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(54) **Title:** COMPOSITIONS COMPRISING PROBIOTIC LACTOBACILLUS STRAINS FOR IMPROVED VAGINAL HEALTH

(57) **Abstract:** The present invention relates to a composition comprising: one or more isolated probiotic bacterial strain selected from the group consisting of: a) Lactobacillus fermentum ssp mucosae TRF#36 deposited under number MTCC 5617; b) Lactobacillus gasserii TRF #8 deposited under number MTCC 5615; and c) Lactobacillus salivarius TRF #30 deposited under number MTCC 5616 or variants thereof; at least one or more pharmaceutically acceptable excipients; optionally one or more herbal extract and/or prebiotics.

COMPOSITIONS COMPRISING PROBIOTIC LACTOBACILLUS STRAINS FOR
IMPROVED VAGINAL HEALTH

TECHNICAL FIELD

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The present invention relates to probiotic lactobacillus strains, compositions comprising said strains and uses thereof.

BACKGROUND

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In course of evolution, some bacteria have evolved to co-exist with human tissues, particularly in cavities such as the gastrointestinal tract and vagina. These non pathogenic friendly bacteria contribute to maintain the health of the tissue on which they are colonized. Doderlein (Zentralbl Gynakol 18: 10-14, 1894), a German scientist
15 observed over a century back the presence of such micro-organisms in the vagina of women. Over time much has been learnt on the characteristics and function of these bacteria classified as "Probiotics".

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Probiotics comprise live microorganisms beneficial to the host organism. According to definition adopted by FAO/WHO, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". As far as the reproductive cavity of females is concerned, probiotic bacteria include the genus of *Lactobacillus* mostly and in postnatal years, bifidobacteria. The pH of normal healthy
25 vagina is maintained around 4 (instead of the pH 7.4 of normal blood) by virtue of lactic acid that these bacteria make and secrete. Also many, *but not all*, strains of Lactobacilli secrete H₂O₂, around them which could be construed to act as a local antiseptic. The acidic pH created by Lactobacilli discourages the growth of several pathogens. Besides that, various lactobacilli also make and secrete peptides, termed as Bacteriocins, with anti-microbial properties. Many, but not all, lactobacilli have the enzyme arginine
30 deiminase, which prevents the making of foul odour derivatives of this amino acid by other microbiota.

Lactobacilli Important for Healthy Vagina

Absence/Depletion of Lactobacilli in vagina disposes them to reproductive tract infections
35 (RTIs). A number of women suffer from recurring episodes of RTIs, vaginosis/vaginitis

caused by a range of aerobic, anaerobic micro-organisms. They have abnormal vaginal discharge with foul odour. Invariably their vaginal pH is above 5, indicating that the lactobacilli normally resident in healthy vagina have disappeared from their vagina.

- 5 Hence, there is a need for a composition comprising probiotic bacterial strains of the genera Lactobacillus, which have; an ability to colonize in human Vagina and produce high levels of lactic acid and have preferably the capability of producing and secreting H_2O_2 and have arginine deiminase to prevent the occurrence of foul odour. The composition is used for treatment and /or prophylaxis of vaginosis/ vaginitis, infections in
- 10 the vagina and sexually transmitted infections.

SUMMARY

An object of the present subject matter is to provide a composition comprising: isolated probiotic bacterial strains of the genera lactobacillus which have the ability to colonize in

15 human vagina and produce high levels of lactic acid to restore the acidic pH range of the vagina akin to that in normal vagina; at least one or more pharmaceutically acceptable excipient and/or prebiotics and/or polyherbal microbicide for treatment of vaginosis/ vaginitis, infections in the vagina, sexually transmitted diseases, infections endangering the foetus in pregnant women, preterm labour, urinary tract infection, and a microbial

20 infection of the reproductive tract.

Another object of the present subject matter is to provide novel probiotic bacterial strains of the genera lactobacillus which have the ability to colonize in human Vagina and produce high levels of lactic acid to restore the acidic pH range of the vagina akin to that

25 in normal vagina.

Another object of the present subject matter is to provide novel probiotic bacterial strains of the genera lactobacillus which in addition to producing high levels of lactic acid have the capability of producing and secreting H_2O_2 and/or have arginine deiminase to prevent

30 the occurrence of foul odour.

Another objective of the present invention is to provide novel probiotic bacterial strains of the genera lactobacillus which can be administered intra-vaginally or orally, and are capable of colonizing in human vagina.

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Another objective of the present invention is to provide novel probiotic bacterial strains of the genera lactobacillus which can be administered intra rectally or orally to benefit the urogenital tract health.

- 5 Another object of the present invention is to provide novel probiotic bacterial strains of the genera lactobacillus which prevent, alleviate the effects of, and/or treat vaginosis/vaginitis, infections in the vagina and sexually transmitted infections.

Accordingly, the subject matter described herein is directed to a composition comprising:

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one or more isolated probiotic bacterial strains selected from the group consisting of:

- a) Lactobacillus fermentum ssp mucosae TRF#36 deposited under number MTCC 5617;
- 15 b) Lactobacillus gasseri TRF #8 deposited under number MTCC 5615; and
- c) Lactobacillus salivarius TRF #30 deposited under number MTCC 5616 or variants thereof;

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at least one pharmaceutically acceptable excipients selected from the group comprising of diluent, binder, disintegrant, lubricant, glidant, stabilizer, surfactant, organic solvent, water, film forming polymer, opacifier, plasticizer, modified release polymer.

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The composition of the present invention can optionally comprise one or more herbal extract selected from the group comprising of curcumin, amla (*Emblica officinalis*) extract , Neem (*Azadirachta indica*) leaves extract , Aloe Vera extract, Sapindus mukerosii , rose water and/or prebiotics.

