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PATENT SPECIFICATION

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(54) LACTOBACILLUS DEODORANT

(71) We, SEIKEN KAI FOUNDATIONAL JURIDICAL PERSON, a legal body organized under the laws of Japan, of 95, Fushimido-cho, Tondabayashi City, Osaka Prefecture, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a culturing composition, a storing composition and a deodorizing composition of a *Lactobacillus* strain.

The inventor of the present invention has already found certain specific strains of Lactobacillus which have the ability to de-odorize unpleasant odors from the excrement of humans and livestock, the breath and the vagina. As a result of these findings, various investigations have been conducted with respect to *Lactobacillus* strains including those found by the inventor of the present invention and, at the same time, the de-odorizing activity of these strains and the relation thereof with their microbiological properties have been further scrutinised. The present invention is based on these investigations.

According to one aspect of the present invention, there is provided a composition useful for culturing a *Lactobacillus* strain comprising (1) *a Lactobacillus* strain whose growth is enabled or promoted in a culture medium comprising S—W (Stephenson-Whetham) medium, S—W medium containing vitamins or S—W medium containing casamino acid, or in a culture medium selected from the above three media and further containing hydrogen sulphide, sodium sulphide, ammonia, a lower fatty acid or a sulfur-containing amino acid, and (2) a culture medium containing, as a main ingredient, a growth promoting amount of one or more of sulfur-containing amino acids, glycine, glutamic acid, lysine, alanine, phenyl-alanine, arginine, aspartic acid, hydrogen sulphide, sodium sulphide, ammonia and lower fatty acids.

According to another aspect of the present invention, there is provided a composition useful for storing a *Lactobacillus* strain comprising (1) a *Lactobacillus* strain whose growth is enabled or promoted in a culture medium comprising S—W medium, S—W medium containing vitamins or S—W medium containing casamino acid, or in a culture medium selected from the above three media and further containing hydrogen sulphide, sodium sulphide, ammonia, a lower fatty acid or a sulfur-containing amino acid, and (2) one or more of sulfur-containing amino acids, glycine, glutamic acid, lysine, alanine, phenylalanine, arginine and aspartic acid, hydrogen sulphide, sodium sulphide, ammonia or lower fatty acids.

According to a further aspect of the present invention, there is provided a deodorizing composition comprising (1) the living cells of a *Lactobacillus* strain whose growth is enabled or promoted in a culture medium comprising S—W medium, S—W medium containing vitamins or S—W medium containing casamino acid, or in a culture medium selected from the above three media and further containing hydrogen sulphide, sodium sulphide, ammonia, a lower fatty acid or a sulfur-containing amino acid, and (2) one or more compounds selected from sulfur-containing amino acids, glycine, glutamic acid, lysine, alanine, phenylalanine, arginine, aspartic acid, hydrogen sulphide, sodium sulphide, ammonia or lower fatty acids.

According to a still further aspect of the present invention, there is provided a composition comprising (1) the living cells of a *Lactobacillus* strain whose growth is enabled or promoted in a culture medium comprising S—W medium, S—W medium containing vitamins or S—W medium containing casamino acid, or in a culture medium selected from the above three media and further containing hydrogen

sulphide, sodium sulphide, ammonia, a lower fatty acid or a sulfur-containing amino acid, and (2) one or more compounds selected from sulfur-containing amino acids, glycine, glutamic acid, lysine, alanine, phenylalanine, arginine, aspartic acid, hydrogen sulphide, sodium sulphide, ammonia or lower fatty acids.

The inventor has now succeeded in isolating many *Lactobacillus* strains having a high de-odorizing activity even when administered orally to humans and livestock or applied directly to their excrement. After having studied the properties of these strains, the inventor has succeeded in ascertaining the relationship between the de-odorizing activity of said strains and the mcrobiological properties thereof. As one of the important facts, it has been ascertained that, with respect to the *Lactobacillus* strains having a de-odorizing activity, the conditions for cultivation, sub-culture and preservation thereof are quite different from those employed for known strains. The results of experiments carried out by the inventor with respect to the de-odorizing *Lactobacillus* strains are described below. The *Lactobacillus* strains which can be recognized organo-leptically to have a de-odorizing activity must have the following characteristic properties.

As essential properties, they must

(1) be resistant to bile,
(2) have less nutritional requirements than known *Lactobacillus* strains,
(3) have a high growth rate even in a medium containing poor nutrients, and
(4) one or a combination of the *Lactobacillus* strains must satisfy the S.N.C.-theory propounded by the inventor and explained in detail hereinafter.

As important properties for effective de-odorizing activity, the *Lactobacillus* strains must

(5) exhibit antibiotic and lactic acid production and
(6) be resistant to antibacterial compounds including spices which are commonly used in food.

Before explaining in detail the above-mentioned properties, the microbiological properties of known *Lactobacilli* will now be described. The morphology of *Lactobacillus* has been defined as follows.

Gram-negative, facultative anaerobic, non-spore forming rods. Depending on the strains they may be spherical rod-like, curved rod-like, coryne-like or thread-like, but do not form many branches. They are usually non-motile, negative to catalase and do not reduce nitrates. They do not decompose gelatin and do not produce indole or hydrogen sulfide. Some of the strains are bipolar-strained. Their ability to decompose protein and fat is very weak, if any. They show better growth under anaerobic or microaerophilic conditions rather than under areobic conditions, have strong ability to decompose sugars, and are acid-fast. When used for glucose fermentation, they produce lactic acid in a yield of more than 50%. They are not pathogenic to animals and plants.

Moreover, it is known that *Lactobacillus* strains having these properties defined above can grow only in highly nutritional media e.g. in media containing amino acids, peptides, nucleic acid analogs, vitamins, salts, fatty acids or their esters and sugars.

The *Lactobacillus* strains which have been recognized by the inventor as having deodorizing activity show the same morphological characteristics as those of known *Lactobacillus* strains, but are quite different from the latter in the properties (1), (2) and (3) mentioned hereinbefore.

The important points among the above are explained in detail in the following:—

(A) As mentioned above, known *Lactobacillus* strains require amino acids, peptides, nucleic acid analogs, vitamins, salts, fatty acids or their esters and sugars for their growth, and they belong to a group of bacteria which show relatively high nutritional requirements. Therefore, highly nutritional media such as Briggs' and MRS media must have been generally used for cultivation of the *Lactobacillus* strains.

(B) The Lactobacillus strains which have been found by the inventor and are used in the present invention (hereinafter simply referred to as "the *Lactobacillus* of the invention", unless otherwise indicated) show quite different characteristics as compared with the known lactobacillus. That is, the *Lactobacillus* of the invention not only shows rapid growth in high nutritional media such as Briggs' medium, but, as shown in Table 1, it also grows well in low nutritional media.

TABLE 1

Strains (FERM–P Nos.)	Media used				
	Briggs	LC	(S–W) + casamino acids + vitamins	(S–W) + vitamins	(S–W) + casamino acids
Lactobacillus available in the market	++	+++	− ~ +	−	−
1946	+++	+++	++	+	++
2742	+++	+++	++	+	++
2779	+++	+++	++	−	+
2780	+++	+++	++	+	+
2781	+++	+++	++	-	+
2782	+++	+++	++	−	+

Note:– S–W means Stephenson-Whetham.

(C) As shown in Table 2, the *Lactobacillus* of the invention when cultivated in Briggs' and MRS media is superior to known Lactobacillus in growth rate and final number of cells thereof.

