2 refers to spore forming bacteria being in a state where its germination has been promoted via the treatment of Step 1. Depending on the conditions described below, bacteria being in an ungerminated state are also included.

[0034]

Means for killing bacteria include means of physically killing bacteria and means of chemically killing bacteria.

In Step 2 mentioned above, the means of physically killing bacteria and the means of chemically killing bacteria may be used alone, or two or more types of physical means and two or more types of chemical means may be combined. Two or more types of physical means and chemical means may be used in combination.

[0035] [Physical Sterilization]

<Step 2A>

Step 2A of the present invention is a step of physically killing spore forming bacteria, for example, spore forming

bacteria promoted to germinate.

[0036]

Examples of the means of physically killing bacteria include heat, pressure, and electromagnetic waves. Examples of such electromagnetic waves include radiation such as X-rays and γ -rays, and ultraviolet rays. From the viewpoints of economical efficiency and convenience, the means for physically killing bacteria is preferably heat.

Step 2A can be commenced by applying physical means to spore forming bacteria having been subjected to Step 1. Step 2A can be quitted by stopping the physical means.

[0037]

From the viewpoints of further improvement in sporicidal effect and economical efficiency, Step 2A of the present invention is preferably a step of killing spore forming bacteria by heat, and more preferably a step of killing spore forming bacteria at a temperature of from 50°C to 250°C.

[0038]

The temperature applied when killing spore forming bacteria by heat is preferably, from the viewpoint of further improvement in sporicidal effect, 50°C or more, more preferably 55°C or more, more preferably 60°C or more, more preferably 65°C or more, more preferably 70°C or more, and more preferably 75°C or more, and from the viewpoints of further improvement in sporicidal effect, economical efficiency, and versatility, the

temperature is preferably 250°C or less, more preferably 200°C or less, more preferably 150°C or less, more preferably 120°C or less, more preferably 100°C or less, more preferably 90°C or less, and more preferably 85°C or less.

[0039]

From the aforementioned points of view, the temperature applied when killing bacteria by heat in Step 2 of the present invention is preferably 50°C to 250°C, more preferably 50°C to 200°C, more preferably 50°C to 150°C, more preferably 50°C to 100°C, more preferably 55°C to 100°C, more preferably 60°C to 100°C, more preferably 65°C to 90°C, more preferably 70°C to 90°C, and more preferably 75°C to 85°C.

[0040]

Usually, it is difficult to kill spore forming bacteria at a temperature of 100°C or less if the bacteria has not germinated. In the present invention, however, a sporicidal effect is exhibited even at a temperature of 100°C or less in Step 2 because germination is promoted by Step 1. It is possible to kill the bacteria at temperatures higher than 100°C also in the present invention, and according to circumstances, it is also possible to kill the bacteria at temperatures higher than 100°C.

[0041]

The time of killing bacteria by heat in Step 2A of the present invention is preferably, from the viewpoint of further

improvement in sporicidal effect, 3 minutes or more, more preferably 5 minutes or more, more preferably 8 minutes or more, more preferably 10 minutes or more, more preferably 20 minutes or more, and more preferably 25 minutes or more, and from the viewpoints of economical efficiency and versatility, the time is preferably 90 minutes or less, more preferably 70 minutes or less, more preferably 50 minutes or less, more preferably 40 minutes or less, and more preferably 35 minutes or less.

[0042]

From the aforementioned points of view, the time of killing bacteria by heat in Step 2A of the present invention is preferably 3 minutes to 90 minutes, more preferably 5 minutes to 70 minutes, more preferably 8 minutes to 50 minutes, more preferably 10 minutes to 50 minutes, more preferably 20 to 40 minutes, and more preferably 25 to 35 minutes.

[0043]

Examples of the method of killing bacteria by heat of Step 2A of the present invention include methods of applying dry heat, wet heat, boiling heat, hot water, steam heat, or the like to spore forming bacteria: e.g., a method of immersing spore forming bacteria in hot water, a method of placing a solid such as a hard surface on which spore forming bacteria is present, a liquid containing spore forming bacteria, or the like in a thermostatic bath, and a method involving heating treatment such as heat cooking for food processing.

[0044]

The pH condition for killing bacteria by heat in Step 2A is not particularly limited, and the sterilization can be performed under any conditions such as pH 1 to 9. Like Step 1, conditions with pH 6 or lower are also available.

[0045]

In the method for killing spores of the present invention, Step 2A can be finished by rapidly cooling the spore forming bacteria killed by heat in Step 2A, with water containing ice, a cooled container, or the like. Alternatively, Step 2 can be finished by removing heat naturally by leaving for a while the spore forming bacteria killed by heat in Step 2A. The spore forming bacteria killed can be in a state where the spore forming bacteria is present in a liquid.

[0046]

When spore forming bacteria is present in a liquid in Step 1 or Step 2A, the method for killing spores of the present invention may have a step of stirring the spore forming bacteria in Step 1 or Step 2A or before or after these steps. Step 1 and Step 2 may be repeated.

[0047] [Chemical Sterilization]

<Step 2B>

Step 2B of the present invention is a step of chemically killing spore forming bacteria.

[0048]

From the viewpoint of economical efficiency, the step of chemically killing bacteria is preferably use of a bactericide.

[0049]

As to the bactericide to be used in the method of killing bacteria with a bactericide in Step 2 of the present invention, examples of a bactericide to be used in the form of a liquid include bactericides containing halides such as chlorine-based antiseptics, such as aqueous solutions of hypochlorous acid or a salt thereof, chlorous acid or a salt thereof, dichloroisocyanuric acid or a salt thereof, trichloroisocyanuric acid or a salt thereof, chlorine dioxide, and the like, and iodine-based antiseptics, such as povidone iodine and iodoform; alcohols such as ethanol, 1-propanol, isopropyl alcohol, and methanol; aldehydes such as formaldehyde and glutaraldehyde; phenols such as lime acid, cresol, xlenol, isopropylmethylphenol, and triclosan; and peroxides, such as peracetic acid, hydrogen peroxide, and ozone. Examples of the bactericide to be used in a gaseous state include ethylene oxide, formaldehyde, hydrogen peroxide, ozone, and chlorine dioxide.

[0050]

In the present invention, since germination of spore forming bacteria having spores is promoted by Step 1, the spore forming bacterial treated in Step 1 can be killed with a bactericide used in an amount smaller than usual. For example, when killing spore forming bacteria having spores with a

chlorine-based antiseptic, preferably with a chlorine-based antiseptic selected from hypochlorous acid and salts thereof, chlorous acid and salts thereof, dichloroisocyanuric acid and salts thereof, trichloroisocyanuric acid and salts thereof, and chlorine dioxide, more preferably with sodium hypochlorite, an effective chlorine concentration of 1000 ppm or more is usually required if Step 1 of the present invention is not performed. In contrast, when performing Step 1 of the present invention, the effective chlorine concentration necessary for sterilization may be lower than 1000 ppm. Specifically, in the present invention, the effective chlorine concentration is preferably, from the viewpoint of further improvement in sporicidal effect, 10 ppm or more, more preferably 50 ppm or more, and more preferably 80 ppm or more, and from the viewpoint of economical efficiency, the concentration is preferably 1000 ppm or less, more preferably 800 ppm or less, more preferably 500 ppm or less, more preferably 300 ppm or less, and more preferably 150 ppm or less. The temperature at which spore forming bacteria is killed by chemical sterilization means, including a chlorine-based antiseptic such as sodium hypochlorite is preferably, from the viewpoint of further improvement in sporicidal effect, 5°C or more, more preferably 10°C or more, more preferably 15°C or more, more preferably 20°C or more, and more preferably 23°C or more, and from the viewpoint of economical efficiency, the temperature is preferably 50°C

or less, more preferably 40°C or less, more preferably 30°C or less, and more preferably 25°C or less.