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Said bacterial strains have been deposited according to the Budapest Treaty at the MTCC in Chandigarh, India on June 1, 2011, and comprise the strain of:

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- (1) *Lactobacillus fermentum* ssp mucosae TRF#36 deposited under number MTCC 5617
- (2) *Lactobacillus gasseri* TRF #8 deposited under number MTCC 5615
- (3) *Lactobacillus salivarius* TRF #30 deposited under number MTCC 5616

BRIEF DESCRIPTION OF DRAWINGS:

These and other features, aspects, and advantages of the present invention will become better understood when the following detailed description is read with reference to the accompanying drawings in which like characters represent like parts throughout the drawings, wherein:

Fig. 1 illustrates a Genus specific PCR product (200bp amplicon) of 6 of the isolates obtained from the vagina of 80 women having healthy vagina.

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Fig. 2 illustrates results of a Species specific PCR for 10 species of Lactobacilli obtained from the vagina of 80 women with healthy vagina. The representative figure shows the profiles of 2 isolates of 6 species and 1 isolate of the rest 4 species viewed on 2% agarose gel.

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Lane S1 and S2 is *L. salivarius* strain's 400bp product. Lane R1 and R2 is *L. reuteri* strains's 300bp product. Lane P1 and P2 is *L. plantarum* strains' 250 bp amplicon. Lane F1 and F2 is *L. fermentum* strain's, 200bp amplicon. Lane G1 and G2 is *L. gasseri* strain's 350bp product. Lane C1 and C2 is *L. crispatus* strain's 500bp PCR product. Lane J1 is *L. Jensen/ls* 700bp amplicon. Lane A1 is *L. acidophilus* strain's 200bp PCR product. Lane PC is *L. paracasei* strain's 300bp product. Lane Rh1 is *L. rhamnosus strain's* 100bp product.

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Fig. 3 illustrates a 350 bp polymorphic sequence from 16S r DNA of one of the isolate *L. fermentum* TRF # 36 obtained from the vagina of 80 women with healthy vagina

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Fig. 4 illustrates a RAPD (Random Amplified Polymorphic DNA) profile of 7 strains of *L. salivarius*, 7 strains of *L. reuteri* and 6 strains of *L. fermentum* obtained from the vagina of 80 women with healthy vagina illustrating the heterogeneity of the strains in each species.

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DESCRIPTION:

The invention relates to a composition comprising probiotic bacterial strains of the genera lactobacillus which in addition to producing high levels of lactic acid have the capability of producing and/or secreting H₂O₂ and have arginine deiminase to prevent the occurrence of foul odour along with at least one pharmaceutically acceptable excipients and/or

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prebiotics and/or vaginal use polyherbal microbicide for treatment and /or prophylaxis of vaginosis/ vaginitis, infections in the vagina, sexually transmitted diseases, infections endangering the foetus in pregnant women, preterm labour, urinary tract infection, and microbial infections of the reproductive tract. The said bacterial strains are chosen from
5 the group comprising of;

(1) *Lactobacillus fermentum* ssp mucosae TRF#36 deposited under number MTCC
5617

(2) *Lactobacillus gasser* TRF #8 deposited under number MTCC 5615

10 (3) *Lactobacillus salivarius* TRF #30 deposited under number MTCC 5616

or a variant thereof

The phrase "*variant thereof*" as used herein in reference to the bacterial strains of the invention, especially those which have been deposited, is defined as a bacteria belonging
15 to the cluster having a RAPD similarity of at least 80 % to said probiotic bacterial strains; and/or having the ability to colonize human vagina; and/or producing high levels of lactic acid and have preferably the capability of producing and secreting H₂O₂ and / or have arginine deiminase.

20 The phrase "*probiotic bacterial strain(s)*" as used refers to bacterial strains which when administered in adequate amounts alone or in combination confer a health benefit on the host. In addition, the bacterial strains must colonize the *vagina* and produce lactic acid in high amounts.

25 In one embodiment of the invention the *probiotic* bacterial strain is *Lactobacillus fermentum* ssp mucosae TRF#36, which was deposited, under the provisions of the Budapest Treaty, at MTCC Chandigarh on June 1, 2011 and has been assigned accession number MTCC 5617, or a variant thereof.

30 In another embodiment of the invention the *probiotic* bacterial strain is *Lactobacillus gasser* TRF #8, which was deposited under the provisions of the Budapest Treaty, at MTCC Chandigarh on June 1, 2011 and has been assigned accession number MTCC 5615, or a variant thereof.

35 In still another embodiment of the invention the *probiotic* bacterial strain is *Lactobacillus*

salivarius TRF #30, which was deposited, under the provisions of the Budapest Treaty, at MTCC Chandigarh on June 1, 2011 and has been assigned accession number MTCC 5616, or a variant thereof.

- 5 In yet another embodiment the bacterial strains described above are used as a medicament.

In another aspect the present invention relates to the use of a bacterial strain as described above or a variant thereof along with an effective microbicide for treatment
10 and /or prophylaxis of vaginosis/ vaginitis, infections in the vagina, sexually transmitted diseases, infections endangering the foetus in pregnant women, preterm labour, urinary tract infection, and a microbial infection of the reproductive tract.

The invention further relates to a composition comprising:

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one or more isolated probiotic bacterial strain selected from the group consisting of:

- a) *Lactobacillus fermentum* ssp *mucosae* TRF#36 deposited under number MTCC 5617;
- 20 b) *Lactobacillus gasseri* TRF #8 deposited under number MTCC 5615; and
- c) *Lactobacillus salivarius* TRF #30 deposited under number MTCC 5616 or variants thereof;

at least one pharmaceutically acceptable excipients selected from the group
25 comprising of diluent, binder, disintegrant, lubricant, glidant, stabilizer, surfactant, organic solvent, water, film forming polymer, opacifier, plasticizer, modified release polymer.

In a preferred embodiment of the present Invention the composition comprises one or
30 more herbal extract selected from the group comprising curcumin , amla (*Emblica officinalis*) extract , Neem (*Azadirachta indica*) leaves extract , Aloe Vera extract , *Sapindus mukerosii* , rose water.