TABLE 2

Strains (FERM–P Nos.)	Media			
	MRS		Briggs	
	Specific growth rate (μ)	Final number of cells (cc)	Specific growth rate (μ)	Final number of cells (cc)
1946	0.516	65×10^8	0.53	40×10^8
2742	0.597	80×10^8	0.57	50×10^8
2779	0.53	60×10^8	0.51	35×10^8
2780	0.47	70×10^8	0.45	40×10^8
2781	0.53	70×10^8	0.51	40×10^8
2782	0.47	60×10^8	0.45	35×10^8
Mean value for known *Lacto-bacillus*	0.40	20×10^8	0.38	15×10^8

(D) Further, as seen from the above-mentioned nutritional requirements, S—W medium (a), (S—W+vitamins) medium (b) and even (S—W+casamino acids) medium (c) are unsuitable for growth of known *Lactobacillus*. With respect to this point, the result of experiments carried out by the inventor are shown in Table 4.

(E) On the contrary, the *Lactobacillus* of the invention can in almost all cases grow in at least one of the media (a), (b) and (c), and at the same time shows good growth rate and good final yield (number of cells/cc) therein.

Concomitantly, a deodorizing *Lactobacillus* of the invention which grows in (S—W+vitamins+amino acids) medium but not in the above-mentioned media (a), (b) and (c) has also been isolated by the inventor.

The LC and (S—W) media described above are composed as follows. LC medium: 10 g of peptone, 10 g of meat extracts, 5 g of NaCl, 3 g of K_2HPO_4, 10 g of glucose, 5 g of yeast extracts, 3 g of $CaCO_3$ and 10 liters of water, pH 7.4. (S—W) medium: 1 g of KH_2PO_4, 0.7 g of $MgSO_4$, $7H_2O$, 1 g of NaCl, 4 g of $(NH_4)_2HPO_4$, 0.03 g of $FeSO_4$ $7H_2O$, 5 g of glucose and 1 liter of water. Briggs' medium: Briggs. M. (1953) can be prepared according to "An Improved Medium For *Lactobacilli*" described in J. Dairy Res. *20*, 36. MRS medium: DEMAN J. C., ROGOSA M & SHARPE, M E (1960) is prepared according to J. Appl. Bact. *23*(1), 130—135.

The facts shown in the above-mentioned paragraphs (D) and (E) are summarized in the following Tables 3—1 and 3—2.

TABLE 3–1

Strains	Media
	Either one of (a), (b) and (c)
Lactobacillus of the invention	+
Known *Lactobacillus*	–

TABLE 3–2

μ and Yield (number of cells/cc)		
Lactobacillus of the invention (Medium used: (c)–medium	≒	Known *Lactobacillus* (Medium used: Briggs' medium)

As is clear from the above, the *Lactobacillus* of the invention when cultivated in a low nutritional medium such as the medium (c) shows almost the same growth rate and yield as those of the known strains cultivated in a high nutritional medium such as Briggs' medium. The particulars of the test results as to the six representative *Lactobacillus* strains of the invention, each of which have different nutritional requirements, are shown as follows:

TABLE 4

Strains (FERM–P Nos.)	Test items	Media (a)	Media (b)	Media (c)
1946	Growth	–	+	++
	μ		0.17	0.53
	Yield		5×10^8	25×10^8
2742	Growth	+	+	++
	μ	0.20	0.40	0.56
	Yield	7×10^8	20×10^8	50×10^8
2779	Growth	-	-	+
	μ			0.35
	Yield			8×10^8
2780	Growth	–	+	+
	μ		0.3	0.35
	Yield		6×10^8	20×10^8
2781	Growth	–	–	+
	μ			0.53
	Yield			30×10^8
2782	Growth	–	–	+
	μ			0.35
	Yield			8×10^8

Note: Yield is shown by the number of cells/cc.

(F) It has never been reported that the growth of known *Lactobacilli* might be enabled or promoted by adding one or more S—, N—, or C— compounds to the above-mentioned media (a), (b) and (c). And in fact, as shown in Table 5, the investigations of the inventor reveal the negative results on this point.

(G) On the contrary, however, Table 6 shows that growth of the *Lactobacillus* of the invention is enabled or promoted in all of or at least one of the media (a), (b) and (c) which contain the S.N.C-compounds and/or sulfur-containing amino acids.

TABLE 5

Summary of paragraphs (F) and (G)

Strains	Growth or promotion of growth: Compounds added to the media: S.N.C.-compounds were added to either one of (a), (b) and (c)
Lactobacillus of the invention	+
Known *Lactobacillus*	–

TABLE 6

Nutritional requirements and growth or growth promotion of the *Lactobacillus* of the invention

Strains (FERM–P Nos.)	Basic medium	Compounds added to the basic medium									
		i	ii	iii	iv	v	vi	vii	viii	ix	x
1946	(a)	−	+	+	+	±	±	±	±	±	±
	(b)	+	++	++	++	++	++	+	++	++	++
	(c)	++	++	++	++	++	++	++	++	++	++
2742	(a)	±	+	+	+	+	+	+	+	+	+
	(b)	+	++	++	++	++	++	++	++	++	++
	(c)	++	++	++	++	++	++	++	++	++	++
2779	(a)	−	−	−	−	−	−	−	−	−	−
	(b)	−	±	±	±	−	−	−	−	−	−
	(c)	+	++	++	++	++	++	+	++	++	+
2780	(a)	−	−	−	−	−	−	−	−	−	−
	(b)	+	+	+	+	+	+	+	+	+	+
	(c)	+	++	++	++	++	++	+	++	++	++
2781	(a)	−	+	+	+	+	+	−	±	±	±
	(b)	−	+	+	+	+	+	+	+	+	+
	(c)	+	++	++	++	++	++	++	±	++	++
2782	(a)	−	−	−	−	−	−	−	−	−	−
	(b)	−	+	±	±	−	−	−	−	−	−
	(c)	+	++	++	++	++	++	+	++	++	++
Lacto-bacillus available in the market	(a)	−	−	−	−	−	−		−	−	−
	(b)	−	−	−	−	−	−	−	−	−	−
	(c)	−	−	−	−	−	−	−	−	−	−

Note: i: No addition ii: cystein iii: cystine iv: methionine V: Na_2S
vi: ammonia vii: scatole viii: acetic acid ix: butyric acid x: propionic acid
In these experiments, Na_2S $9H_2O$ was employed as Na_2S, glacial acetic acid as acetic acid and 37% aqueous ammonia as ammonia.

The particulars of the S.N.C-compounds suggested by the inventor and used herein are explained in the following.

According to the inventor's investigations, a wide variety of foul smelling compounds in excrement are divided into the following three groups: S-compounds, N-compounds and C-compounds. Moreover, it has been found that, for studying the de-odorization of foul smelling compounds in excrement, it is convenient to examine the de-odorizing effects of the *Lactobacillus* of the invention upon H_2S or Na_2S (S-compounds), NH_3, indole or scatole (N-compounds) and lower fatty acids (C-compounds) because they are representative of said S-, N-, C-compounds, respectively. In this connection, P-compounds can be considered to be of secondary importance. Further, it has been ascertained that the growth of the *Lactobacillus* of the invention is enabled or promoted in an appropriately selected lower nutritional medium containing S-, N-, C-compounds as foul smelling ingredients. Taking all of the above into consideration, therefore, the inventor has designated them as the S—N—C-theory concerning micro-organisms and deodorization of excrement.

