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(54) Title: NODULE SPECIFIC MEDICAGO PEPTIDES HAVING ANTIMICROBIAL ACTIVITY AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME

(57) Abstract: The present invention relates to the use of at least one peptide originated from *Medicago truncatula* nodules, comprising the SEQ IDs NO: 1 - 463 or at least one peptide having a sequence derived from said SEQ IDs NO: 1 - 463 by deletion of about 9 to about 44 contiguous amino acids, from the N-terminal part of the peptide, in particular peptides having the SEQ IDS NO: 464 to 925, for the preparation of a drug intended for the treatment of human, animal or plant diseases induced by microorganisms, wherein said peptides have a broad-spectrum and fast antibiotic activity, in particular killing of the bacteria within 1 to 3 hours.



WO 2010/146067 A1

NODULE SPECIFIC MEDICAGO PEPTIDES HAVING ANTIMICROBIAL ACTIVITY AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME

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The invention relates to nodule-specific Medicago peptides having antimicrobial activity and pharmaceutical compositions containing the same.

Symbiotic nitrogen fixation takes place in particular plant root organs named nodules. Nodule formation on plants of the Leguminosae family is a result of consecutive interactions with
10 bacteria of the Rhizobiaceae family (rhizobia). The interaction is considered mutually beneficial.

The bacteria within the nodule cells gain the ability to fix nitrogen gas by means of their nitrogenase enzyme complex and supply the host plant with the reduced nitrogen for plant growth. The plant provides photosynthates to the bacteria and a microaerobic niche for the oxygen-sensitive nitrogenase. Nodule development is induced by lipochitooligosaccharide
15 signals of rhizobia, called Nod factors.

Nodules are formed on a particular host only in response to compatible rhizobia producing Nod factors with the adequate chemical structure (Dénarié, J., Debellé, F. & Promé, J.-C. (1996) *Annu. Rev. Biochem.* **65**, 503–535). This is one of the major causes of the generally pronounced host specificity in *Rhizobium*–legume symbiosis. Nod factors induce cell divisions in the root
20 cortex and successive divisions lead to the formation of the nodule primordium. Simultaneously, the rhizobia enter the host plant via the root hairs through the formation of tubular structures called infection threads which traverse the root epidermis and cortex and then the nodule primordium.

Rhizobia are released from infection threads in the cytoplasm of postmitotic nondividing
25 cells by endocytosis. The term “bacteroid” refers to these intracellular membrane encapsulated bacteria. In legumes of the Papilionoideae subfamily, the nodules can be of either the determinate or the indeterminate type (Franssen, H. J., Vijn, I., Yang, W. C. & Bisseling, T. (1992) *Plant Mol. Biol.* **19**, 89–107). In the case of determinate nodules, the initial cell division activity required for nodule primordium formation ceases rapidly and therefore the determinate nodules
30 contain no meristem. Differentiation of infected cells occurs synchronously and the mature nodule contains symbiotic cells with a homogenous population of nitrogen-fixing bacteroids

(Franssen, H. J., Vijn, I., Yang, W. C. & Bisseling, T. (1992) *Plant Mol. Biol.* **19**, 89–107). Legumes such as bean (*Phaseolus vulgaris*) or *Lotus japonicus* form this type of nodules.

In contrast, cell division activity in the indeterminate nodules is maintained and forms an apical meristem (nodule zone I). Because the size of the meristem is constant, cell division activity and production of new sets of meristematic cells are balanced with the exit of the same number of cells from the mitotic cell cycle. These postmitotic cells are unable to divide and enter the nodule differentiation program. The infection thread penetrates into the submeristematic cells and liberates the rhizobia. In the infected cells, both partners differentiate progressively along the 12–15 cell layers of the infection zone (or zone II), ending in the formation of nitrogen fixing cells that will constitute the constantly growing nodule zone III (2). Legumes of the galegoid clade (such as *Medicago* spp., *Vicia sativa*, and *Pisum sativum*) are examples of plants forming indeterminate nodules.