In a preferred embodiment of the present Invention the composition comprises herbal
35 extract as under:

Ingredient	Preferable Amount
curcumin	0.18 to 1.5 %
amla (<i>Embllica officinalis</i>) extract	1.5 to 15 %
Neem (<i>Azadirachta indica</i>) leaves extract	5 to 15 %
Aloe Vera extract	1.5 to 15 %
Sapindus mukerosii	0.5 to 2 %
Rose water	1.5 to 5 %

In another preferred embodiment the composition is administered along with BASANT, a polyherbai anti microbial composition comprising; Aloe vera, *Embllica officinalis extract*,
5 *Sapindus mukerosii*, Curcumin and Rose water.

In another preferred embodiment the composition further comprises prebiotics.

The preferred embodiment of the invention further relates to a composition comprising:

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at least two lyophilized probiotic bacterial strains selected from the group consisting of:

- a) Lactobacillus fermentum ssp mucosae TRF#36 deposited under number MTCC 5617;
- 15 b) Lactobacillus gasseri TRF #8 deposited under number MTGC 5615; and
- c) Lactobacillus salivarius TRF #30 deposited under number MTCC 5616 or variants thereof;

at least one pharmaceutically acceptable excipients selected from the group
20 comprising of diluent, binder, disintegrant, lubricant, glidant, stabilizer, surfactant, organic solvent, water, film forming polymer, opacifier, plasticizer, modified release polymer; and

one or more herbal extract selected from the group comprising of curcumin , amla
25 (*Embllica officinalis*) extract , Neem (*Azadirachta indica*) leaves extract , Aloe Vera extract Sapindus mukerosii , rose water and/or prebiotics.

The most preferred embodiment of the invention relates to a composition comprises:

at least 3 lyophilized probiotic bacterial strains selected from the group consisting of:

- 5
- a) *Lactobacillus fermentum* ssp *mucosae* TRF#36 deposited under number MTCC 5617;
 - b) *Lactobacillus gasseri* TRF #8 deposited under number MTCC 5615; and
 - c) *Lactobacillus salivarius* TRF #30 deposited under number MTCC 5616 or variants thereof;

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at least one pharmaceutically acceptable excipients selected from the group comprising of diluent, binder, disintegrant, lubricant, glidant, stabilizer, surfactant, organic solvent, water, film forming polymer, opacifier, plasticizer, modified release polymer; and

15

one or more herbal extract selected from the group comprising of curcumin , amla (*Emblica officinalis*) extract , Neem (*Azadirachta indica*) leaves extract , Aloe Vera extract, *Sapindus mukerosii* , rose water and/or prebiotics.

20 In another preferred embodiment the composition of the present invention further comprises at least an herbal extract selected from the group comprising purified curcumin , purified amla (*Emblica officinalis*) extract , Neem (*Azadirachta indica*) leaves extract , Aloe Vera extract, rose water or combination of these.

25 In another aspect of the present invention the diluents are selected from microcrystalline cellulose, directly compressible grade microcrystalline cellulose, lactose, starch, pregelatinized starch, calcium carbonate, calcium sulfate, sugar, dextrans, dextrin, dextrose, dibasic calcium phosphate dihydrate, tribasic calcium phosphate, magnesium carbonate, magnesium oxide, maltodextrin, mannitol and the like, preferably starch.

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In another aspect of the present invention the surfactant are selected from anionic surfactant, cationic surfactants and nonionic surfactant. Examples of the above-mentioned nonionic surfactant are, for instance, polyoxyethylene oxides, alkylamine oxides, polyoxyethylene alkyl ethers, polyoxyethylene alkyl phenyl ethers,

35 polyoxyethylene fatty acid esters, sorbitan fatty acid esters, polyoxyethylene sorbitan

fatty acid esters, glycerine esters, polyoxyethylene alkylamine, the derivatives thereof, and the like. Anionic surfactants are selected from higher fatty acid and its salt, alkyl sulfate, alkyl sulfonate, alkyl aryl sulfonate, alkyl phosphoric acid ester and the like, preferably Sodium lauryl sulphate.

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In another aspect of the present invention the lubricants are selected from Magnesium stearate, Sodium stearyl fumarate, Stearic acid, Talc, Colloidal silicon dioxide and the like, preferably Magnesium stearate.

10 In another aspect of the present invention the disintegrants are selected from alginic acid, croscarmellose sodium, sodium starch glycolate, crospovidone, polacrillin potassium, powdered cellulose, pregelatinized starch, sodium alginate and starch, preferably sodium starch glycolate or crospovidone .

15 In another aspect of the present invention the solvents are selected from purified water, alcohols, ketones, esters, ethers, halogenated solvents, hydrocarbons, nitrites, or mixtures thereof.

In yet another aspect of the invention, at least one *probiotic* bacterial strain is
20 encapsulated or coated, and preferably present in an amount giving, an effective daily dose of 10^3 - 10^{10} CFU. In yet another aspect of the invention, pharmaceutically acceptable excipients are selected from the group consisting of; Citric Acid; Sorbitol; Microcrystalline Cellulose; Sodium Starchglycolate; Starlac; Crospovidones; Sodium alginate and Magnesium Stearate.

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In still another preferred embodiment of the present invention, the composition comprises at least one of the lactobacilli selected from the group consisting of:

- 30 a) *Lactobacillus fermentum* ssp mucosae TRF#36 deposited under number MTCC 5617
b) *Lactobacillus gasseri* TRF #8 deposited under number MTCC 5615
c) *Lactobacillus sal/varius* TRF #30 deposited under number MTCC 5616

35 in the range of from 1×10^6 to 10×10^9 , preferably a total of 3×10^9 along with the following ingredients;

Ingredients	Range
curcumin	0.18 to 1.5 %
amla (<i>Embllica officinalis</i>) extract	1.5 to 15 %
Neem (<i>Azadirachta indica</i>) leaves extract	5 to 15 %
Aloe Vera extract	1.5 to 15 %
Sapindus mukerosii	0.5 to 2 %
Rose water	1.5 to 5 %
Citric Acid	2-6%
Sorbitol	2-10%
Microcrystalline Cellulose	15-30%
Sodium Starchglycolate	5-15%
Starlac	15-30%
Crospovidones	0.5-5%
Sodium alginate	1-10%
Magnesium Stearate	0.25-2%

In one embodiment said pharmaceutical composition is administered vaginally along with
5 pharmaceutically acceptable excipients and one or more herbal extracts.