[0051]

Also in the present invention, sterilization can be performed with a usual dosage of a bactericide. For example, sterilization can be performed with a chlorine-based antiseptic, preferably a chlorine-based antiseptic selected from hypochlorous acid and salts thereof, chlorous acid and salts thereof, dichloroisocyanuric acid and salts thereof, trichloroisocyanuric acid and salts thereof, and chlorine dioxide, more preferably sodium hypochlorite, having an effective chlorine concentration of 1000 ppm or more.

[0052]

Step 2 can be performed after finishing Step 1 (Embodiment 1).

When Step 2 is Step 2A of killing spore forming bacteria physically, there may be a period in which Step 1 and Step 2A are performed simultaneously (Embodiment 2). Embodiment 2 is divided as follows.

Embodiment 2-1: Step 1 is started first.

Embodiment 2-2: Step 1 and Step 2A are started at the same time.

Embodiment 2-3: Step 2A is started first.

Of Embodiments 2, Embodiment 2-1 is preferred from the viewpoint of further improvement in sporicidal effect.

Step 2A of Embodiment 2 is preferably a step of killing bacteria by heat.

[0053] [Embodiment 1: an embodiment in which Step 2 is performed after the finish of Step 1]

In the present invention, Step 2 can be performed after the finish of Step 1. Here, Step 1 can be finished with the completed promotion of the germination of spore forming bacteria. Even if Step 2 is either of Step 2A (physical sterilization) and Step 2B (chemical sterilization), preferred conditions for Step 1 and Step 2 of this embodiment 1 are as previously described. Step 1 can be finished by bringing the pH to greater than 6 or by removing dipicolinic acid. The embodiment thereof is as previously described.

Here, "to perform Step 2 after the finish of Step 1" means to perform Step 2 at the finish of Step 1, and means to transfer to Step 2 preferably within 1 hour, more preferably within 30 minutes, more preferably within 15 minutes, more preferably within 5 minutes, more preferably within 1 minute, of the finish of Step 1 at the latest.

[0054] [Embodiment 2: an embodiment having a period in which Step 1 and Step 2A are performed simultaneously]

In the present invention, there can be a period in which Step 1 and Step 2A are performed simultaneously.

The conditions of time and temperature where Step 1 and Step 2A overlap each other during the period in which Step 1

and Step 2A are performed simultaneously are the same as the conditions of Step 2A previously described when Step 2A is a step of killing bacteria by heat. When Step 2A is a step of performing physical sterilization other than heating, the condition of temperature at the time of performing Step 1 and Step 2A simultaneously is the same as the condition of Step 1 previously described.

Conditions other than the time and the temperature during the period in which Step 1 and Step 2A are performed simultaneously, the conditions of, for example, dipicolinic acid content and pH are the same as the conditions of Step 1 previously described.

Preferable conditions at the time of performing only Step 1 other than the above are as previously described.

[0055] [Embodiment 2-1: an embodiment where Step 1 is started first]

In the method for killing spores of the present invention having a period in which Step 1 and Step 2 are performed simultaneously, Step 1 can be started first. That is, the sterilization of Step 2A can be started by applying physical means at the middle of Step 1. For example, when Step 2A is a step of killing spore forming bacteria by heat and in the case where the step of contacting dipicolinic acid or a salt thereof with the spore forming bacteria in Step 1 is performed in a solution, the solution can be subjected to Step 2A as it is and

the temperature of the solution can be transferred to the temperature zone of Step 2A.

[0056]

As to the finish of the individual steps, any of the following is available: to finish Step 1 first, to finish Step 1 and Step 2A at the same time, and to finish Step 2A first. The methods of finishing Step 1 and Step 2A are as previously described. When Step 2A is finished earlier than Step 1, the time of Step 1 from the finish of Step 2A to the finish of Step 1 is not included in the "sum total of the contact time" as mentioned in the present specification.

[0057]

In Embodiment 2-1, the time for which spore forming bacteria is contacted with dipicolinic acid or a salt thereof in Step 1 before starting Step 2A is preferably, from the viewpoint of further improvement in sporicidal effect, 30 seconds or more, more preferably 1 minute or more, more preferably 3 minutes or more, more preferably 5 minutes or more, more preferably 8 minutes or more, more preferably 10 minutes or more, more preferably 15 minutes or more, more preferably 20 minutes or more, and more preferably 25 minutes or more, and from the viewpoint of economical efficiency, the contact time is preferably 60 minutes or less, more preferably 50 minutes or less, more preferably 40 minutes or less, more preferably 35 minutes or less, more preferably 30 minutes or less, more

preferably 25 minutes or less, more preferably 20 minutes or less, more preferably 15 minutes or less, more preferably 10 minutes or less. From the viewpoint of further improvement in sporicidal effect, the contact time is preferably 30 seconds to 60 minutes, more preferably 1 minute to 60 minutes, more preferably 3 minutes to 60 minutes, more preferably 5 minutes to 60 minutes, more preferably 5 minutes to 50 minutes, more preferably 5 minutes to 40 minutes, more preferably 8 minutes to 40 minutes, more preferably 10 minutes to 40 minutes, more preferably 15 minutes to 40 minutes, more preferably 20 minutes to 40 minutes, more preferably 25 minutes to 40 minutes. From the viewpoint of further improvement in sporicidal effect and the viewpoint of economical efficiency, the contact time is preferably 25 minutes to 35 minutes.

[0058]

In Embodiment 2-1, the sum total of the contact time of spore forming bacteria and dipicolinic acid or a salt thereof in Step 1 is preferably, from the viewpoint of further improvement in sporicidal effect, 30 minutes or more, more preferably 35 minutes or more, more preferably 40 minutes or more, more preferably 45 minutes or more, more preferably 50 minutes or more, more preferably 55 minutes or more, more preferably 60 minutes or more, and from the viewpoint of economical efficiency, the sum total is preferably 90 minutes or less, more preferably 80 minutes or less, more preferably

70 minutes or less, more preferably 65 minutes or less.

When Step 2A is finished earlier than Step 1, the time from the finish of Step 2A to the finish of Step 1 is not included in the sum total of the contact time.

[0059]

Conditions of temperature, pH, etc. used during the period in which Step 1 or Step 2 is performed alone are the same as the conditions in the embodiment where Step 2 is performed after the finish of Step 1.

[0060] [Embodiment 2-2: Step 1 and Step 2A are started at the same time]

In the method for killing spores of the present invention having a period in which Step 1 and Step 2A are performed simultaneously, Step 1 and Step 2A can be started at the same time. That is, the contact of spore forming bacteria with dipicolinic acid or a salt thereof at a pH of 6 or less can be started concurrently with the application of physical means. When using heat as physical means, the temperature used in the case of performing Step 1 and Step 2A simultaneously is the same as the temperature of the step of killing bacteria by heat in Step 2A. That is, dipicolinic acid or a salt thereof is contacted with spore forming bacteria, from the beginning, within a temperature range of the step of killing bacteria by heat of Step 2A, under the condition of a pH of 6 or less.

[0061]

As to the finish of the individual steps, any of the following is available: to finish Step 1 first, to finish Step 1 and Step 2A at the same time, and to finish Step 2A first. The methods of finishing Step 1 and Step 2A are as previously described. When Step 2A is finished earlier than Step 1, the time of Step 1 from the finish of Step 2A to the finish of Step 1 is not included in the "sum total of the contact time" as mentioned in the present specification.

[0062]

Conditions of temperature, pH, etc. used during the period in which Step 1 or Step 2A is performed alone are the same as the conditions in the embodiment where Step 2 is performed after the finish of Step 1.