In the accompanying drawings, Figs. 1 to 9 show the growth rate of the *Lactobacillus* of the present invention and known *Lactobacilli* at their log phase. The results of Figure 1 were obtained in LC-medium (basic medium) containing acetic acid. The results of Figure 2 were obtained in said basic medium containing Na_2S and those of Figure 3 in said basic medium containing NH_3. Figure 4 shows the results which were obtained by adding acetic acid to S—W (+vitamins+casamino acids) and the results of Figures 5 and 6 were obtained by adding Na_2S and NH_3 thereto, respectively. Figure 7 shows the results which were obtained in a now nutritional medium containing acetic acid and, in Figures 8 and 9, Na_2S and NH_3 were employed, respectively, instead of said acetic acid. (1) shows the growth rate of the *Lactobacillus* of the present invention in a medium containing an additive, and (1′) that of the known *Lactobacillus* in the same medium. On the other hand, (2) and (2′) show the results of control tests for the *Lactobacillus* of the present invention and the known *Lactobacillus*, respectively. The control tests were carried out in a medium which did not contain the additive.

In Figure 1, (1) relates to the growth rate of the Lactobacillus strain of the invention in a medium containing 5 g per liter of acetic acid and (2) and (2′) show, respectively, the growth rate of the *Lactobacillus* strain of the invention and the known *Lactobacillus* strain in a medium containing 0.1 to 1 g per liter of acetic acid. As can be seen from Figure 1, the growth of the *Lactobacillus* strain of the present invention was promoted only slightly in the log phase and the degree of growth could be barely observed even by counting the number of living cells, whereas the known *Lactobacillus* strain showed a very much more apparent promotion of growth as a result of addition of acetic acid.

Similarly, the results which were obtained by cultivating the strains in the L—C medium containing Na_2S (2 g per liter) or NH_3 (2 g per liter) are shown in Figure 2 (Na_2S) and Figure 3 (NH_3), respectively. The known Lactobacillus strains were more inhibited in their growth at lower concentrations than the *Lactobacillus* strains of the invention, i.e. the *Lactobacillus* strains of the invention showed a higher resistance towards growth inhibition. Curves 2 and 2′ in Figures 2 and 3 show the results of control tests which were carried out in a medium containing only 0.1 g per liter of Na_2S or NH_3.

The influence of S.N.C-compounds upon the growth of the *Lactobacillus* of the invention and the known *Lactobacillus* are shown in Figures 4 to 6. In these experiments, the strains were cultivated in high, medium, and low nutritional media and, in the log phase of the strains, the S.N.C-compounds were added thereto. As will be seen from the results of these experiments, addition of some concentrations of acetic acid, Na_2S and NH_3 to the medium nutritional medium were effective to promote growth of the Lactobacillus of the invention, whereas the growth of the known *Lactobacillus* was not promoted by addition of Na_2S and NH_3 to the medium nutritional medium.

Furthermore, as shown in Figures 7 to 9, the known *Lactobacillus* does not grow in a low nutritional medium or even in said medium containing S and N compounds. However, the Lactobacillus of the invention, including the one which does not grow in (S—W) medium is always capable of growth in any medium containing Na_2S. This indicates that Na_2S is essential for growth of such a *Lactobacillus*.

In Figures 1 to 9, the straight line (——) stands for the *Lactobacillus* of the invention, and the dotted line (- - -) the known *Lactobacillus*. Concomitantly, the above-mentioned relationship can be summarized as Table 7.

TABLE 7

Conditions of experiments			*Lactobacillus* Known *Lactobacillus*	*Lactobacillus* of the invention
High nutritional medium	Degree of growth		++	+++
	Compounds added	acetic acid butyric acid	B	
		Na_2S	D	D
		NH_3	D	D
Middle nutritional medium	Degree of growth		+ ~ −	++
	Compounds added	acetic acid butyric acid	C − D	B
		Na_2S	E	B
		NH_3	E	B
Low nutritional medium	Degree of growth		−	+
	Compounds added	acetic acid butyric acid	D	A
		Na_2S	D	A
		NH_3	D	A

Note: A means that the compound is essential for growth of the strain or promotes the growth thereof strongly.

B means that the compound stimulates the growth of the strain fairly well.

C means that the compound stimulates the growth of the strain slightly.

D means that the compound does not stimulate the growth of the strain.

E means that the compound inhibits the growth of the strain.

For the *Lactobacillus* of the invention to show its deodorizing activity more efficiently, it should preferably have the following characteristics in addition to the aforementioned ones.

(1) First of all, the *Lactobacillus* strains should have a high antibiotic production. The *Lactobacillus* of the present invention has been found to include those having high, low and no antibiotic production.

(2) As another preferable characteristic, the *Lactobacillus* should be resistant to antibacterial compounds, such as antibiotics and spices commonly used in cooking. Namely, as suggested from the bacteriology thereof, the *Lactobacillus* strains of the present invention may not show their effects sufficiently if not made resistant to the the antibacterial compounds encountered in use. This fact may be seen from the result of experiments using the *Lactobacillus* strains of the invention as anti-inflammatory agents or deodorants, and one example of such experiments using them as the deodorants is shown in Table 8.

TABLE 8

Tetracycline 250 ml 4 tablets/day (p.o.)	Effects of deodorization	
	Strain sensitive to tetracycline	Strain resistant to tetracycline
Degree of deodorization of excrement	3' — 4	1

The *Lactobacillus* strain of the present invention was subcultured in the presence of tetracycline, and the resultant tetracycline-resistant strain was administered orally to men. Then, tetracycline (4 tablets per day) was further administered thereto. On the other hand, the sensitive strain was employed for the control group. As seen in Table 8, the resistant strain showed its deodorizing activity as expected, whereas the sensitive strains barely showed any such activity.

(3) The other preferable characteristic is bile-resistance. Bile has strong antibacterial activity and was used as a disinfectant in the ancient times. Therefore, the *Lactobacillus* strains of the invention should be rendered resistant to bile when used for deodorization of excrement by proliferating them in intestines. Further, this fact was confirmed by the experiments which were conducted by administration of the strains to men and animals, and one example of such experiments is shown in Table 9.

TABLE 9

Lactobacillus of the invention	Degree of deodorization (2 days after oral administration)
Strains sensitive to bile	3'
Strains not sensitive to 25% bile powder	1

As seen therein, the *Lactobacillus* of the present invention which was made resistant to 25% bile powder showed sufficient deodorizing activity, whereas the strains sensitive to bile barely showed any deodorizing activity.

Through the inventor's investigations as to the conditions for cultivation, subculture and proliferation of the *Lactobacillus* strains of the invention, it has been found that said strains, depending on the media employed, decrease their deodorizing activity during the subculture or proliferation thereof. In order to further investigate this phenomenon, therefore, the deodorizing activity of the strains of the invention was examined by subculturing them in high, middle or low nutritional media which optionally contained milk powder (which is known to encourage the growth of *Lactobacilli*) or bile acids. In these experiments, media which consisted mainly of the MRS, LC-medium and/or milk powder were employed as the high nutritional media. The medium which was obtained by adding 0.5 g per liter of Na_2S, 0.5 g per liter of scatole and 1 g/liter of butyric acid (scatole and butyric acid are ingredients of excrement referred to hereinafter as "F-ingredients) to MRS, LC-medium and/or milk powder was also employed as one of the high nutritional media. The middle nutritional media employed consist mainly of (S—W)medium, casamino acids and vitamins, and said F-ingredients and peptone were further added thereto. The low nutritional media are mainly a mixture of (S—W)medium, amino acids and vitamins, and a medium which was obtained by adding said F-ingredients and/or milk powder to said mixture was also employed as one of the low nutritional media. Examples of compositions of these media are shown as follows; the weights given are per liter of medium:

(i) Low nutritional media: (1) (S—W) medium+1 g of casamino acids+10 g of bile powder; (2) (1)+F-ingredients; (3) (1)+30 g of milk powder; (4) (3)+F-ingredients; (5) (S—W)medium+0.1 g of vitamins+10 g of bile powder; (6) (5)+F-ingredients; (7) (5)+30 g of milk powder; (8) (7)+F-ingredients.