In a further embodiment said pharmaceutical composition is administered orally.

In another embodiment said pharmaceutical composition is administered in the form of
10 vaginal tablets, vaginal cellulose capsules, vaginal cream, gel, ointment, lotion, pessaries.

In another embodiment of the invention the composition is administered via a rectal
suppository for treatment and relief from the symptoms of urogenital infections.

15 Another embodiment of the present invention relates to a hygiene product comprising
composition as described above. In one embodiment said hygiene product is chosen from
the group comprising tampons, sanitary napkins, sanitary pads, diapers.

In one embodiment said *probiotic* bacterial strain is viable. In still another embodiment it is genetically modified.

DESCRIPTION OF EMBODIMENT

5 With the consent of women and approval of the Institutional Ethics Committee, swabs were taken from the vagina of 80 women with healthy vagina in Delhi attending the Antenatal Clinics. These were cultured in MRS Broth.

Morphologically the isolates were rods. All of them were Gram positive, catalase negative.

10 They were identified at molecular level by Polymerase Chain Reaction (PCR) employing genus specific primers. Figure 1 illustrates the result of a Genus specific PCR product of 6 of the isolates. All 80 isolates gave ~ 200 bp amplicon. The isolates were further identified by a Polymerase Chain Reaction (PCR) employing species specific primers. Figure 2 shows the profiles of 2 isolates of each of 6 species and 1 isolate of 4 species
15 viewed on 2% agarose gel.

The identification of the species as revealed by the above investigation was confirmed by gene sequencing of 16s rDNA and Fig 3 gives as an illustration of such confirmation at genetic level of *L fermentum* TRF#36.

20

These investigations revealed that the most predominant Lactobacilli in Indian women are *Lfermentum*, *Lreuteri*, *L.salivarius* and *Lplantarum* belonging to Group IV (Garg et al, Ind. J. Med. Res. 2009, 129(6), 652-657), whereas Group II consisting of *L crispatus*, *L. jensenii*, and Group III consisting of *L. rhamnosus* are the most predominant
25 Lactobacilli reported from vagina of women in USA, Canada, and Sweden {Vasquez et al, J Clin Microbiol (2002) 40(8): 2746-2749; Vallor et al, J Infect Diseas (2001) 184: 1431-1436; Burton et al, Appl Environ Microbiol (2003) 69: 97-101}.

Thus, the probiotics for replenishment of vagina in India may be preferably chosen from
30 the species prevalent in the country, although there is no reason to believe that once exposed, women elsewhere cannot benefit from colonization of Lactobacilli strains of the present invention.

After conducting extensive studies on 80 isolates form ecohealthy vagina of women, the
35 following 3 strains were found highly beneficial:

(1) *Lactobacillus fermentum* ssp mucosae TRF#36 deposited under number MTCC 5617

(2) *Lactobacillus gasser*/TRF #8 deposited under number MTCC 5615

5 (3) *Lactobacillus salivarius*IR? #30 deposited under number MTCC 5616

They have good growth rates. They are high producers of D lactic acid. *Lactobacillus fermentum* and *L. gasser* make and secrete H_2O_2 . They are highly hydrophobic as measured by Microbial adhesion to hydrocarbon (MATH) assay. They have arginine deiminase to prevent the occurrence of foul odour.

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L. fermentum TRF #36 and *L. gasser* TRF #8 are H_2O_2 producers. H_2O_2 has antiseptic and anti-viral properties. Peroxidase, H_2O_2 and halides constitute a strong anti-microbial system in phagocytes and tissue fluids (Klebanoff, 3 Bacteriol 1968; 95:2131-2138).

15 Peroxidase and halide ions are already present in the vaginal fluid. In vitro, H_2O_2 producing *Lactobacillus* strains have strong inhibitory action against *Gardnerella vaginalis*, *Prevotella bivia*, *Neisseria gonorrhoeae* and HIV-1 (Klebanoff et al, J Infect Diseases 1991; 164: 94-100).

20 The strains *L. fermentum* TRF#36, *L. salivarius*IR? #30 and *L. gasser* TRF #8 produce arginine deiminase which prevents the production of foul smelling derivatives of arginine. The enzyme has anti-viral activity against HIV-1 (Kubo et al, J General Virol 2006; 87:1589-1593). Furthermore, it prevents the formation of polyamines. Polyamines like spermine, spermidine, putrescine and cadaverine are found in elevated concentration in

25 vaginal discharge of women with bacterial vaginosis contributing to elevated vaginal pH. These polyamines account for transudation and exfoliation of the epithelial cells thereby causing copious discharge.

All the three strains namely, *L. fermentum* TRF # 36, *L. salivarius* TRF # 30 and *L. gasser* TRF # 8 produce high amount of lactic acid sufficient to acidify the vagina. Healthy vagina has a pH of 3.8-4.5. Vaginal pH of women with bacterial vaginosis is elevated >5 because of depletion of resident lactobacilli of good properties.

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All the three strains namely, *L. fermentum* TRF # 36, *L. salivarius* TRF # 30 and *L. gasser* TRF # 8 are resistant to antibiotics metronidazole, clotrimazole, vancomycin,

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ciprofloxacin up to concentrations usually employed for therapy against banal infections. They are also tolerant to Polyherbal microbicide Praneem, Basant and Nauroz. Hence, these polyherbal microbicides can be employed for prevention of STIs without affecting the presence of these lactobacilli in the vagina. The selected strains are however sensitive to antibiotics, clindamycin, amoxicillin, erythromycin.

Clinical Evaluation of Colonization Potential and Efficacy of bacterial strains to Protect against RTIs.

Clinical trials were conducted with the permission of the Drugs Controller General of India and Institutional Ethics committees in 2 premiere Medical Institutions viz The All India Institute of Medical Sciences and Sir Gangaram Hospital, New Delhi. These trials were conducted in women with recurring episodes of vaginosis/vaginitis with vaginal pH > 5. The objective of the trials was to determine the colonization potential of the selected probiotics and their ability to maintain the pH of vagina and protect women from getting recurrence of reproductive tract infections such as vaginosis /vaginitis.