In *Medicago truncatula* nodules several hundreds of peptide coding genes are expressed (Kevei et al., 2002, *Mol.Plant-Microbe Interact.*, 15, 922-931; Mergaert et al., 2003, *Plant Physiol.* 132, 161-173; Alunni et al., 2007, *Mol. Plant-Microbe Interact.* 20, 1138-1148). Production of these peptides in the infected plant cells and their targeting to the bacteroids (Van de Velde et al., 2010) correlate with irreversible terminal differentiation of bacteroids involving genome amplification, cell elongation and branching, loss of cell division capacity and increase in membrane permeability (Mergaert et al., 2006, *Proc. Natl. Acad. Sci. USA*, 103(13):5230-5).

Natural products are the sources of most antibiotics, however the antimicrobial potential of plant peptides has been largely unexplored.

Nowadays, we face the danger of the emergence of antibiotic resistant bacteria as a consequence of misuse of traditional antibiotics that places selection pressure on the bacterium strains. Antimicrobial peptides are rarely used as antibiotics. Moreover, antibiotics such as polymyxins (cyclic peptide with hydrophobic tail) have adverse effects such as nephrotoxic and neurotoxic (Falagas ME, Kasiakou SK; February 2006; "Toxicity of polymyxins: a systematic review of the evidence from old and recent studies". *Crit Care* **10** (1): R27) and, though effective, are therefore used only if less toxic antibiotics are ineffective or are contraindicated. Its typical use cases are infections with strains of *Pseudomonas aeruginosa* or Enterobacteriaceae species that are highly resistant to other types of antibiotics such as cephalosporins. Further, polymyxins

are not absorbed from the gastrointestinal tract and therefore must be administered intravenously and active only against Gram - bacteria.

Thus there is a growing need for the discovery of new safe antibiotics with improved efficacy against multiple strains of bacteria, in particular the antibiotic resistant ones, that are administrable by oral route, nasal inspiration or topical (local surface) treatments for example in case of skin infections.

One of the aims of the invention is to provide peptides having broad-spectrum antibiotic activity.

Another aim of the invention is to provide peptides having further fungicidal and antiviral activities.

Still another aim of the invention is to provide pharmaceutical compositions comprising said peptides presenting no adverse side effects.

The present invention relates to the use of at least one peptide originated from *Medicago truncatula* nodules, comprising the SEQ IDs NO: 1 – 463 or at least one peptide having a sequence derived from said SEQ IDs NO: 1 – 463 by deletion of about 9 to about 44 contiguous amino acids, from the N-terminal part of the peptide, in particular peptides having the SEQ IDS NO: 464 to 925, for the preparation of a drug intended for the treatment of diseases induced by microorganisms, wherein said peptides have a broad-spectrum and fast antibiotic activity, in particular killing of the bacteria within 1 to 3 hours.

In an advantageous embodiment, the present invention relates to the use of at least one peptide originated from *Medicago truncatula* nodules, comprising the SEQ IDs NO: 1 – 463 or at least one peptide having a sequence derived from said SEQ IDs NO: 1 – 463 by deletion of about 9 to about 44 contiguous amino acids, in particular the SEQ IDS NO: 464 to 925, for the preparation of a drug intended for the treatment of human, animal or plant diseases induced by microorganisms, wherein said peptides have a broad-spectrum and fast antibiotic activity, in particular killing of the bacteria within 1 to 3 hours.

By the expression “peptide originated from *Medicago* nodules”, it is meant peptides that have been isolated from the nodules of legume of the galeoid clade such as *Medicago truncatula* but it also covers synthetic peptides having the sequence of said isolated peptides.

Isolated peptides from *Medicago* nodules are free of the nodule environment, i.e. of compounds, other than said peptides, that are present in the nodule.

Isolated peptides from *Medicago* nodules or synthesized peptides have a purity being equal or more than about 90%, in particular more than about 95%.

The sequence of said isolated peptides covers the sequence of amino acids given in the sequence listing as well as the modified peptide after the translation, such as for instance a glycosylated peptide.