[0063] [Embodiment 2-3: Step 2A is started first]

In the method for killing spores of the present invention having a period in which Step 1 and Step 2A are performed simultaneously, Step 2A can be started first. That is, physical means of Step 2A is applied to spore forming bacteria first, and then the contact of the spore forming bacteria with dipicolinic acid or a salt thereof is started while applying the physical means. When using heat as the physical means, the temperature of the spore forming bacteria is brought into the temperature zone of Step 2A beforehand, and dipicolinic acid or a salt thereof is contacted with the spore forming bacteria at a pH of 6 or less while keeping the temperature.

[0064]

As to the finish of the individual steps, any of the following is available: to finish Step 1 first, to finish Step 1 and Step 2A at the same time, and to finish Step 2A first. The methods of finishing Step 1 and Step 2A are as previously described. When Step 2A is finished earlier than Step 1, the time of Step 1 from the finish of Step 2A to the finish of Step 1 is not included in the "sum total of the contact time" as mentioned in the present specification.

[0065]

Conditions of temperature, pH, etc. used during the period in which Step 1 or Step 2A is performed alone are the same as the conditions in the embodiment where Step 2 is performed after the finish of Step 1.

[0066] <Sporicidal Assistant Composition>

The sporicidal assistant composition of the present invention is a composition including dipicolinic acid or a salt thereof. The sporicidal assistant composition of the present invention is used for treatment performed for the purpose of germination. The sporicidal assistant composition is distinguished from bactericide compositions, which is used at normal temperature (20°C to about 38°C) for the purpose of killing spores. In particular, there is referred to a composition capable of exerting a sporicidal effect or a bactericidal effect by killing bacteria by physical or chemical

means after Step 1 of the present invention or currently with Step 1. In the present invention, the sporicidal assistant composition can be used in Step 1.

[0067]

The sporicidal assistant composition may be a liquid composition or a solid composition.

[0068]

The liquid sporicidal assistant composition includes dipicolinic acid or a salt thereof and a solvent.

[0069]

When the sporicidal assistant composition is a liquid, the solvent contained may be water or a hydrophilic solvent. Examples of the hydrophilic solvent include alcohols such as ethanol, methanol, and isopropanol, polyhydric alcohols, such as glycerol, ethylene glycol, and propylene glycol, carbitols, such as methylcarbitol and ethylcarbitol. From the viewpoint of further improvement in sporicidal effect and the viewpoint of economical efficiency, the solvent is preferably water or a mixture of water and a hydrophilic solvent, more preferably water.

[0070]

From the viewpoint of convenience, the liquid sporicidal assistant composition is preferably a solution of dipicolinic acid or a salt thereof, more preferably an aqueous solution of dipicolinic acid or a salt thereof.

[0071]

The liquid sporicidal assistant composition can further include a pH adjuster. Examples of the pH adjuster contained in the liquid sporicidal assistant composition include conventional acids and bases, for example, inorganic acids such as hydrochloric acid and sulfuric acid, organic acids such as lactic acids, citric acid, and salts thereof, inorganic bases such as sodium hydroxide and potassium hydroxide, and organic bases such as triisopropanolamine. As to the pH adjuster, from the viewpoint of versatility, inorganic acids such as hydrochloric acid and sulfuric acid are preferred as an acid and inorganic bases such as sodium hydroxide and potassium hydroxide are preferred as a base.

[0072]

From the viewpoint of safety, the pH at 24°C of a liquid sporicidal assistant composition, preferably a solution of dipicolinic acid or a salt thereof, more preferably an aqueous solution of dipicolinic acid or a salt thereof, is preferably 1 or more, more preferably 1.2 or more, and from the viewpoint of further improvement in sporicidal effect in the case of contacting spore forming bacteria with a solution of dipicolinic acid or a salt thereof, the pH is preferably 6 or less, more preferably 5 or less, more preferably 4 or less, more preferably 3 or less, more preferably 2.5 or less, more preferably 1.8 or less. Totally considering the

aforementioned points of view, the pH of the solution of dipicolinic acid or a salt thereof at 24°C is preferably 1 to 6, more preferably 1 to 5, more preferably 1 to 4, more preferably 1 to 3, more preferably 1 to 2.5, more preferably 1.2 to 1.8.

[0073]

The content of dipicolinic acid in a liquid sporicidal assistant composition, preferably in a solution of dipicolinic acid or a salt thereof, more preferably in an aqueous solution of dipicolinic acid or a salt thereof is preferably, from the viewpoint of further improvement in sporicidal effect, 0.05 mM or more, more preferably 0.5 mM or more, more preferably 3 mM or more, more preferably 5 mM or more, more preferably 8 mM or more, and from the viewpoints of the stability of a solution of dipicolinic acid or a salt thereof and economical efficiency, the content is preferably 30 mM or less, more preferably 25 mM or less, more preferably 20 mM or less, more preferably 15 mM or less, more preferably 12 mM or less. Totally considering the aforementioned points of view, the content of dipicolinic acid in the solution of dipicolinic acid or a salt thereof is preferably 0.05 to 30 mM, more preferably 0.5 to 30 mM, more preferably 3 to 30 mM, more preferably 3 to 25 mM, more preferably 3 to 20 mM, more preferably 3 to 15 mM, more preferably 5 to 15 mM, more preferably 8 to 12.

[0074]

The sporicidal assistant composition of the present

invention can be a composition including dipicolinic acid or a salt thereof and a pH adjuster, water, or another solvent and not containing any one of peracetic acid, sulfoperoxy-carboxylic acid and dicarboxylic acid diester. "Not containing" as referred to herein means that the sporicidal assistant composition does not have sporicidal effects, and preferably indicates 1000 ppm or less, more preferably 100 ppm or less, more preferably 10 ppm or less, more preferably 1 ppm or less, more preferably substantially 0 ppm. "Substantially" means being at or lower than the detection limit.

[0075]

The liquid sporicidal assistant composition of the present invention is preferably a solution of dipicolinic acid or a salt thereof, more preferably an aqueous solution of dipicolinic acid or a salt thereof. The sporicidal assistant composition of this case can be prepared by mixing, preferably dissolving, powdery dipicolinic acid or a salt thereof in water or another solvent.

[0076]

Examples of the solid sporicidal assistant composition include a composition containing dipicolinic acid or a salt thereof itself, or dipicolinic acid or a salt thereof and a firming agent.

[0077]

The firming agent which the solid sporicidal assistant

composition contains may be, but is not limited to, polyethylene glycol having a number average molecular weight of 1,000 to 100,000; a wax, such as carnauba wax, candelilla wax, jojoba oil, cera flava, and lanolin; a hydrocarbon having 15 or more carbon atoms, such as paraffin, vaseline, ceresin, and microcrystalline wax; a higher fatty acid having 12 to 22 carbon atoms, such as lauric acid, myristic acid, and stearic acid; or a higher alcohol having 14 to 22 carbon atoms, such as cetyl alcohol and stearyl alcohol.

[0078]

The solid sporicidal assistant composition can further include a pH adjuster. Examples of the pH adjuster contained in the solid sporicidal assistant composition include conventional acids and bases, for example, inorganic acids such as hydrochloric acid and sulfuric acid, organic acids such as lactic acids, citric acid, and salts thereof, inorganic bases such as sodium hydroxide and potassium hydroxide, and organic bases such as triisopropanolamine. As to the pH adjuster, from the viewpoint of versatility, inorganic acids such as hydrochloric acid and sulfuric acid are preferred as an acid and inorganic bases such as sodium hydroxide and potassium hydroxide are preferred as a base.