To restore the Reproductive Health of women, a composition comprising the isolated probiotic bacterial strain was administered intra-vaginally in women. The composition comprised 3 billion Probiotics of either one or more than one selected strains of Lactobacillus TRF # 36, TRF # 30 and TRF # 8 along with purified diferuloyl methane {(E,E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione} commonly known as curcumin, purified extract of Embilca officinalis (Amla), purified extracts of Neem leaves and Aloe vera. These ingredients are formulated in pharmaceutically approved excipients selected from the group comprising of: Citric Acid, Sorbitol, Microcrystalline Cellulose, Sodium Starchglycolate, Starlac, Crospovidones and Sodium alginate. This formulation is easily insertable in the vagina, with washed fingers, when dispensed in a cellulose capsule, the preferred mode of delivery.

The composition comprises 3×10⁹ lactobacilli alone or combination of 2 or 3 of selected strains of Lactobacillus TRF # 36, TRF # 30 and TRF # 8 along with;

Ingredients	Range	Preferably
Curcumin	0.25-1.5 %	1%

Amla	2.5-15%	5%
Neem leaves extract	5-15%	6%
Aloe vera	2.5-15%	5%
Citric Acid	2-6%	4%
Sorbitol	2-10%	5%
Microcrystalline Cellulose	15-30%	28%
Sodium Starchglycolate	5-15%	10%
Starlac	15-30%	24%
Crospovidones	0.5-5%	3%
Sodium alginate	1-10%	6%
Magnesium Stearate	0.25-2%	1%

Typical observations on efficacy and utility of the Formulation are indicated by the observations given below in women suffering from recurring episodes of vaginosis/vaginitis, whose vaginal pH was above 5 at the time of enrolment.

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A few illustrative examples of efficacy of administration of these bacilli given alone or in combination are given below.

10 **Table 1: Follow up chart of subject (APB) who after treatment with Polyherbal microbicide BASANT was given a single Probiotic selected strain.**

Day	Bacterial vaginosis	pH of vagina	Nugent Score	Product Administered	Administered Probiotic (s) detectable in vagina
Day 1	+	6.0	8	Basant 14 tablets	-
Day 19	-	4.5	5	<i>L.fermentum</i> TRF#36 (3×10^9 lyophilized bacilli)	-
Day 22	-	4.5	0		+

Day 26	-	4.5	1		+
Day 29	-	4.5	0		+
Day 36	-	4.5	1		+
Day 53	-	4.5	2		+
Day 54	-	4.5	2		+

Table 2: Follow up chart of subject (Asukh)

Day	BV	pH	Nugent Score	Product Administered	Probiotic detectable in vagina
Day 1	+	7.1	8	Basant 7 tablets	-
Day 8	+	4.5	4	<i>L.gasseri</i> TRF#8 (3×10^9 lyophilized bacilli)	-
Day 12	-	4.5	2		+
Day 20	-	4.5	1		+
Day 27	-	4.5	2		+
Day 55		4.5	0		+
Day 83		4.5	1		+

Table 3: Follow up chart of subject (G)

Day	BV	pH	Nugent Score	Product Administered	Probiotic detectable in vagina
Day 1	+	5.0	7	Basant 14 tablets	-

Day 22	-	5.0	4	<i>L. salivarius</i> TRF#30 (3x10 ⁹ lyophilized bacilli)	-
Day 40	-	4.5	2		+
Day 53	-	4.3	2		+
Day 82	-	4.5	3		-

Table 4: Follow up chart of subject (RD)Day	BV	pH	Nugent Score	Product Administered	Probiotic detectable in vagina
Day 1	+	6.1	7	Basant 14 tablets	-
Day 26	-	4.5	3	Double Combination <i>L.fermentum</i> TRF#36 + <i>L.gasseri</i> TRF#8 (Total 3x10 ⁹ lyophilized bacilli)	-
Day 40	-	4.5	1		+(<i>L.fermentum</i> TRF#36 & <i>L.gasseri</i> TRF#8)
Day 68	-	4.5	0		+(<i>L.fermentum</i> TRF#36 & <i>L.gasseri</i> TRF#8)

Day 83	-	4.5	0		+(<i>L.fermentum</i> TRF#36& <i>L.gasseri</i> TRF#8)
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Table 5: Follow up chart of subject (UD):

Day	BV	pH	Nugent Score	Product Administered	Probiotic detectable in vagina
1 day	+	5.0	8	Basant 14 tablets	-
22 day	-	4.5	5	Triple Combination <i>L.fermentum</i> TRF#36 + <i>L.gasseri</i> TRF#8 and <i>L. Salivarius</i> TRF#30 (Total 3×10 ⁹ lyophilized bacilli)	-
28 day	-	4.5	3		<i>TRF#36</i> & <i>TRF#8</i> & <i>TRF#30</i>
49 day	-	4.5			<i>L.fermentum</i> TRF#36 & <i>L.gasseri</i> TRF#8

5 **Table 6: Follow up chart of Subject with Code: BV-138/IIB**

	Initial at Enrollment (Day 1)	Follow up after giving polyherbal BASANT along with Probiotics Triple combination Capsules.
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		After 20 days	After 30 days
Symptom Abnormal Vaginal Discharge	++	-	-
Discharge-Amount	++	-	-
Colour	-	-	-
Odour	-	-	-
Quality	Watery	-	-
Itching/Burning	+	-	-
pH of vagina	6.0	4.5	4.5
Subjective Relief from Itching, Burning and Discharge		90% Relief highly satisfied	>90% Relief fully satisfied
Side Effects		None	None

- These trials have amply shown the merit of 3 of these selected *Lactobacillus* strains to colonize for periods of 1-3 months in the vagina lacking lactobacillus after a single intra-vaginal administration of 3×10^9 lyophilized bacilli. The strains are *Lactobacillus fermentum* strain TRF#36, *Lactobacillus gasseri* strain TRF #8 and *Lactobacillus salivarius* strain TRF #30. The combination of TRF#36 and TRF#8 maintained the vagina healthy at pH 4 free of recurrence of vaginosis in women getting recurring episodes of infections for longer than 1 month and in some recipients up to 3 months of observation period.
- 10 Although each one of the three selected Probiotics strain have ability to colonize vagina of women suffering from recurring episodes of vaginosis/vaginitis, combination of two is more effective. The most effective results were obtained with a triple combination of the three selected strains. The Table given below provides a summary statement:
- 15 **Table 7: Table shows properties of Probiotic bacterial strains of the present invention when administered alone or in combination for their ability to**

colonize and restore pH to acidic range in women suffering recurrently from vaginosis.