Synthetic peptides can be prepared by chemical (peptides synthesis) or biological ways well known from a man skilled in the art (recombinant production).

Peptides from *Medicago truncatula* nodules are cysteine rich peptides (4 or 6 cysteines) and are synthesized in the cell with a signal peptide which is a short (from 9 to 60 amino acids long) peptide chain that directs the transport of the said peptides.

The amino acids comprising the signal peptide are cleaved off the protein (corresponding to the deletion above cited) in the endoplasmic reticulum leading thus to the mature peptide.

By the expression "contiguous amino acids" is meant amino acids touching or connected throughout in an unbroken sequence.

SEQ IDs NO: 1 to 463 corresponds thus to the mature peptides containing the signal peptide and SEQ IDs NO: 464-925 corresponds to the mature peptides after cleavage of said signal peptide being 9-44 contiguous amino acids long.

Table I give the correlation between SEQ IDs NO: 1-463 and SEQ IDs: 464- 925 (for the reader's convenience, the numbering of the present document and of the priority document EP 09 305 547.3 are indicated) and their corresponding NCR (Nodule Cystein Rich) number that is a numbering given by the inventors:

TABLE I

MATURE PEPTIDE AND SIGNAL PEPTIDE SEQ ID NO: (Present document)	MATURE PEPTIDE AND SIGNAL PEPTIDE SEQ ID NO: (Priority document EP 09 305 547.3)	NCR NUMBER	MATURE PEPTIDE SEQ ID NO: (Priority document EP 09 305 547.3)	MATURE PEPTIDE SEQ ID NO: (Present document)
SEQ ID NO:1	SEQ ID NO:1	NCR001	SEQ ID NO:523	SEQ ID NO:464
SEQ ID NO:2	SEQ ID NO:2	NCR002	-	SEQ ID NO:465