(ii) Middle nutritional media: (9) (S—W)medium+1 g of casamino acids+1 g of yeast extracts+10 g of bile powder; (10) (9)+F-ingredients; (11) (9)+30 g of milk powder; (12) (11)+F-ingredients; (13) 2 g of peptone+0.005 g of $MgSO_4 7H_2O$+0.5 g of KH_2PO_4+1 g of NaCl+1.0 g of bile powder; (14) (13)+F-ingredients; (15) 1/10 MRS-medium; (16) (15)+F-ingredients; (17) 1/3 MRS-medium; (18) (17)+F-ingredients.

(iii) High nutritional media: (19) MRS-medium; (20) (19)+F-ingredients; (21) MRS+10 g of bile powder; (22) (21)+F-ingredients; (23) MRS+30 g of milk powder; (24) (23)+F-ingredients; (25) MRS+30 g of milk powder+10 g of bile powder; (26) (25)+F-ingredients; (27) milk powder medium; (28) (27)+F-ingredients; (29) LC-medium; (30) (29)+F-ingredients; (31) LC-medium+10 g of bile powder; (32) (31)+F-ingredients.

The deodorizing activity of the strains which were examined by subculturing them for an average time in these media are shown in the following:

(i) First it was observed that the deodorizing *Lactobacillus* strains increase their growth rate in the initial and middle stages of growth or further over all stages of their growth by adding said F-ingredients to either one of the low nutritional media (Nos. 1 to 8), though the specific growth rate of the strains varied depending on the media used. Moreover, whereas the deodorizing activity of the strains did not change by subculturing them for a period in the low nutritional media not containing said F-ingredients, the strains sometimes increased their deodorizing activity by repeating subculture thereof in the presence of said F-ingredients.

(ii) The strains showed good growth in the high nutritional media (Nos. 19 to 32), and the growth promoting effect or said F-ingredients was undetected and could not be observed even by counting the number of the living cells. Moreover, when the strains were subcultured in the high nutritional media, they showed rapid decrease in their deodorizing activity by repeating said subculture irrespective of whether or not F-ingredients had been added to the media. *Lactobacillus* strains (e.g., those employed in preparing commercial lactic beverages) which neither grew in middle nutritional media nor showed any substantial deodorizing activity were inhibited in their growth by adding said F-ingredients to the low and middle nutritional media, and at the same time they showed no sensitivity in the high nutritional media as was the case with the *Lactobacillus* strains of the present invention.

As is clear from the above, in order to prevent the deodorizing *Lactobacillus* strains from losing their activity, such a medium as the growth of the strains are stimulated by addition of said F-ingredients thereto as well as a medium containing F-ingredients must be employed for cultivation thereof. The decrease in activity of the deodorizing *Lactobacillus* strains was observed during the experiments therefor. Moreover, since such phenomenon was also observed during the subculture and storage of said strains, it became of great importance to seek conditions for subculturing the strains while retaining their deodorizing activity. Therefore, in view of the fact that the deodorizing activity of the *Lactobacillus* strains sometimes increases by subculturing them in low nutritional media containing said F-ingredients and that their activity decreases rapidly by subculturing in high nutritional media, the inventor carried out experiments by using the basic low nutritional media with and without the S.N.C-compounds, i.e., foul smelling compounds in excrement classified by the inventor. Table 10 shows typical and illustrative examples of such experiments. The amounts given are per liter of the medium.

TABLE 10

Compounds added to the culture medium	3rd-generations	6th-generations	9th-generations
0.5 g of Na_2S + 0.5 g of NH_3 + 1 g of acetic acid	A	A	A
1 g of acetic acid	A – B	B	C
5 g of skim milk	A – C	C – D	D

Further, while in the above-mentioned experiments Na_2S, NH_3 and acetic acid were employed representatively as the compounds which serve to maintain the activity of the strains, it was simultaneously made clear that any one of compounds designated as S.N.C-compounds by the inventor such as methyl sulfide, mercaptane, scatole, indole, butyric acid and propionic acid can in all of the cases give almost the same results as above insofar as they are used in combination with said S.N.C-compounds. Then, the changes in the deodorizing activity of the strains which were obtained by adding various amino acids and proteins to the nutritional or culture medium are shown in the following Table 11.

TABLE 11(A)

	Compounds added to the culture medium (i.e., S-W + vitamins)	Results of subculture		
		3rd-	6th- generations	9th-
Protein Peptide	Peptone	D	E	E
	Meat extracts	D	E	E
	Skim milk	D	E	E
Amino acids	Cystine	A	A	A
	Cystein	A	B	C
	Methionine	A	B	C
	Alanine	D	D	E
	Phenylalanine	D	D	E
	Arginine	D	D	E
	Asparagine	D	D	D
	Aspartic acid	D	D	D
	Glycine	D	D	D
	Glutamic acid	A	B	C
	Aminobutyric acid	D	D	E
	Leucine	D	D	E
	Isoleucine	D	D	E
	Histidine	D	D	D
	Proline	D	E	E
	Lysine	A	C	D
	Tyrosine	D	D	E
	Tryptophan	D	E	E
	Threonine	D	E	E
	Serine	D	E	E

Note: A : No decrease in activity
B : Slight decrease in activity
C : Moderate decrease in activity
D : Decrease in activity
E : Substantial decrease in activity

(S—W) refers to the Stephenson-Whetham medium. As will be clear from Table 11(A), the specific four to five amino acids were effective to maintain the activity of the strains during the subculture thereof. On the contrary, other amino acids such as proline and tyrosine served to decrease the deodorizing activity of the strains rapidly. That is, it was first confirmed by these experiments that amino acids are mainly divided into three groups: the first group of amino acids which serve to maintain the activity of the deodorizing Lactobacillus strains; the second group of amino acids such as glycine, glutamic acid and lysine which induce slight decrease in the activity of the strains; and the third group which induce a substantial decrease in said activity. Thus, although it was observed heretofore that the deodorizing activity of *Lactobacillus* strains decreases when cultivated in a highly nutritional medium to proliferate well therein, the inventor has succeeded in overcoming this disadvantage. Moreover, the above-mentioned experiments were also meaningful in that some compounds other than the foul smelling compounds in excrement, such as the S.N.C-compounds have been found to maintain the activity of the strains during the subculture thereof. Additionally, it was found that even a single compound may be effective to maintain the activity of the *Lactocillus* of the invention insofar as said compound contains S.N.C. This finding may have great importance from a practical point of view.

Further, as shown in Table 11(B), good results may also be obtained by the use of a mixture of S.N.C-compounds and specific amino acids. The amounts given are per liter of the medium.