[0079]

The content of the dipicolinic acid in the solid sporicidal assistant composition is preferably, from the

viewpoint of further improvement in sporicidal effect, 0.1% by mass or more, more preferably 1% by mass or more, more preferably 5% by mass or more, more preferably 10% by mass or more, more preferably 20% by mass or more, more preferably 30% by mass or more, more preferably 40% by mass or more, more preferably 50% by mass or more, and from the viewpoint of economical efficiency, the content is preferably 100% by mass or less, more preferably 90% by mass or less, more preferably 80% by mass or less, more preferably 70% by mass or less, more preferably 60% by mass or less, more preferably 50% by mass or less.

[0080]

The content of the firming agent in the solid sporicidal assistant composition is preferably, from the viewpoint of convenience, 0% by mass or more, more preferably 10% by mass or more, more preferably 20% by mass or more, more preferably 30% by mass or more, more preferably 40% by mass or more, more preferably 50% by mass or more, and from the viewpoint of further improvement in sporicidal effect and from the viewpoint of economical efficiency, the content is preferably 99.9% by mass or less, more preferably 99% by mass or less, more preferably 95% by mass or less, more preferably 90% by mass or less, more preferably 80% by mass or less, more preferably 70% by mass or less, more preferably 60% by mass or less, more preferably 50% by mass or less.

[0081]

In use in Step 1 of the method for killing spores of the present invention, the sporicidal assistant composition of the present invention is preferably used such that the pH at 24°C of the solution containing the spore forming bacteria and the sporicidal assistant composition is preferably, from the viewpoint of safety, 1 or more, more preferably 1.2 or more, and from the viewpoint of further improvement in sporicidal effect, the pH is preferably 5 or less, more preferably 4 or less, more preferably 3 or less, more preferably 2.5 or less, more preferably 1.8 or less. Totally considering the aforementioned points of view, in use in Step 1 of the method for killing spores of the present invention, the sporicidal assistant composition of the present invention is preferably used such that the pH at 24°C of the solution containing the spore forming bacteria and the sporicidal assistant composition is preferably 1 to 6, more preferably 1 to 5, more preferably 1 to 4, more preferably 1 to 3, more preferably 1 to 2.5, more preferably 1.2 to 1.8.

[0082]

In use in Step 1 of the method for killing spores of the present invention, the sporicidal assistant composition of the present invention is preferably used such that the content of dipicolinic acid in the solution containing the spore forming bacteria and the sporicidal assistant composition is preferably, from the viewpoint of further improvement in sporicidal effect,

0.05 mM or more, more preferably 0.5 mM or more, more preferably 3 mM or more, more preferably 4 mM or more, more preferably 6 mM or more, and more preferably 8 mM or more, and from the viewpoints of the stability of a solution of dipicolinic acid or a salt thereof and economical efficiency, the content is preferably 30 mM or less, more preferably 25 mM or less, more preferably 20 mM or less, more preferably 15 mM or less, more preferably 12 mM or less, more preferably 10 mM or less. Totally considering the aforementioned points of view, the sporicidal assistant composition of the present invention is preferably used such that the content of dipicolinic acid in the solution containing the spore forming bacteria and the sporicidal assistant composition is preferably 0.05 to 30 mM, more preferably 0.5 to 30 mM, more preferably 3 to 30 mM, more preferably 3 to 25 mM, more preferably 3 to 20 mM, more preferably 3 to 15 mM, more preferably 4 to 15 mM, more preferably 6 to 12, and more preferably 8 to 10 mM, in use in Step 1 of the method for killing spores of the present invention.

[0083]

As the dipicolinic acid or a salt thereof of the present invention, either a synthesized product or a product prepared by use of a fermentation method using a method of extracting the dipicolinic acid or a salt thereof from a microorganism can be employed.

[0084]

Regarding the embodiments described above, the present invention discloses the sporicidal method and the sporicidal assistant composition described below.

[0085] <Item 1>

A method for killing spores in which Step 2 defined below is performed after the completion of Step 1 defined below,

Step 1: a step of contacting spore forming bacteria with dipicolinic acid or a salt thereof under conditions with a pH of 6 or less; and

Step 2: a step of killing the spore forming bacteria.

[0086] <Item 2>

The method for killing spores set forth in <Item 1>, wherein the Step 2 is Step 2A defined below,

Step 2A: a step of killing the spore forming bacteria physically.

[0087] <Item 3>

The method for killing spores set forth in <Item 2>, wherein the Step 2A is a step of killing spore forming bacteria by heat, pressure, or electromagnetic waves.

[0088] <Item 4>

The method for killing spores set forth in <Item 2> or <Item 3>, wherein the spore forming bacteria is killed at a temperature of 50°C to 250°C in the Step 2A.

[0089] <Item 5>

The method for killing spores set forth in any one of <Item

2> to <Item 4>, wherein the spore forming bacteria is killed in the Step 2A preferably at a temperature of 50°C or more, more preferably 55°C or more, more preferably 60°C or more, more preferably 65°C or more, more preferably 70°C or more, more preferably 75°C or more, and preferably 250°C or less, more preferably 200°C or less, more preferably 150°C or less, more preferably 120°C or less, more preferably 100°C or less, more preferably 90°C or less, more preferably 85°C or less, and is killed preferably at a temperature of 50°C to 250°C, more preferably 50°C to 200°C, more preferably 50°C to 150°C, more preferably 50°C to 100°C, more preferably 55°C to 100°C, more preferably 60°C to 100°C, more preferably 65°C to 90°C, more preferably 70°C to 90°C, more preferably 75°C to 85°C.

[0090] <Item 6>

The method for killing spores set forth in any one of <Item 2> to <Item 5>, wherein the time of killing the spore forming bacteria in the Step 2A is preferably 3 minutes or more, more preferably 5 minutes or more, more preferably 8 minutes or more, more preferably 10 minutes or more, preferably 20 minutes or more, more preferably 25 minutes or more, and preferably 90 minutes or less, more preferably 70 minutes or less, more preferably 50 minutes or less, more preferably 40 minutes or less, more preferably 35 minutes or less.

[0091] <Item 7>

The method for killing spores set forth in any one of <Item

1> to <Item 6>, wherein the Step 2 is Step 2B defined below,

Step 2B: a step of killing the spore forming bacteria chemically.

[0092] <Item 8>

The method for killing spores set forth in <Item 7>, wherein the Step 2B is a step of killing the spore forming bacteria with a bactericide.

[0093] <Item 9>

The method for killing spores set forth in <Item 8>, wherein the bactericide is used in the form of a liquefied material.

[0094] <Item 10>

The method for killing spores set forth in any one of <Item 8> to <Item 9>, wherein the bactericide is one or more species selected from halides, alcohols, aldehydes, phenols, and peroxides, or one or more species selected from hypochlorous acid or a salt thereof, chlorous acid and salts thereof, dichloroisocyanuric acid and salts thereof, trichloroisocyanuric acid and salts thereof, chlorine dioxide, povidone iodine, iodoform, ethanol, 1-propanol, isopropyl alcohol, methanol, formaldehyde, glutaraldehyde, lime acid, cresol, xylenol, isopropylmethylphenol, triclosan, peracetic acid, hydrogen peroxide, and ozone.

[0095] <Item 11>

The method for killing spores set forth in any one of <Item

8> to <Item 10>, wherein the bactericide is an aqueous solution of one or more species selected from hypochlorous acid or a salt thereof, chlorous acid and salts thereof, dichloroisocyanuric acid and salts thereof, trichloroisocyanuric acid and salts thereof, and chlorine dioxide.