SEQ ID NO:3	SEQ ID NO:3	NCR003	-	SEQ ID NO:466
SEQ ID NO:4	SEQ ID NO:4	NCR004	SEQ ID NO:516	SEQ ID NO:467
SEQ ID NO:5	SEQ ID NO:5	NCR005		SEQ ID NO:468
SEQ ID NO:6	SEQ ID NO:6	NCR006	SEQ ID NO:355	SEQ ID NO:469
SEQ ID NO:7	SEQ ID NO:7	NCR007	SEQ ID NO:539	SEQ ID NO:470
SEQ ID NO:8	SEQ ID NO:8	NCR008	SEQ ID NO:524	SEQ ID NO:471
SEQ ID NO:9	SEQ ID NO:9	NCR009	SEQ ID NO:395	SEQ ID NO:472
SEQ ID NO:10	SEQ ID NO:10	NCR010	SEQ ID NO:495	SEQ ID NO:473
SEQ ID NO:11	SEQ ID NO:11	NCR011	SEQ ID NO:356	SEQ ID NO:474
SEQ ID NO:12	SEQ ID NO:12	NCR012	SEQ ID NO:414	SEQ ID NO:475
SEQ ID NO:13	SEQ ID NO:13	NCR013	SEQ ID NO:455	SEQ ID NO:476
SEQ ID NO:14	SEQ ID NO:14	NCR014	SEQ ID NO:525	SEQ ID NO:477
SEQ ID NO:15	SEQ ID NO:15	NCR015	-	SEQ ID NO:478
SEQ ID NO:16	-	NCR016	-	SEQ ID NO:479
SEQ ID NO:17	-	NCR017	-	SEQ ID NO:480
SEQ ID NO:18	SEQ ID NO:16	NCR018	SEQ ID NO:415	SEQ ID NO:481
SEQ ID NO:19	SEQ ID NO:17	NCR019	SEQ ID NO:561	SEQ ID NO:482
SEQ ID NO:20	SEQ ID NO:18	NCR020	SEQ ID NO:424	SEQ ID NO:483
SEQ ID NO:21	SEQ ID NO:19	NCR021	SEQ ID NO:329	SEQ ID NO:484
SEQ ID NO:22	SEQ ID NO:20	NCR022	SEQ ID NO:471	SEQ ID NO:485
SEQ ID NO:23	SEQ ID NO:21	NCR023	SEQ ID NO:499	SEQ ID NO:486
SEQ ID NO:24	SEQ ID NO:22	NCR024	SEQ ID NO:472	SEQ ID NO:487
SEQ ID NO:25	SEQ ID NO:23	NCR025	SEQ ID NO:509	SEQ ID NO:488
SEQ ID NO:26	SEQ ID NO:24	NCR026	SEQ ID NO:436	SEQ ID NO:489
SEQ ID NO:27	SEQ ID NO:25	NCR027	-	SEQ ID NO:490
SEQ ID NO:28	SEQ ID NO:26	NCR028	SEQ ID NO:548	SEQ ID NO:491
SEQ ID NO:29	SEQ ID NO:27	NCR029	SEQ ID NO:357	SEQ ID NO:492
SEQ ID NO:30	SEQ ID NO:28	NCR030	SEQ ID NO:396	SEQ ID NO:493
SEQ ID NO:31	SEQ ID NO:29	NCR031	SEQ ID NO:337	SEQ ID NO:494
SEQ ID NO:32	SEQ ID NO:30	NCR032	-	SEQ ID NO:495
SEQ ID NO:33	SEQ ID NO:31	NCR033	-	SEQ ID NO:496
SEQ ID NO:34	SEQ ID NO:32	NCR034	SEQ ID NO:485	SEQ ID NO:497
SEQ ID NO:35	SEQ ID NO:33	NCR035	SEQ ID NO:385	SEQ ID NO:498
SEQ ID NO:36	SEQ ID NO:34	NCR036	SEQ ID NO:397	SEQ ID NO:499
SEQ ID NO:37	SEQ ID NO:35	NCR037	SEQ ID NO:358	SEQ ID NO:500
SEQ ID NO:38	SEQ ID NO:36	NCR038	-	SEQ ID NO:501
SEQ ID NO:39	SEQ ID NO:37	NCR039	-	SEQ ID NO:502
SEQ ID NO:40	SEQ ID NO:38	NCR040	-	SEQ ID NO:503
SEQ ID NO:41	SEQ ID NO:39	NCR041	-	SEQ ID NO:504