Probiotic Strain	Properties					
	D-Lactic Acid Production	H ₂ O ₂ Secretion	Ability to clear Foul odour	Hydrophobicity	Colonization	pH Restoration
<i>Lactobacillus fermentum</i> TRF#36	+	+	+	+	++	+
<i>Lactobacillus salivarius</i> TRF#30	++	-	+	+	+	+
<i>Lactobacillus gasseri</i> TRF#8	+	+	+	+	++	+
TRF#36 and TRF#8	++	+	+	+	++	++
TRF#36 and TRF#30 and TRF#8	+++	+	+	+	+++	+++

5 In table 1 and 7

"-" indicates- "Negative result"

"+" indicates- "Positive result"

"++" indicates- "a positive result that is better than "+"

"+++" indicates- "a positive result that is better than "++"

10 The best results were obtained when the 3 probiotic strains of the invention were used in combination.

Although the invention has been described with reference to specific embodiments, this description is not meant to be construed in a limiting sense. Various modifications of the disclosed embodiments, as well as alternate embodiments of the invention, will become

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apparent to persons skilled in the art upon reference to the description of the invention. It is therefore contemplated that such modifications can be made without departing from the spirit or scope of the present invention as defined.

We claim:

1  A composition comprising:

5 one or more isolated probiotic bacterial strain selected from the group consisting of:

- a) Lactobacillus fermentum ssp mucosae TRF#36 deposited under number MTCC 5617;
- b) Lactobacillus gasseri TRF #8 deposited under number MTCC 5615; and
- 10 c) Lactobacillus salivarius TRF #30 deposited under number MTCC 5616 or variants thereof;

at least one or more pharmaceutically acceptable excipients selected from the group comprising of diluent, binder, disintegrant, lubricant, glidant,

15 stabilizer, surfactant, organic solvent, water, film forming polymer, opacifier, plasticizer, modified release polymer; and

optionally one or more herbal extract or combination selected from the group comprising of curcumin, amla (*Emblica officinalis*) extract , Neem (*Azadirachta indica*) leaves extract , Aloe Vera extract , Sapindus mukerosii , rose water and/or prebiotics.

20

2. The composition as claimed in claim 1, wherein the probiotic bacterial strains are present in the range of from 1×10^6 to 10×10^9 .

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3. The composition as claimed in claim 1, wherein any two of said lyophilized probiotic bacterial strains are in consortium.

4. The composition as claimed in claim 1, wherein all 3 of said lyophilized probiotic bacterial strains are in consortium.

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5. The composition as claimed in any of the claims 1 to 4, wherein curcumin is present in the in the range of from 0.18 to 1.5 %, amla (*Emblica officinalis*) extract is present in the range of from 1.5 to 15 %, Neem (*Azadirachta indica*) leaves extract is present in the of from 5 to 15 %, Aloe Vera extract is present in

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the range of from 1.5 to 15 %, Sapindus mukerosii is present in the range of from 0.5 to 2 %, rose water is present in the range of from 1.5 to 5 % .

- 5 6. The composition as claimed in any of the claims 1 to 4, wherein the diluents are selected from the group comprising of; microcrystalline cellulose, directly compressible grade microcrystalline cellulose, lactose, starch, pregelatinized starch, calcium carbonate, calcium sulfate, sugar, dextrans, dextrin, dextrose, dibasic calcium phosphate dihydrate, tribasic calcium phosphate, magnesium carbonate, magnesium oxide, maltodextrin, mannitol.
- 10 7. The composition as claimed in any of the claims 1 to 4, wherein the binders are selected from the group comprising of; acacia, alginic acid, carbomer, carboxymethylcellulose sodium, dextrin, ethyl cellulose, guar gum, hydrogenated vegetable oil, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, microcrystalline cellulose, liquid glucose, magnesium aluminum silicate, maltodextrin, methylcellulose, polymethacrylates, povidone, pregelatinized starch, sodium alginate, sorbitol, and starch.
- 15 8. The composition as claimed in any of in any of the claims 1 to 4, wherein the surfactant may be selected from the group comprising of anionic surfactant, cationic surfactants and non ionic surfactant.
- 20 9. The composition as claimed in any of the claims 1 to 4, wherein the lubricants are selected from magnesium stearate, sodium stearyl fumarate, stearic acid, talc, colloidal silicon dioxide.
- 25 10. The composition as claimed in any of the claims 1 to 4, wherein the disintegrants and stablizer are selected from alginic acid, croscarmellose sodium, sodium starch glycdlate, crospovidone, polacrilin potassium, powdered cellulose, pregelatinized starch, sodium alginate, starlac and starch.
- 30 11. The composition as claimed in any of the claims 1 to 4, wherein the solvents are selected from purified water, alcohols, ketones, esters, ethers, halogenated solvents, hydrocarbons, nitrites, or mixtures thereof.

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12. The composition as claimed in claim 1, wherein said one or more of the lactobacilli are in the range of from 1×10^6 to 10×10^9 ;

5 Said pharmaceutically acceptable excipients are, citric acid preferably in the range of from 2 to 6%; sorbitol preferably in the range of from 2 to 10%; microcrystalline cellulose preferably in the range of from 15 to 30% ; sodium starchglycolate preferably in the range of from 5 to 15%; starlac preferably in the range of from 15 to 30%; crospovidones preferably in the range of from 0.5 to 5%; sodium alginate preferably in the range of from 1 to 10% and magnesium stearate preferably in the range of from 0.25 to 2%;

15 Said herbal extracts are, curcumin preferably in the range of from 0.18 to 1.5 %, amla (*Emblica officinalis*) extract preferably in the range of from 1.5 to 15 %, neem (*Azadirachta indica*) leaves extract preferably in the of from 5 to 15 %, Aloe Vera extract preferably in the range of from 1.5 to 15 % optionally also Sapindus mukerosii preferably in the range of from 0.5 to 2 %, rose water preferably in the range of from 1.5 to 5 %.