[0096] <Item 12>

The method for killing spores set forth in any one of <Item 8> to <Item 11>, wherein the effective chlorine concentration of the bactericide is preferably 10 ppm or more, more preferably 50 ppm or more, more preferably 80 ppm or more, and preferably 1000 ppm or less, more preferably 800 ppm or less, more preferably 500 ppm or less, more preferably 300 ppm or less, more preferably 150 ppm or less.

[0097] <Item 13>

The method for killing spores set forth in any one of <Item 8> to <Item 12>, wherein the bactericide is in a gaseous form and is one or more species selected from ethylene oxide, formaldehyde, hydrogen peroxide, ozone, and a chlorine dioxide.

[0098] <Item 14>

The method for killing spores set forth in any one of <Item 8> to <Item 13>, wherein the pH at 24°C at the time of the step of contacting in the Step 1 is preferably 1 or more, more preferably 1.2 or more, and preferably 5 or less, more preferably 4 or less, more preferably 3 or less, more preferably 2.5 or less, more preferably 1.8 or less, and preferably 1 to

6, more preferably 1 to 5, more preferably 1 to 4, more preferably 1 to 3, more preferably 1 to 2.5, more preferably 1.2 to 1.8.

[0099] <Item 15>

The method for killing spores set forth in any one of <Item 1> to <Item 14>, wherein the spore forming bacteria is contacted with dipicolinic acid or a salt thereof in a liquid in the Step 1.

[0100] <Item 16>

The method for killing spores set forth in any one of <Item 1> to <Item 15>, wherein the content of dipicolinic acid in the liquid is 0.05 mM to 30 mM.

[0101] <Item 17>

The method for killing spores set forth in any one of <Item 1> to <Item 16>, wherein the content of dipicolinic acid in the liquid is preferably 0.05 mM or more, more preferably 0.5 mM or more, more preferably 3 mM or more, more preferably 4 mM or more, more preferably 6 mM or more, more preferably 8 mM or more, and preferably 30 mM or less, more preferably 25 mM or less, more preferably 20 mM or less, more preferably 15 mM or less, more preferably 12 mM, more preferably 10 mM or less, and preferably 0.05 to 30 mM, more preferably 0.5 to 30 mM, more preferably 3 to 30 mM, more preferably 3 to 25 mM, more preferably 3 to 20 mM, more preferably 3 to 15 mM, more preferably 4 to 15 mM, more preferably 6 to 12 mM, more preferably 8 to 10 mM.

[0102] <Item 18>

The method for killing spores set forth in any one of <Item 1> to <Item 17>, wherein the Step 1 is performed at a temperature of 15°C or more and less than 50°C.

[0103] <Item 19>

The method for killing spores set forth in any one of <Item 1> to <Item 18>, wherein the Step 1 is performed preferably at a temperature of 15°C or more, more preferably 20°C or more, and preferably less than 50°C, more preferably 49°C or less, more preferably 45°C or less, more preferably 40°C or less, more preferably 30°C or less, and is performed preferably at a temperature of 15°C or more and less than 50°C, more preferably 15°C to 49°C, more preferably 15°C to 40°C, more preferably 15°C to 30°C.

[0104] <Item 20>

The method for killing spores set forth in any one of <Item 1> to <Item 19>, wherein the Step 1 is performed for a time of 5 minutes to 60 minutes.

[0105] <Item 21>

The method for killing spores set forth in any one of <Item 1> to <Item 20>, wherein the Step 1 is performed preferably for a time of 30 seconds or more, more preferably 1 minute or more, more preferably 3 minutes or more, more preferably 5 minutes or more, more preferably 8 minutes or more, more preferably 10 minutes or more, more preferably 15 minute or more, more preferably 20 minute or more, more preferably 25 minutes or more,

and 60 minutes or less, more preferably 50 minutes or less, more preferably 40 minutes or less, more preferably 35 minutes or less, and is performed preferably for a time of 30 seconds to 60 minutes, more preferably 1 minute to 60 minutes, more preferably 3 minutes to 60 minutes, more preferably 5 minutes to 60 minutes, more preferably 5 minutes to 50 minutes, more preferably 5 minutes to 40 minutes, more preferably 8 minutes to 40 minutes, more preferably 8 minutes to 35 minutes, more preferably 10 minutes to 35 minutes, more preferably 15 minutes to 35 minutes, more preferably 20 minutes to 35 minutes, more preferably 25 minutes to 35 minutes.

[0106] <Item 22>

A method for killing spores, the method having a period in which Step 1 defined below and Step 2A defined below are performed simultaneously,

Step 1: a step of contacting spore forming bacteria with dipicolinic acid or a salt thereof under conditions with a pH of 6 or less; and

Step 2A: a step of killing the spore forming bacteria physically.

[0107] <Item 23>

The method for killing spores set forth in <Item 22>, wherein the Step 2A is a step of killing spore forming bacteria by heat, pressure, or electromagnetic waves.

[0108] <Item 24>

The method for killing spores set forth in <Item 22> or <Item 23>, wherein the spore forming bacteria is killed at a temperature of 50°C to 250°C in the Step 2A.

[0109] <Item 25>

The method for killing spores set forth in any one of <Item 22> to <Item 24>, wherein the spore forming bacteria is killed in the Step 2A preferably at a temperature of 50°C or more, more preferably 55°C or more, more preferably 60°C or more, more preferably 65°C or more, more preferably 70°C or more, more preferably 75°C or more, and preferably 250°C or less, more preferably 200°C or less, more preferably 150°C or less, more preferably 120°C or less, more preferably 100°C or less, more preferably 90°C or less, more preferably 85°C or less, and is killed preferably at a temperature of 50°C to 250°C, more preferably 50°C to 200°C, more preferably 50°C to 150°C, more preferably 50°C to 100°C, more preferably 55°C to 100°C, more preferably 60°C to 100°C, more preferably 65°C to 90°C, more preferably 70°C to 90°C, more preferably 75°C to 85°C.

[0110] <Item 26>

The method for killing spores set forth in any one of <Item 22> to <Item 25>, wherein the time of killing the spore forming bacteria at a temperature of 50°C to 250°C in the Step 2A is 3 minutes to 90 minutes.

[0111] <Item 27>

The method for killing spores set forth in any one of <Item

22> to <Item 26>, wherein the time of killing the spore forming bacteria in the Step 2A is preferably 3 minutes or more, more preferably 5 minutes or more, more preferably 8 minutes or more, more preferably 10 minutes or more, preferably 20 minutes or more, more preferably 25 minutes or more, and preferably 90 minutes or less, more preferably 70 minutes or less, more preferably 50 minutes or less, more preferably 40 minutes or less, more preferably 35 minutes or less.

[0112] <Item 28>

The method for killing spores set forth in any one of <Item 22> to <Item 27>, wherein the spore forming bacteria is contacted with dipicolinic acid or a salt thereof in a liquid in the Step 1.

[0113] <Item 29>

The method for killing spores set forth in any one of <Item 22> to <Item 28>, wherein the pH at 24°C at the time of the contact of the Step 1 is preferably 1 or more, more preferably 1.2 or more, and preferably 5 or less, more preferably 4 or less, more preferably 3 or less, more preferably 2.5 or less, more preferably 1.8 or less, and preferably 1 to 6, more preferably 1 to 5, more preferably 1 to 4, more preferably 1 to 3, more preferably 1 to 2.5, more preferably 1.2 to 1.8.

[0114] <Item 30>

The method for killing spores set forth in any one of <Item 22> to <Item 29>, wherein the concentration of dipicolinic acid

in the liquid is 0.05 mM to 30 mM.