SEQ ID NO:42	-	NCR042	-	SEQ ID NO:505
SEQ ID NO:43	SEQ ID NO:40	NCR043	SEQ ID NO:398	SEQ ID NO:506
SEQ ID NO:44	SEQ ID NO:41	NCR044	SEQ ID NO:399	SEQ ID NO:507
SEQ ID NO:45	SEQ ID NO:42	NCR045	SEQ ID NO:386	SEQ ID NO:508
SEQ ID NO:46	SEQ ID NO:43	NCR046	-	SEQ ID NO:509
SEQ ID NO:47	SEQ ID NO:44	NCR047	SEQ ID NO:400	SEQ ID NO:510
SEQ ID NO:48	SEQ ID NO:45	NCR048	-	SEQ ID NO:511
SEQ ID NO:49	SEQ ID NO:46	NCR049	-	SEQ ID NO:512
SEQ ID NO:50	SEQ ID NO:47	NCR050	-	SEQ ID NO:513
SEQ ID NO:51	SEQ ID NO:48	NCR051	SEQ ID NO:473	SEQ ID NO:514
SEQ ID NO:52	SEQ ID NO:49	NCR052	SEQ ID NO:345	SEQ ID NO:515
SEQ ID NO:53	SEQ ID NO:50	NCR053	SEQ ID NO:437	SEQ ID NO:516
SEQ ID NO:54	SEQ ID NO:51	NCR054	SEQ ID NO:425	SEQ ID NO:517
SEQ ID NO:55	SEQ ID NO:52	NCR055	SEQ ID NO:438	SEQ ID NO:518
SEQ ID NO:56	SEQ ID NO:53	NCR056	-	SEQ ID NO:519
SEQ ID NO:57	SEQ ID NO:54	NCR057	SEQ ID NO:486	SEQ ID NO:520
SEQ ID NO:58	SEQ ID NO:55	NCR058	SEQ ID NO:359	SEQ ID NO:521
SEQ ID NO:59	SEQ ID NO:56	NCR059	SEQ ID NO:387	SEQ ID NO:522
SEQ ID NO:60	SEQ ID NO:57	NCR060	SEQ ID NO:360	SEQ ID NO:523
SEQ ID NO:61	SEQ ID NO:58	NCR061	-	SEQ ID NO:524
SEQ ID NO:62	SEQ ID NO:59	NCR062	SEQ ID NO:338	SEQ ID NO:525
SEQ ID NO:63	SEQ ID NO:60	NCR063	SEQ ID NO:324	SEQ ID NO:526
SEQ ID NO:64	-	NCR064	-	SEQ ID NO:527
SEQ ID NO:65	SEQ ID NO:61	NCR065	SEQ ID NO:339	SEQ ID NO:528
SEQ ID NO:66	SEQ ID NO:62	NCR066	-	SEQ ID NO:529
SEQ ID NO:67	SEQ ID NO:63	NCR067	SEQ ID NO:426	SEQ ID NO:530
SEQ ID NO:68	SEQ ID NO:64	NCR068	SEQ ID NO:427	SEQ ID NO:531
SEQ ID NO:69	SEQ ID NO:65	NCR069	SEQ ID NO:456	SEQ ID NO:532
SEQ ID NO:70	SEQ ID NO:66	NCR070	-	SEQ ID NO:533
SEQ ID NO:71	SEQ ID NO:67	NCR071	-	SEQ ID NO:534
SEQ ID NO:72	SEQ ID NO:68	NCR072	SEQ ID NO:346	SEQ ID NO:535
SEQ ID NO:73	SEQ ID NO:69	NCR073	SEQ ID NO:439	SEQ ID NO:536
SEQ ID NO:74	SEQ ID NO:70	NCR074	SEQ ID NO:347	SEQ ID NO:537
SEQ ID NO:75	SEQ ID NO:71	NCR075	-	SEQ ID NO:538
SEQ ID NO:76	SEQ ID NO:72	NCR076	SEQ ID NO:487	SEQ ID NO:539
SEQ ID NO:77	SEQ ID NO:73	NCR077	SEQ ID NO:540	SEQ ID NO:540
SEQ ID NO:78	SEQ ID NO:74	NCR078	SEQ ID NO:416	SEQ ID NO:541
SEQ ID NO:79	SEQ ID NO:75	NCR079	SEQ ID NO:348	SEQ ID NO:542
SEQ ID NO:80	SEQ ID NO:76	NCR080	SEQ ID NO:361	SEQ ID NO:543