20 13. The composition as claimed in claim 12, comprising

3×10^9 of said lactobacilli strains alone or in combination;

25 4 % citric Acid; 5% sorbitol; 28% microcrystalline cellulose; 10 % sodium starchglycolate; 24 % starlac; 3% crospovidones; 6% sodium alginate; 1% magnesium stearate;

30 1% curcumin; 5 % amla extract; 6 % Neem leaves extract and 5 % Aloe Vera extract.

14. The composition as claimed in claim 12, comprising 3×10^9 lactobacilli alone or combination of said three selected strains of Lactobacillus.

35 15. The composition as claimed in claim 1 to 14, wherein the probiotic bacterial strains are encapsulated or coated.

16. The composition as claimed in any of the preceding claims, wherein the probiotic bacterial strains are present in an amount giving an effective dose of from 10^3 to 10^{10} CFU.
- 5
17. The composition as claimed in any of the preceding claims, wherein said composition is capable of vaginal or oral administration.
18. The composition as claimed in any of the preceding claims, wherein said composition is in a form selected from the group consisting of vaginal tablets, vaginal vegetarian (cellulose) capsules, cream, gel, ointment, lotion, pessaries.
- 10
19. The composition as claimed in claims 1 to 17, wherein said composition is in the form of a rectal suppository.
- 15
20. A hygiene product comprising the composition claimed in any one of the preceding claims.
21. The hygiene product as claimed in claim 20, wherein said hygiene product is selected from the group comprising of tampons, sanitary napkins, sanitary pads, diapers.
- 20
22. An isolated *Lactobacillus fermentum* ssp mucosae TRF#36 deposited under number MTCC 5617 or variants thereof.
- 25
23. An isolated *Lactobacillus gasseri*R? #8 deposited under number MTCC 5615 or variants thereof.
24. An isolated *Lactobacillus salivarius*IK? #30 deposited under number MTCC 5616 or variants thereof.
- 30
25. A consortium of one or more of the isolated bacteria claimed in claim 22 to 24.