TABLE 11 (B)

S.N.C-compounds	Amino acids added to S.N.C.-compounds	Results of subculture 3rd-	6th-generations	9th-
0.2 g of Na_2S + 0.2 g of NH_3 + 0.4 g of acetic acid	Cystine	A	A	A
	Cystein	A	A	A
	Methionine	A	A	A
	Glycine	A	A	B
	Glutamic acid	A	A	B
	Alanine	A	A	B
	Aspartic acid	A	A	B
	Lysine	A	A	B
	Arginine	A	A	B
	Phenylalanine	A	A	C
0.5 g of Na_2S + 0.5 g of NH_3 + 1 g of acetic acid	Casamino acids	A	B	C

With respect to methods of storage and pharmaceutical compositions:

Various methods for storage of strains and pharmaceutical compositions containing them are known. The *Lactobacillus* strains of the present invention can be stored by various known methods such as refrigeration, drying at atmospheric pressure, drying *in vacuo* or in a liquid medium. However, as has already been made clear by the inventor's experiments, in storing the strains special care may have to be paid to prevent any decrease of the deodorizing activity which is a characteristic property of the *Lactobacillus* strains of the present invention. Namely, when the strains are stored at a low e.g. 8°C medium or e.g. 28°C temperature, in a liquid medium or by

refrigeration or in the dry state in the presence of a compound which, in subculturing the strains, serves to decrease the activity thereof, the strains would also decrease their deodorizing activity as in the case of the subculture thereof.

Tables 12 and 13 show the results of experiments carried out by the inventor.

TABLE 12

Compounds added to the culture medium (i.e., S-W + vitamins)	Effects of compounds upon storage of the strains					
	28°C			8°C		
	dried form	semi-dried form	moist-ened form	dried form	semi-dried form	moist-ened form
Na_2S	A	A	A	A	A	A
Methyl sulfide	B	B	B	B	B	B
NH_4Cl	B	B	B	B	B	B
Mercaptan	B	B	B	B	B	B
Scatole	B	B	B	B	B	B
Indole	B	B	B	B	B	B
Sodium acetate	A	A	A	A	A	A
Sodium butyrate	A	A	A	A	A	A
Sodium propionate	B	B	B	B	B	B

Note: (S-W) : Same as defined hereinbefore.

Table 12 shows the result of experiments which were carried out in the presence of a suitable amount of either one of S.N.C-compounds, and this result demonstrates that said compounds are effective to maintain the deodorizing activity of the strains.

TABLE 13

Compounds added to the basic medium (i.e., S-W + vitamins)		Effects of compounds upon storage of the strains					
		28°C			8°C		
		dried form	semi-dried form	moistened form	dried form	semi-dried form	moistened form
Protein + Peptide	Peptone	E	E	E	E	E	E
	Meat extracts	E	E	E	E	E	E
	Skim milk	D	D	D	D	D	D
Amino acids	Cystine	A	A	A	A	A	A
	Cystein	B	B	C	B	B	C
	Methionine	B	B	C	A	B	C
	Alanine	D	D	D	D	D	D
	Phenylalanine	D	D	D	D	D	D
	Arginine	C	D	D	C	C	D
	Asparagine	D	D	D	D	D	D
	Glycine	C	D	D	C	D	D
	Glutamic acid	C	C	D	B	C	D
	Aminobutyric acid	D	D	D	D	D	D
	Leucine	D	D	D	D	D	D
	Isoleucine	D	D	D	D	D	D
	Histidine	C	D	D	C	D	D
	Proline	D	D	D	D	D	D
	Lysine	C	C	D	C	C	D
	Tyrosine	D	D	D	D	D	D
	Tryptophan	D	D	D	D	D	D
	Threonine	D	D	D	D	D	D
	Serine	D	D	D	D	D	D

Note: Dried form: water content = about 8% Semi-dried form: water content = about 15% Moistened form: a fermentation broth of the strain, or a mass of living cells of the strain. The strains stored under dried conditions keep their deodorizing activity for a longer period of time as compared with those stored under moistened conditions.

Concomitantly, when stored by lyophilization or at an extremely low temperature, even the strains coated with milk do not lose their deodorizing activity but are kept in good condition. Also in such cases, however, it is preferred to coat the strains with sulfur-containing amino acids.

As in the case of the aforementioned subculture thereof, good results can also be obtained under either dried, semi-dried or moistened conditions when the strains are stored in the presence of a mixture of S.N.C-compounds and amino acids.

From Table 13, it has been found that the coating of the strains with amino acids such as cystine or methionine is effective for the storage thereof. This indicates that the strains of the present invention can be administered directly to a living body. This also indicates that various methods other than lyophilization and storage at a low temperature can be employed in making the pharmaceutical compositions of various forms. For example, when the strains are cultivated in a medium containing a large amount of Na_2S, NH_3 and butyric acid and said compounds are not digested completely, the strains must be washed thoroughly. This washing detracts from the storage conditions of the strains. However, from the above-mentioned facts it is clear that, in such cases, cystine or methionine, which have been recognized to be useful for the living body, can be employed as a coating agent for the *Lactobactillus* strains.

Various properties of the deodorizing strains of the invention such as the biochemical property and deodorizing activity thereof are shown in the following lines, using the six strains as the representative examples thereof.

Table 14 shows the biochemical properties. On the other hand, the relationship between the nutritional requirements and growth of the strains is shown in Tables 15 and 16. Further, Table 17 shows the effects of the strains upon deodorization of excrement. The experiments of Table 17 were carried out by adding a few loopfuls of the strains or a fermentation broth thereof to fresh excrement or a 5-fold dilution thereof, and then cultivating the mixture.

TABLE 14

Microscopic observation and morphological characteristics

	FERM–P Nos.					
	1946	2742	2779	2780	2781	2782
Gram	+	+	+	+	+	+
Shape	short rod, rounded ends	*cocco-bacilli*	short rod, rounded ends	*cocco-bacilli*	*cocco-bacilli*	short rod, rounded ends
Fragella Capsule	–	–	–	–	–	–
Motility	–	–	–	–	–	–
Culti-vation	anaerobic to microaerophilic					
In a medium of (Agar + sugar + vitamins)	round middle colonies					
Projection	semi-spherical, thick	semi-spherical, thick	semi-spherical, average	semi-spherical, thick	thin	semi-spherical, thick
Surface	smooth, moistened					
Circum-ference	plain					
Color	milky white, not trans-parent, mucous	milky white, not trans-parent, mucous	milky white, not trans-parent, mucous	milky white, not trans-parent, mucous	white, not trans-parent mucous	milky white, not trans-parent mucous

TABLE 15

(General properties)

	FERM–P Nos.					
	1946	2742	2779	2780	2781	2782
Ammonia-production	–	–	–	–	–	–
H_2S-production	–	–	–	–	–	–
Indole-production	–	–	–	–	–	–
Catalase-production	–	–	–	–	–	–
Pigment-production	–	–	–	–	–	–
Gelatin-liquefaction	–	–	–	–	–	–
Utilization of citric acid	–	–	–	–	–	–
Decomposition of urea	–	–	–	–	–	–
M.R. reaction	+	+	+	+	+	+
V.P. reaction	–	–	–	–	–	–
Reduction of nitrates	–	–	–	–	–	–

TABLE 16

(Ability to decompose sugars)

	FERM–P Nos.					
	1946	2742	2779	2780	2781	2782
Ribsose	–	+	+	+	+	+
Galactose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+
Raffinose	–	+	–	–	–	–
Melezitose	–	+	–	–	–	–
Mannitol	–	–	–	–	–	–