[0115] <Item 31>

The method for killing spores set forth in any one of <Item 22> to <Item 30>, wherein the content of dipicolinic acid in the liquid is preferably 0.05 mM or more, more preferably 0.5 mM or more, more preferably 3 mM or more, more preferably 4 mM or more, more preferably 6 mM or more, more preferably 8 mM or more, and preferably 30 mM or less, more preferably 25 mM or less, more preferably 20 mM or less, more preferably 15 mM or less, more preferably 12 mM, more preferably 10 mM or less, and preferably 0.05 to 30 mM, more preferably 0.5 to 30 mM, more preferably 3 to 30 mM, more preferably 3 to 25 mM, more preferably 3 to 20 mM, more preferably 3 to 15 mM, more preferably 4 to 15 mM, more preferably 6 to 12 mM, more preferably 8 to 10 mM.

[0116] <Item 32>

The method for killing spores set forth in any one of <Item 22> to <Item 31>, wherein the method has a period of performing the Step 1 at a temperature of 15°C or more and less than 50°C before the commencement of the Step 2A.

[0117] <Item 33>

The method for killing spores set forth in any one of <Item 22> to <Item 32>, wherein the Step 1 is performed before the commencement of the Step 2A preferably at a temperature of 15°C or more, more preferably 20°C or more, and preferably less than 50°C, more preferably 49°C or less, more preferably 45°C or less,

more preferably 40°C or less, more preferably 30°C or less, and is performed preferably at a temperature of 15°C or more and less than 50°C, more preferably 15°C to 49°C, more preferably 15°C to 40°C, more preferably 15°C to 30°C.

[0118] <Item 34>

The method for killing spores set forth in any one of <Item 22> to <Item 33>, wherein the method has a period of performing the Step 1 for a time of 30 seconds to 60 minutes before the commencement of the Step 2A.

[0119] <Item 35>

The method for killing spores set forth in any one of <Item 22> to <Item 34>, wherein the Step 1 is performed before the commencement of the Step 2A preferably for 30 seconds or more, more preferably 1 minute or more, more preferably 3 minutes or more, more preferably 5 minutes or more, more preferably 8 minutes or more, more preferably 10 minutes or more, more preferably 15 minutes or more, more preferably 20 minutes or more, more preferably 25 minutes or more, and preferably 60 minutes or less, more preferably 50 minutes or less, more preferably 40 minutes or less, more preferably 35 minutes or less, and is performed preferably for a time of 30 seconds to 60 minutes, more preferably 1 minute to 60 minutes, more preferably 3 minutes to 60 minutes, more preferably 5 minutes to 60 minutes, more preferably 5 minutes to 50 minutes, more preferably 5 minutes to 40 minutes, more preferably 8 minutes

to 40 minutes, more preferably 10 minutes to 40 minutes, more preferably 15 minutes to 40 minutes, more preferably 20 minutes to 40 minutes, more preferably 25 minutes to 40 minutes, more preferably 25 minutes to 35 minutes.

[0120] <Item 36>

The method for killing spores set forth in any one of <Item 22> to <Item 35>, wherein the Step 1 is performed for a time of 30 minutes to 90 minutes in total.

[0121] <Item 37>

The method for killing spores set forth in any one of <Item 22> to <Item 36>, wherein the Step 1 is performed preferably for 30 minutes or more, more preferably 40 minutes or more, more preferably 45 minutes or more, more preferably 50 minutes or more, preferably 55 minutes or more, more preferably 60 minutes or more, and preferably 90 minutes or less, more preferably 80 minutes or less, more preferably 70 minutes or less, more preferably 65 minutes or less, in total.

[0122] <Item 38>

A sporicidal assistant composition including dipicolinic acid or a salt thereof and having a pH of 6 or less.

[0123] <Item 39>

The sporicidal assistant composition set forth in <Item 38>, wherein the pH thereof at 24°C is preferably 1 or more, more preferably 1.2 or more, and preferably is 5 or less, more preferably 4 or less, more preferably 3 or less, more preferably

2.5 or less, more preferably 1.8 or less.

[0124] <Item 40>

The sporicidal assistant composition set forth in <Item 38> or <Item 39>, which is a liquid composition containing 0.05 mM to 30 mM of dipicolinic acid.

[0125] <Item 41>

The sporicidal assistant composition set forth in any one of <Item 38> to <Item 40>, wherein the content of dipicolinic acid is preferably 0.05 mM or more, more preferably 0.5 mM or more, more preferably 3 mM or more, more preferably 5 mM or more, more preferably 8 mM or more, and preferably 30 mM or less, more preferably 25 mM or less, more preferably 20 mM or less, more preferably 15 mM or less, more preferably 12 mM or less, and preferably 0.05 to 30 mM, more preferably 0.5 to 30 mM, more preferably 3 to 30 mM, more preferably 3 to 25 mM, more preferably 3 to 20 mM, more preferably 3 to 15 mM, more preferably 5 to 15 mM, more preferably 8 to 12 mM.

[0126] <Item 42>

The sporicidal assistant composition set forth in any one of <Item 38> to <Item 41>, which is a liquid composition including dipicolinic acid or a salt thereof and a solvent or a liquid composition including dipicolinic acid or a salt thereof, a pH adjuster, and a solvent.

[0127] <Item 43>

The sporicidal assistant composition set forth in any one

of <Item 38> to <Item 42>, wherein the solvent is preferably water or a mixture of water and a hydrophilic solvent, more preferably is water.

[0128] <Item 44>

The sporicidal assistant composition set forth in any one of <Item 38> to <Item 43>, wherein the pH adjuster is preferably selected from inorganic salts such as hydrochloric acid and sulfuric acid and organic acids such as lactic acid, citric acid or salts thereof, preferably from inorganic bases such as sodium hydroxide and potassium hydroxide and organic bases such as triisopropanolamine.

[0129] <Item 45>

The sporicidal assistant composition set forth in any one of <Item 38> to <Item 44>, which includes dipicolinic acid or a salt thereof, a pH adjuster, water, or another solvent, and wherein the content of peracetic acid, sulfoperoxycarboxylic acid and dicarboxylic acid diester is preferably 1000 ppm or less, more preferably 100 ppm or less, more preferably 10 ppm or less, more preferably 1 ppm or less, more preferably substantially 0 ppm.

[0130] <Item 46>

Use of a sporicidal assistant composition including dipicolinic acid or a salt thereof and having a pH of 6 or less, as a sporicidal assistant.

EXAMPLES

[0131]

Test 1: Examples 1-1 to 1-4 and Comparative Example 1-1

Sporicidal Test

With the use of a 10 mM aqueous solution of dipicolinic acid (henceforth, DPA solution) adjusted to pH given in Table 1 and *B. cereus* NBRC 13494 strain as a strain to be tested, a test was performed in accordance with the following procedure to evaluate a sporicidal effect. The results of the evaluation are given in Table 1. In all the following tests, spore forming bacteria was used for tests after confirming that 95% or more thereof had formed spores by microscopic observation.

In Step 1-1, Step 1-2 and Step 2A-1 described below, spore forming bacteria was contacted with dipicolinic acid (henceforth DPA) under conditions with a pH of 6 or less and, therefore, the steps are included in Step 1. Step 2A is included in Step 2. This test corresponds to Embodiment 2-1 described in paragraph 0052.

[0132]

Aqueous solution of dipicolinic acid: The dipicolinic acid used was a reagent (produced by Wako Pure Chemical Industries, Ltd., production code 165-05342), the water used was ion-exchanged water, and the pH adjuster used was hydrochloric acid or sodium hydroxide. The preparation of the aqueous solution of dipicolinic acid was performed at 24°C. The

pH adjuster was dropped into the aqueous solution of dipicolinic acid while measuring the pH of the aqueous solution of dipicolinic acid with a benchtop pH meter Model 9611 (manufactured by HORIBA, Ltd.), thereby adjusting the pH of the aqueous solution of dipicolinic acid. In Examples, any pH is a pH at 24°C unless otherwise stated.