Fig 1

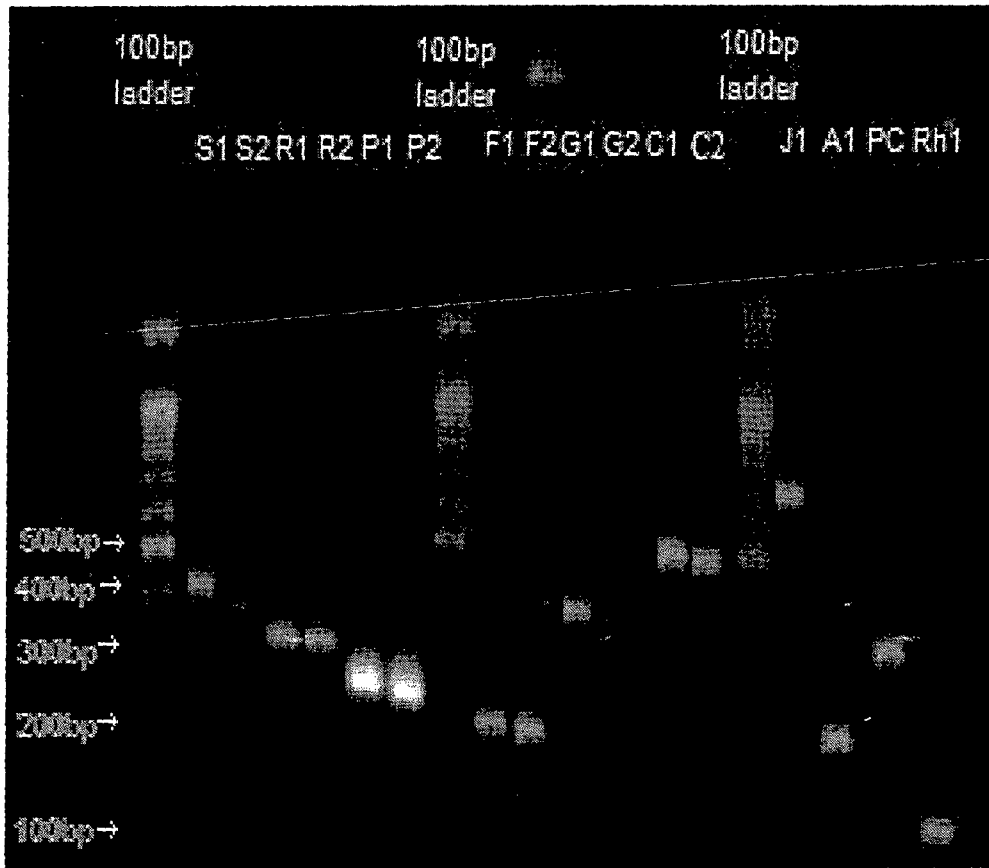


Fig 2

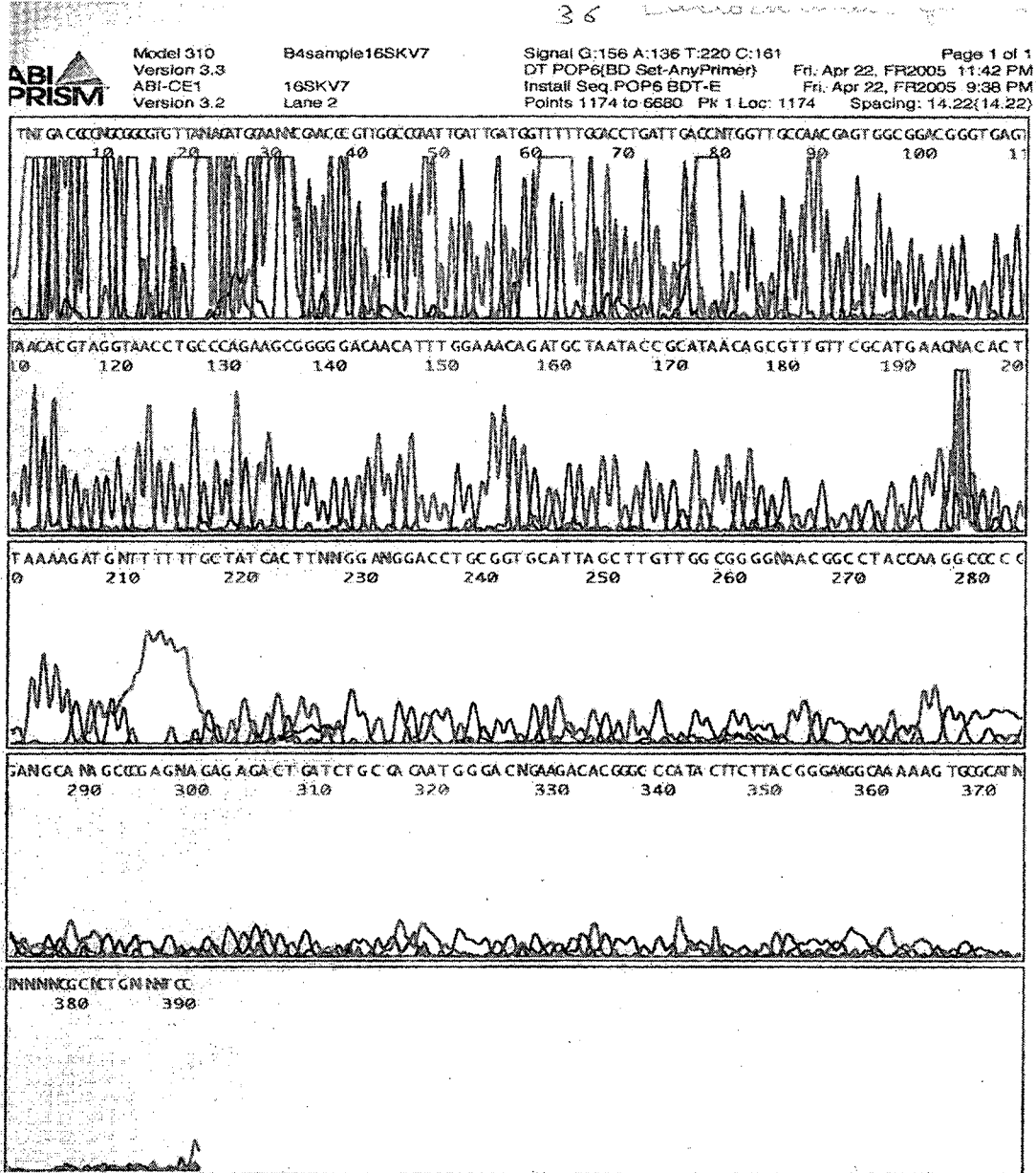


Fig 3

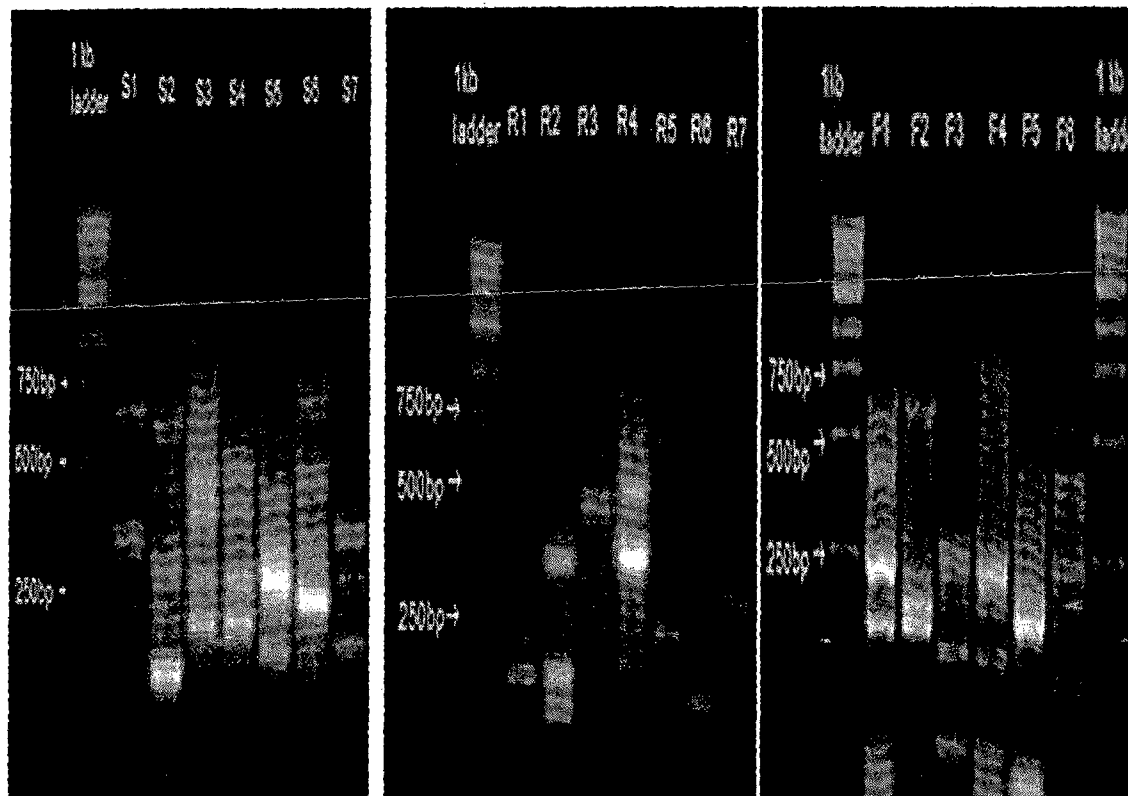


Fig 4