TABLE 17–1

Deodorization of animal excement
(directly applied to the excrement)

Strains	Animals	Fresh excrement Total weight: 2 g (%)	Control (no strain was added)	Strains added to excrement: mass of living cells (3 loopfuls) Period of cultivation after addition — hrs 24	48	72
	dogs	100	4	2'	2	1'
		20	4	2'	1	1'
	cows	100	4	1'	1'	1'
		20	4	1'	1'	1'
1946	pigs	100	4	2	2	1'
		20	4	2	2	1'
	hens	100	4	2	2	1'
		20	4	2	2	1'
	men	100	4	2'	2	1'
		20	4	2'	2	1'
	dogs	100	4	3	2'	2
		20	4	3	3	2'
	cows	100	4	2	1'	1'
		20	4	2	1'	1'
2742	pigs	100	4	3	3	2'
		20	4	3	3	2'
	hens	100	4	3	3	2'
		20	4	3	3	2'
	men	100	4	3	2'	2
		20	4	3	3	2
	dogs	100	4	3	2'	2
		20	4	3	3	2'
	cows	100	4	2	1'	1'
		20	4	2	1'	1'
2779	pigs	100	4	3	3	2
		20	4	3	3	2
	hens	100	4	3	3	2
		20	4	3	3	2
	men	100	4	3	2'	2
		20	4	3	3	3

TABLE 17–1 (Continued)

Strains	Animals	Fresh excrement Total weight: 2 g (%)	Control (no strain was added)	Strains added to excrement: mass of living cells (3 loopfuls) Period of cultivation after addition – hrs 24	48	72
	dogs	100	4	2'	2	1'
		20	4	2'	2	1'
	cows	100	4	2	1'	1'
		20	4	2	1'	1
2780	pigs	100	4	2'	2	1'
		20	4	2'	2	1'
	hens	100	4	2'	2	1'
		20	4	2'	2	1'
	men	100	4	2'	2	1'
		20	4	2'	2	1'
	dogs	100	4	3	2'	2
		20	4	3	2'	2
	cows	100	4	2	2	2
		20	4	2'	2'	2
2781	pigs	100	4	3	3	2
		20	4	3	3	2
	hens	100	4	3	3	2
		20	4	3	3	2
	men	100	4	3	2'	2
		20	4	2'	2'	2
	dogs	100	4	2'	2	1'
		20	4	2	2	1'
	cows	100	4	2	2	1'
		20	4	2	1'	1'
2782	pigs	100	4	3	2	1'
		20	4	2	2	1'
	hens	100	4	3	2	1'
		20	4	2	2	2'
	men	100	4	2'	2'	2
		20	4	3	2'	2

Note: Excrement (%) 100% : 100% of excrements
20% : 20% of excrements, 80% of water

Living cells: Three loopfuls of the strain cultivated on petri dishes were added to the excrement

Fresh excrement: excrement just discharged

TABLE 17–2

Deodorization of animal excrement
(directly applied to the excrement)

Strains	Animals	Fresh excrement Total weight: 2 g (%)	Control (no strain was added)	Strains added to excrement: Fermentation broth (5 cc) Period of cultivation after addition – hrs 24	48	72
1946	dogs	100	4	2	2	1'
		20	4	2	1'	1'
	cows	100	4	1	1'	1'
		20	4	2	2	1'
	pigs	100	4	2'	2	1'
		20	4	2'	2	1'
	hens	100	4	2'	2	1'
		20	4	2'	2	1'
	men	100	4	2'	2	1'
		20	4	2	2	1'
2742	dogs	100	4	3	3	2'
		20	4	3	3	3
	cows	100	4	2	1'	1'
		20	4	2	2	2
	pigs	100	4	2'	2	2
		20	4	2'	2	2
	hens	100	4	3'	3	2'
		20	4	3'	3	2'
	men	100	4	3	2'	2
		20	4	3	2'	2
2779	dogs	100	4	3	3	2'
		20	4	3'	3	3
	cows	100	4	2	1'	1
		20	4	2	2	2
	pigs	100	4	2'	2	2
		20	4	2'	2	2
	hens	100	4	3'	2'	2
		20	4	3'	2'	2'
	men	100	4	3	2'	2
		20	4	3	2'	2

TABLE 17–2 (Continued)

Strains	Animals	Fresh excrement Total weight: 2 g (%)	Control (no strain was added)	Strains added to excrement: Fermentation broth (5 cc) Period of cultivation after addition – hrs 24	48	72
2780	dogs	100	4	2'	2'	2
		20	4	2'	2'	2
	cows	100	4	2	1'	1'
		20	4	2	2	1'
	pigs	100	4	2'	2	1'
		20	4	2'	2	1'
	hens	100	4	2'	2	1'
		20	4	2	2	1'
	men	100	4	2'	2	2
		20	4	2	1'	1'
2781	dogs	100	4	3'	3	2'
		20	4	2	2	2'
	cows	100	4	2	2	2
		20	4	2	2	2
	pigs	100	4	2'	2	1'
		20	4	2'	2	2
	hens	100	4	2'	2	2
		20	4	3'	2	2'
	men	100	4	2'	2	2
		20	4	3	2	2
2782	dogs	100	4	2	1	1'
		20	4	2	2	1'
	cows	100	4	2	1'	1
		20	4	2	2	1'
	pigs	100	4	2'	2	1'
		20	4	2'	2	1'
	hens	100	4	2'	2	1'
		20	4	2'	2	2'
	men	100	4	2	2	1'
		20	4	2	2	2

Note: Fermentation broth: the strains were cultivated for 48 hours in test tubes, and 5 ml of the fermentation broth thereof were added to the excrement.

Table 18 shows the effects of the strains upon deodorization of excrement. The experiments were carried out by cultivating the representative six *Lactobacillus* strains of the present invention in the following medium and administering them orally to various animals and men.

Compositions for the medium employed: KH_2PO_4, $MgSO_4$ $7H_2O$, NaCl, $(NH_4)_2HPO_4$, $FeSO_4$ $7H_2O$, starch, $CaCO_3$, casamino acids, yeast extracts, Na_2S $9H_2O$, acetic acid, butyric acid, propionic acid, ammonia, indole, scatole, cystine and vitamins. After the strains had been cultivated in the medium, the living cells thereof were collected by centrifugation. The strains thus collected were in the form of wet cake and were mixed with bread or with butter, and administered orally at the dose of 0.5 g/kg (body weight). The effects upon deodorization of the excrements were estimated from the following of the administration.

The degree of deodorization is as follows through the present invention, 0. no odor; 1. slight odor; 1'. very little odor; 2. a little odor is noticed initially but soon fades away; 2'. a little odor; 3. the odor is less than that of the control group; 3'. the odor is only a little weaker than the strong odor of the excrement in the control group; 4. the odor of excrement *per se* in the control group.