[0133]

- Procedure -

(1) Step 1-1: A test liquid was prepared by mixing 100 µL of an aqueous dispersion with a content of spore forming bacteria having spores (*B. cereus* NBRC 13494 strain) of 10^8 CFU/mL (henceforth, spore solution) and 900 µL of a 10 mM DPA solution with a pH given in the table.

(2) Step 1-2: The test liquid of the above (1) was left to stand at 24°C for 30 minutes.

(3) Step 2A: The test liquid was heat treated at 80°C for 30 minutes after the above (2). An aluminum block thermostatic bath MD-01N-110 available from Major Science was used for the heating step.

(4) The test liquid heat treated in (3) was diluted stepwise with sterilized water, and individual diluted liquids (100 µL) were smeared to an LB agar medium (produced by BD) and then cultivated at 30°C for 16 hours.

(5) After the cultivation of (4), the number of surviving bacteria, X (CFU/mL) was determined on the basis of the number

of grown colonies.

(6) The sporicidal effect was evaluated on the basis of the sporicidal effect index calculated according to the formula given below.

A larger sporicidal effect index indicates higher sporicidal effect.

[0134]

$$(\text{Sporicidal effect index}) = \text{Log}_{10}[(\text{the number of surviving bacteria of control})/(\text{the number of surviving bacteria X})]$$

[0135]

The number of surviving bacteria (CFU/mL) of control is the number of surviving bacteria determined by performing the above (1) to (6) using sterilized water with individually adjusted pH instead of the DPA solution. In the table, the number of surviving bacteria X and the number of surviving bacteria of control are expressed by a logarithm taken with 10 as the base. Since the number of surviving bacteria X of Example 1-1 was less than the detection limit, calculation was performed with the number of surviving bacteria X assumed as being 1 for convenience.

[0136]

As shown in Table 1, comparison of Examples 1-1 to 1-4 with Comparative Example 1-1 reveals that the method for killing spores of the present invention is superior in sporicidal effect

and bactericidal effect.

[0137]

[Table 1]

		Examples				Comparative Examples
		1-1	1-2	1-3	1-4	
	pH of DPA solution	1.5	2.2	3.5	4.8	6.7
Result	Number of surviving bacteria, X (Log10[cfu/mL])	Detection limit	1.90	5.96	8.63	9.37
	Number of surviving bacteria of control (Log10[cfu/mL])	5.18	6.86	9.37	9.52	9.41
	Sporicidal effect index	5.18*	4.96	3.41	0.89	0.04

* Calculated by taking the number of surviving bacteria X as 1.

[0138]

Test 2: Examples 2-1 to 2-3:

A sporicidal effect was evaluated by performing a test under the same conditions as Example 1-2 except having used a DPA solution with a concentration given in Table 2 instead of the 10 mM aqueous solution of dipicolinic acid. The results of the evaluation are given in Table 2.

[0139]

[Table 2]

		Example	Example	Example	Example
		2-1	2-2	2-3	1-2
DPA concentration [mM]		0.1	1	5	10
Result	Number of surviving bacteria, X (Log10[cfu/mL])	6.19	5.45	2.00	1.90
	Number of surviving bacteria of control (Log10[cfu/mL])	6.57	6.57	5.66	6.86
	Sporicidal effect index	0.38	1.12	3.66	4.96

[0140]

Test 3: Example 3-1:

A sporicidal effect was evaluated by performing the same test as Example 1-2 except having changed the resting time of Step 1-2 to the time given in Table 3. The results of the evaluation are shown in Table 3. That the resting time of Step 1-2 was 0 means that Steps 1 and 2 were started at the same time.

[0141]

[Table 3]

		Examples	
		3-1	1-2
Still standing time of Step 1-2 [minute]		0	30
Result	Number of surviving bacteria, X (Log10[cfu/mL])	3.71	1.90
	Number of surviving bacteria of control (Log10[cfu/mL])	6.47	6.86
	Sporicidal effect index	2.76	4.96

[0142]

Test 4: Examples 4-1 to 4-3:

A sporicidal effect was evaluated by performing the same test as Example 1-2 except having changed the temperature of the heating treatment of Step 2A to the heating temperature given in Table 4. The results of the evaluation are given in Table 4.

[0143]

[Table 4]

		Examples			
		4-1	4-2	1-2	4-3
Heating temperature [°C]		50	60	80	100
Result	Number of surviving bacteria, X (Log10[cfu/mL])	6.30	4.00	1.90	3.56
	Number of surviving bacteria of control (Log10[cfu/mL])	6.52	6.34	6.86	4.30
	Sporicidal effect index	0.22	2.34	4.96	0.74

[0144]

In Example 4-3, since the sporicidal effect was relatively high even in the control, the value of the bactericidal effect index was small.

[0145]

Test 5: Examples 5-1 to 5-2:

A sporicidal effect was evaluated by performing the same test as Example 1-2 except having changed the time of the heating treatment of Step 2A to the heating time given in Table 5. The results of the evaluation are shown in Table 5.

[0146]

[Table 5]

		Examples		
		5-1	5-2	1-2
Heating time [minute]		5	10	30
Result	Number of surviving bacteria, X (Log10[cfu/mL])	6.63	4.62	1.90
	Number of surviving bacteria of control (Log10[cfu/mL])	7.58	6.02	6.86
	Sporicidal effect index	0.95	1.40	4.96

[0147]

Test 6: Comparative Examples 6-1 to 6-4:

A sporicidal effect was evaluated by performing the same test as Test 1 using the aqueous solutions given in Table 6. Specifically, a sporicidal effect was evaluated by performing

a test using agents with the concentrations given in Table 6 instead of DPA. The results of the evaluation are shown in Table 6. The water used was ion-exchanged water and the pH adjuster used was hydrochloric acid or sodium hydroxide. The agents given in Table 6 are as follows.

[0148]

- DPA-Ca: prepared using dipicolinic acid Ca salt, DPA (produced by Wako Pure Chemical Industries, Ltd., production code 165-05342), and calcium chloride (produced by Wako Pure Chemical Industries, Ltd., production code 039-00475).
- L-alanine: produced by Wako Pure Chemical Industries, Ltd., production code 010-01042
- Inosine: produced by Wako Pure Chemical Industries, Ltd., production code 095-00233

[0149]

[Table 6]

		Examples		Comparative Examples			
		1-2	1-4	6-1	6-2	6-3	6-4
Agent	DPA	10mM	10mM	-	-	-	-
	DPA-Ca	-	-	10mM	-	-	-
	L-Alanine	-	-	-	10mM	5mM	10mM
	Inosine	-	-	-	-	5mM	10mM
pH		2.2	4.8	7.8	7.5	7.5	7.5
Result	Number of surviving bacteria, X (Log10 [cfu/mL])	1.90	8.63	7.81	7.86	9.34	7.99
	Number of surviving bacteria of control (Log10 [cfu/mL])	6.86	9.52	8.01	7.96	9.35	7.96
	Sporicidal effect index	4.96	0.89	0.20	0.10	0.01	-0.03

[0150]

As shown in Table 6, Examples 1-2 and 1-4 exhibit superior sporicidal effects as compared with Comparative Examples 6-1 to 6-4. That is, the method for killing bacteria of the present invention using a DPA solution with a pH of 6 or less has a superior sporicidal effect as compared with the case of using usual DPA-Ca in a usual pH or the case of using L-alanine or L-alanine in combination with inosine.

[0151]

Test 7: Examples 7-1, 7-2:

Sporicidal Test

With the use of a 10 mM aqueous solution of dipicolinic acid (henceforth, DPA solution) the pH of which had been adjusted to 2.2 and *B. cereus* NBRC 13494 strain as a strain to be tested, a sporicidal test was performed using the following procedure and a sporicidal effect was evaluated. The results of the evaluation are given in Table 7.

[0152]

In Step 1-1 and Step 1-2 described below, spore forming bacteria was contacted with dipicolinic acid (henceforth DPA) under conditions with a pH of 6 or less and, therefore, the steps are included in Step 1. Step 2B is included in Step 2. This test corresponds to Embodiment 1 described in paragraph 0052.

[0153]

- Procedure -

(1) Step 1-1: A test liquid was prepared by mixing 100 μL of an aqueous dispersion with a content of spore forming bacteria having spores (*B. cereus* NBRC 13494 strain) of 10^8 CFU/mL (henceforth, spore solution) and 900 μL of a 10 mM DPA solution with a pH of 2.2.

(2) Step 1-2: The test liquid of the above (1) was left to stand at 24°C for the time given in Table 7.

(3) Step 1-3: The test liquid of Step 1-2 was subjected to centrifugal separation with a centrifuge (Table Top Micro Refrigerated Centrifuge 3500, manufactured by KUBOTA Corporation) at 12 krpm for 10 minutes, and the supernatant was removed.

(4) Step 2B: After Step 1-3, the supernatant was removed, and immediately thereafter, 1 mL of an aqueous solution of sodium hypochlorite with a concentration given in Table 7 was added, followed by being left to stand at 24°C for 10 minutes.

(5) The test liquid of Step 2B was diluted ten times by adding 4.5 mL of an LP solution containing 0.1% sodium thiosulfate to 0.5 mL of the test liquid of Step 2B, and the resultant was left to stand for 10 minutes, and thus the bactericidal action of sodium hypochlorite was stopped.

(6) The test liquid of (5) was diluted stepwise with sterilized water, and individual diluted liquids (100 μL) were smeared to an LB agar medium (produced by BD) and then cultivated at 30°C for 16 hours.

(7) After the cultivation of (6), the number of surviving bacteria, X (CFU/mL) was determined on the basis of the number of grown colonies.

(8) The sporicidal effect was evaluated on the basis of the sporicidal effect index calculated according to the formula given below.

A larger sporicidal effect index indicates higher sporicidal effect.

[0154]

$$(\text{Sporicidal effect index}) = \text{Log}_{10}[(\text{the number of surviving bacteria of control})/(\text{the number of surviving bacteria X})]$$

[0155]

The number of surviving bacteria (CFU/mL) of control is the number of surviving bacteria determined by performing the above (1) to (8) using sterilized water adjusted to a pH of 2.2 instead of the DPA solution. In the table, the number of surviving bacteria X and the number of surviving bacteria of control are expressed by a logarithm taken with 10 as the base.

[0156]

The agents used in Test 7 are as follows.

- Sodium hypochlorite: produced by Nankai Chemical Co., Ltd.
- LP solution containing 0.1% sodium thiosulfate: prepared in accordance with a manual of an LP diluent "DAIGO" using an LP diluent "DAIGO" produced by Wako Pure Chemical Industries, Ltd.

and sodium thiosulfate produced by Wako Pure Chemical Industries, Ltd.

[0157]

[Table 7]

		Examples	
		7-1	7-2
Step 1-2	DPA contact time [minute]	10	30
Step 2B	Sodium hypochlorite Effective chlorine concentration [ppm]	100	100
Result	Number of surviving bacteria, X (Log10 [cfu/mL])	4.3	2.0
	Number of surviving bacteria of control (Log10 [cfu/mL])	8.0	8.0
	Sporicidal effect index	3.7	6.0

[0158]

Usually, an effective chlorine concentration as high as about 1000 ppm is required for killing spores, but as shown in Table 7, it has been revealed that the sporicidal effect index was high even in the case of using a lower concentration of sodium hypochlorite and a superior sporicidal effect and a superior bactericidal effect were achieved by performing Step 1 even with a low concentration of sodium hypochlorite.

[0159]

<Check of Germination>

In the present description, "germination" can be

confirmed, for example, by checking the darkening of spores. The method for the confirmation may be to check the presence of darkened spores after Step 1 and Step 2. That is, the presence of darkening is checked with a phase contrast microscope. Where spores have not germinated, spores looking white will darken on germination, and darkened spores indicate to have germinated.

[0160]

The method for killing spores of the present invention can be used for a wide variety of applications, such as linen washing, prevention of food decomposition, and environmental purification.

CLAIMS

1. A method for killing spores in which Step 2 defined below is performed after the completion of Step 1 defined below,

Step 1: a step of contacting spore forming bacteria with dipicolinic acid or a salt thereof under conditions with a pH of 4 or less; and

Step 2: a step of killing the spore forming bacteria.

2. The method for killing spores according to claim 1, wherein the Step 2 is Step 2A defined below,

Step 2A: a step of killing the spore forming bacteria physically.

3. The method for killing spores according to claim 1, wherein the Step 2 is Step 2B defined below,

Step 2B: a step of killing the spore forming bacteria chemically.

4. The method for killing spores according to claim 2, wherein the Step 2A is a step of killing the bacteria by heat.

5. The method for killing spores according to claim 3, wherein the Step 2B is a step of killing the spore forming

bacteria with a bactericide.

6. The method for killing spores according to any one of claims 1 to 5, wherein the spore forming bacteria is contacted with dipicolinic acid or a salt thereof in a liquid in the Step 1.

7. The method for killing spores according to claim 6, wherein the content of dipicolinic acid in the liquid is 0.05 mM to 30 mM.

8. The method for killing spores according to any one of claims 1 to 7, wherein the Step 1 is performed at a temperature of 15°C or more and less than 50°C.

9. The method for killing spores according to any one of claims 1 to 8, wherein the Step 1 is performed for a time of 5 minutes to 60 minutes.

10. A method for killing spores, the method having a period in which Step 1 defined below and Step 2A defined below are performed simultaneously,

Step 1: a step of contacting spore forming bacteria with dipicolinic acid or a salt thereof under conditions with a pH

of 4 or less; and

Step 2A: a step of killing the spore forming bacteria physically.

11. The method for killing spores according to claim 10, wherein the Step 2A is a step of killing the bacteria by heat.

12. The method for killing spores according to claim 10 or 11, wherein the spore forming bacteria is killed at a temperature of 50°C to 250°C in the Step 2A.

13. The method for killing spores according to claim 12, wherein the time of killing the spore forming bacteria at a temperature of 50°C to 250°C in the Step 2A is 3 minutes to 90 minutes.

14. The method for killing spores according to any one of claims 10 to 13, wherein the spore forming bacteria is contacted with dipicolinic acid or a salt thereof in a liquid in the Step 1.

15. The method for killing spores according to claim 14, wherein the content of dipicolinic acid in the liquid is 0.05 mM to 30 mM.

16. The method for killing spores according to any one of claims 10 to 15, wherein method has a period of performing the Step 1 at a temperature of 15°C or more and less than 50°C before the commencement of the Step 2A.

17. The method for killing spores according to any one of claims 10 to 16, wherein the Step 1 is performed for a time of 30 seconds to 60 minutes before the commencement of the Step 2A.

18. The method for killing spores according to any one of claims 10 to 17, wherein the Step 1 is performed for a time of 30 minutes to 90 minutes in total.

19. A sporicidal assistant composition comprising dipicolinic acid and having a pH of 4 or less.

20. The sporicidal assistant composition according to claim 19, which is a liquid composition containing dipicolinic acid in an amount of 0.05 mM to 30 mM.