TABLE 18–1

Deodorizing effects

Animals	Strains (FERM–P Nos.) 1946	2742
Dogs	The deodorizing effects appeared 2 days after the administration and continued for about 30 days. Thereafter the excrement recovered its peculiar odor gradually	The deodorizing effects appeared 2 days after the administration and continued for about 5 days. Thereafter the excrement recovered its peculiar odor gradually
Pigs	The deodorizing effects appeared 2 days after the administration and continued for about 20 days. Thereafter the excrement recovered its odor in the same manner as above	The deodorizing effects appeared 2 days after the administration and continued for about 3 to 4 days. Thereafter the excrement recovered its odor in the same manner as above
Hens	The deodorizing effects appeared 2 days after the administration and continued for about 15 days. Thereafter the excrement recovered its odor in the same manner as above	Same as above
Men	The deodorizing effects appeared 2 days after the administration and continued for about 15 days. Thereafter the excrement recovered its odor in the same manner as above	Same as above

Note: dogs: mean value of 50 dogs
pigs: mean value of 20 pigs
hens: mean value of 30 hens
men: mean value of 30 men

TABLE 18–2

Deodorizing effects

Animals	Strains (FERM–P Nos.) 2779	2780
Dogs	The strain showed almost the same effects as those of Strain No. 2742.	The deodorizing effects appeared 2 days after the administration, but was not strong and disappeared in 7 to 10 days.
Pigs	Same as above	Same as above
Hens	Same as above	The deodorizing effects appeared 2 days after the administration, but was not strong and disappeared in about 5 days.
Men	Same as above	Same as above

TABLE 18–3

Deodorizing effects

Animals	Strains (FERM–P Nos.) 2781	2782
Dogs	The strain showed almost the same deodorizing effects as Strain No. 2779, but the period of deodorization was a little shorter.	The strain showed almost the same deodorizing effects for almost the same period as those of Strain No. 2779.
Pigs	Same as above	Same as above
Hens	Same as above	Same as above
Men	Same as above	Same as above

From the above-mentioned biological properties and nutritional requirements thereof it is clear that the *Lactobacillus* strains of the present invention, except the properties common to the genus *Lactobacillus,* differ over a wide range in their properties such as nutritional requirements and the ability to decompose sugars. For example, some *Lactobacillus* strains of the invention may grow in (S—W) medium, and others may show a good growth in (S—W+vitamins) medium. It is generally known that once the technique for isolation of strains has been established, it becomes relatively easy to isolate many similar strains. The inventor has succeeded in isolating many deodorizing *Lactobacillus* strains and only those strains having different properties have been described herein. Other strains have been isolated by the inventor but these have not been described as they have the same properties as the described strains. Nevertheless, the *Lactobacillus* strains of the present invention isolated by the inventor and described in detail herein have common properties of biologically fundamental importance. Such common properties are the remarkably lower nutritional

requirements and faster growth rate as compared with the known *Lactobacillus* strains, the final yields, the peculiar sensitivity to S.N.C-compounds and such reactions to various amino acids and are not observed in the known Lactobacillus strains. Thus, the present invention is very significant in that the strains which are individually different in their properties have been found to have an extremely important common feature, namely that of deodorizing activity. Accordingly, with respect to the strains having this common property, the inventor has herein designated them as "the deodorizing *Lactobacillus* strains".

Attention is drawn to the Specification and claims of our co-pending British Patent Application No. 21343/77 (Serial No. 1,584,693) which relates to a deodorant composition utilising autotrophic bacteria.

WHAT WE CLAIM IS:—

1. A composition useful for culturing a *Lactobacillus* strain comprising (1) a *Lactobacillus* strain whose growth is enabled or promoted in a culture medium comprising S—W (Stephenson-Weltham) medium, S—W medium containing vitamins or S—W medium containing casamino acid, or in a culture medium selected from the above three media and further containing hydrogen sulphide, sodium sulphide, ammonia, a lower fatty acid or a sulfur-containing amino acid, and (2) a culture medium containing, as a main ingredient, a growth promoting amount or one or more of sulfur-containing amino acids, glycine, glutamic acid, lysine, alanine, phenyl-alanine, arginine, aspartic acid, hydrogen sulphide, sodium sulphide, ammonia and lower fatty acids.

2. A composition as claimed in claim 1, wherein the culture medium (2) includes S—W medium, S—W medium containing vitamins, S—W medium containing casamino acid or S—W medium containing vitamins and amino acids.

3. A composition as claimed in claim 1, wherein said sulfur-containing amino acid is selected from cystine, cystein and methionine.

4. A composition as claimed in any preceding claim, wherein said *Lactobacillus* strain is selected from FERM—P Nos: 1946, 2742, 2779, 2780, 2781 and 2782.

5. A composition useful for storing a *Lactobacillus* strain comprising (1) a *Lactobacillus* strain whose growth is enabled or promoted in a culture medium comprising S—W medium, S—W medium containing vitamins or S—W medium containing casamino acid, or in a culture medium selected from the above three media and further containing hydrogen sulphide, sodium sulphide, ammonia, a lower fatty acid or a sulfur-containing amino acid, and (2) one or more of sulfur-containing amino acids, glycine, glutamic acid, lysine, alanine, phenylalanine, arginine and aspartic acid, hydrogen sulphide, sodium sulphide, ammonia or lower fatty acids.

6. A composition as claimed in claim 5, wherein said *Lactobacillus* strain is stored in the presence of a sulfur-containing amino acid.

7. A deodorizing composition comprising (1) the living cells of a *Lactobacillus* strain whose growth is enabled or promoted in a culture medium comprising S—W medium, S—W medium containing vitamins or S—W medium containing casamino acid, or in a culture medium selected from the above three media and further containing hydrogen sulphide, sodium sulphide, ammonia, a lower fatty acid or a sulfur-containing amino acid, and (2) one or more compounds selected from sulfur-containing amino acids, glycine, glutamic acid, lysine, alanine, phenylalanine, arginine, aspartic acid, hydrogen sulphide, sodium sulphide, ammonia or lower fatty acids.

8. A composition as claimed in claim 1, 5 or 7, wherein said strain of *Lactobacillus* exhibits resistance to antibacterial compounds and spices.

9. A composition as claimed in claim 1, 5 or 7, wherein said strain of *Lactobacillus* exhibits antibiotic production.

10. A composition as claimed in claim 8, wherein said anti-bacterial compound is bile.

11. A composition as claimed in claim 5 or 7, wherein said strain is in a medium selected from S—W medium, S—W medium containing vitamins, S—W medium containing casamino acid or S—W medium containing vitamins and amino acids.

12. A composition comprising (1) the living cells of a *Lactobacillus* strain whose growth is enabled or promoted in a culture medium comprising S—W medium, S—W medium containing vitamins, or S—W medium containing casamino acid, or in a culture medium selected from the above three media and further containing hydrogen sulphide, sodium sulphide, ammonia, a lower fatty acid or a sulfur-containing amino acid, and (2) one or more compounds selected from sulfur-containing amino acids, glycine, glutamic acid, lysine, alanine, phenylalanine, arginine, aspartic acid, hydrogen sulphide, sodium sulphide, ammonia or lower fatty acids.

13. A composition as claimed in any one of claims 5 to 12, wherein the *Lactobacillus* strain is selected from FERM—P Nos. 1946, 2742, 2779, 2780, 2781 and 2782.

14. A composition as claimed in claim 5, wherein the *Lactobacillus* strain is one which has been obtained by lyophilization.

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5 SHEETS This drawing is a reproduction of the Original on a reduced scale
Sheet 1

FIG. 1

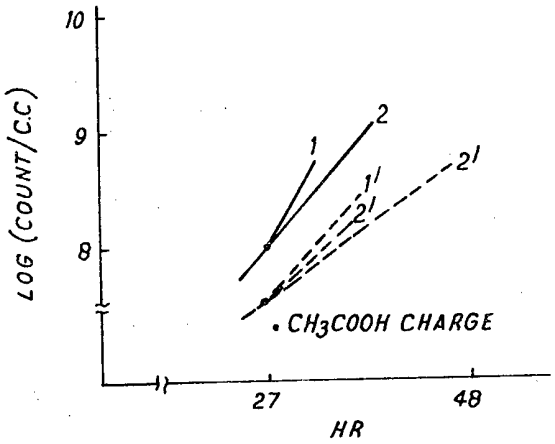


FIG. 2

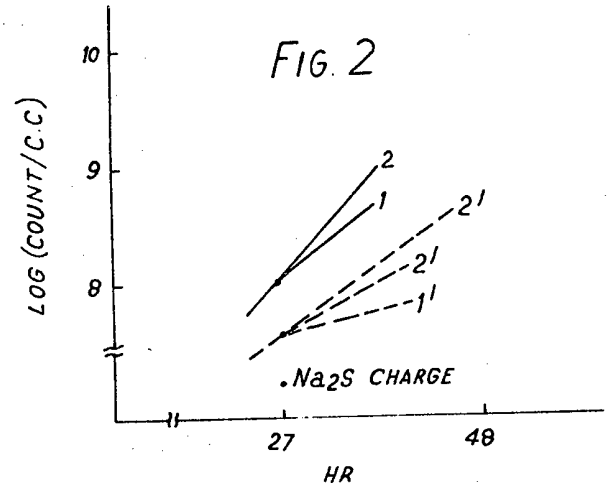


FIG. 3

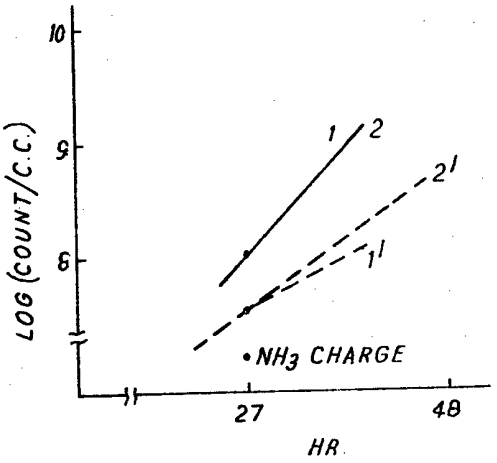

10
9
8
LOG (COUNT/C.C.)
1
2
2¹
1¹
•NH₃ CHARGE
27
48
HR.

FIG. 4

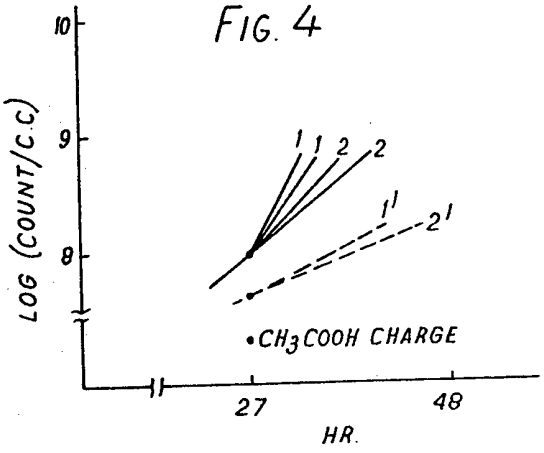

10
9
8
LOG (COUNT/C.C)
1
1
2
2
1¹
2¹
•CH₃COOH CHARGE
27
48
HR.

FIG. 5

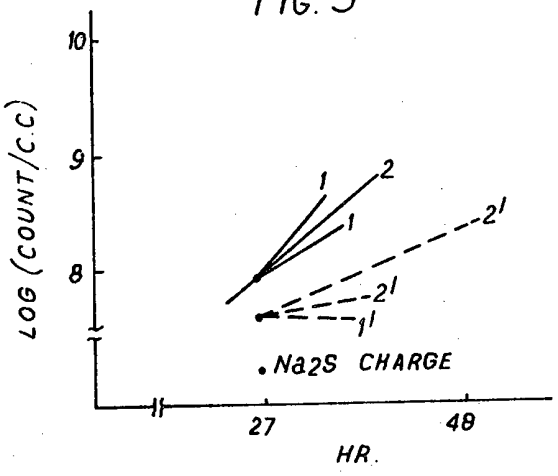

10
9
8
LOG (COUNT/C.C.)
1
2
1
2'
2'
1'
• Na2S CHARGE
27
48
HR.

FIG. 6

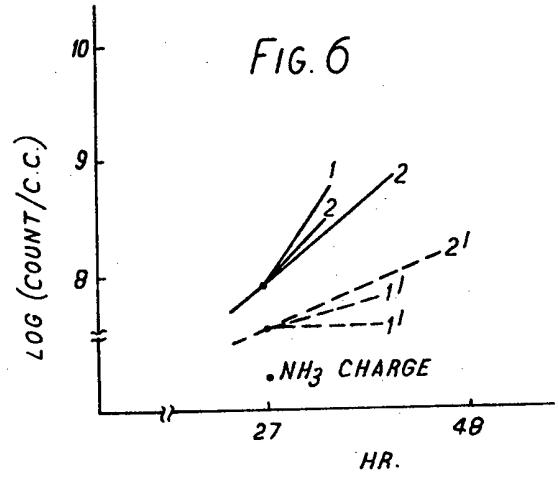

10
9
8
LOG (COUNT/C.C.)
1
2
2
2'
1'
1'
• NH3 CHARGE
27
48
HR.

FIG. 7

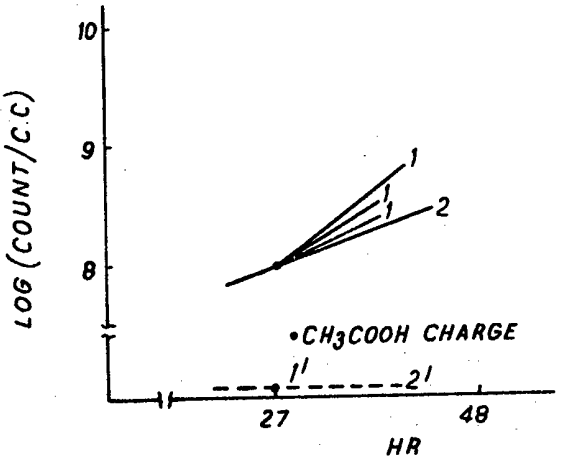
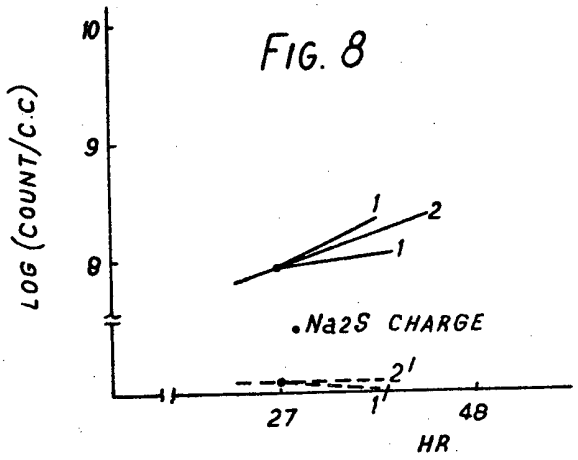

10
9
8
LOG (COUNT/C.C)
1
1
1
2
•CH3COOH CHARGE
1'
2'
27
48
HR

FIG. 8


10
9
8
LOG (COUNT/C.C)
1
2
1
•Na2S CHARGE
2'
1'
27
48
HR

1584694 COMPLETE SPECIFICATION
5 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 5

FIG. 9