SEQ ID NO:81	SEQ ID NO:77	NCR081	SEQ ID NO:362	SEQ ID NO:544
SEQ ID NO:82	SEQ ID NO:78	NCR082	SEQ ID NO:349	SEQ ID NO:545
SEQ ID NO:83	SEQ ID NO:79	NCR083	SEQ ID NO:363	SEQ ID NO:546
SEQ ID NO:84	SEQ ID NO:80	NCR084	SEQ ID NO:488	SEQ ID NO:547
SEQ ID NO:85	SEQ ID NO:81	NCR085	SEQ ID NO:417	SEQ ID NO:548
SEQ ID NO:86	SEQ ID NO:82	NCR086	SEQ ID NO:364	SEQ ID NO:549
SEQ ID NO:87	SEQ ID NO:83	NCR087	-	SEQ ID NO:550
SEQ ID NO:88	SEQ ID NO:84	NCR088	SEQ ID NO:440	SEQ ID NO:551
SEQ ID NO:89	SEQ ID NO:85	NCR089	SEQ ID NO:536	SEQ ID NO:552
SEQ ID NO:90	SEQ ID NO:86	NCR090	SEQ ID NO:418	SEQ ID NO:553
SEQ ID NO:91	SEQ ID NO:87	NCR091	-	SEQ ID NO:554
SEQ ID NO:92	-	NCR092	-	SEQ ID NO:555
SEQ ID NO:93	SEQ ID NO:88	NCR093	SEQ ID NO:500	SEQ ID NO:556
SEQ ID NO:94	SEQ ID NO:89	NCR094	SEQ ID NO:441	SEQ ID NO:557
SEQ ID NO:95	SEQ ID NO:90	NCR095	SEQ ID NO:350	SEQ ID NO:558
SEQ ID NO:96	SEQ ID NO:91	NCR096	SEQ ID NO:442	SEQ ID NO:559
SEQ ID NO:97	SEQ ID NO:92	NCR097	SEQ ID NO:322	SEQ ID NO:560
SEQ ID NO:98	SEQ ID NO:93	NCR098	SEQ ID NO:517	SEQ ID NO:561
SEQ ID NO:99	SEQ ID NO:94	NCR099	SEQ ID NO:474	SEQ ID NO:562
SEQ ID NO:100	SEQ ID NO:95	NCR100	SEQ ID NO:532	SEQ ID NO:563
SEQ ID NO:101	SEQ ID NO:96	NCR101	SEQ ID NO:419	SEQ ID NO:564
SEQ ID NO:102	SEQ ID NO:97	NCR102	SEQ ID NO:489	SEQ ID NO:565
SEQ ID NO:103	SEQ ID NO:98	NCR103	SEQ ID NO:475	SEQ ID NO:566
SEQ ID NO:104	SEQ ID NO:99	NCR104	SEQ ID NO:518	SEQ ID NO:567
SEQ ID NO:105	SEQ ID NO:100	NCR105	SEQ ID NO:551	SEQ ID NO:568
SEQ ID NO:106	SEQ ID NO:101	NCR106	SEQ ID NO:510	SEQ ID NO:569
SEQ ID NO:107	SEQ ID NO:102	NCR107	SEQ ID NO:351	SEQ ID NO:570
SEQ ID NO:108	SEQ ID NO:103	NCR108	SEQ ID NO:542	SEQ ID NO:571
SEQ ID NO:109	SEQ ID NO:104	NCR109	SEQ ID NO:325	SEQ ID NO:572
SEQ ID NO:110	SEQ ID NO:105	NCR110	-	SEQ ID NO:573
SEQ ID NO:111	SEQ ID NO:106	NCR111	SEQ ID NO:559	SEQ ID NO:574
SEQ ID NO:112	SEQ ID NO:107	NCR112	-	SEQ ID NO:575
SEQ ID NO:113	SEQ ID NO:108	NCR113	SEQ ID NO:501	SEQ ID NO:576
SEQ ID NO:114	SEQ ID NO:109	NCR114	SEQ ID NO:526	SEQ ID NO:577
SEQ ID NO:115	SEQ ID NO:110	NCR115	SEQ ID NO:330	SEQ ID NO:578
SEQ ID NO:116	SEQ ID NO:111	NCR116	SEQ ID NO:511	SEQ ID NO:579
SEQ ID NO:117	SEQ ID NO:112	NCR117	SEQ ID NO:443	SEQ ID NO:580
SEQ ID NO:118	SEQ ID NO:113	NCR118	SEQ ID NO:537	SEQ ID NO:581
SEQ ID NO:119	SEQ ID NO:114	NCR119	SEQ ID NO:365	SEQ ID NO:582

SEQ ID NO:120	SEQ ID NO:115	NCR120		SEQ ID NO:583
SEQ ID NO:121	SEQ ID NO:116	NCR121	SEQ ID NO:401	SEQ ID NO:584
SEQ ID NO:122	SEQ ID NO:117	NCR122	SEQ ID NO:402	SEQ ID NO:585
SEQ ID NO:123	-	NCR123	-	SEQ ID NO:586
SEQ ID NO:124	SEQ ID NO:118	NCR124	SEQ ID NO:327	SEQ ID NO:587
SEQ ID NO:125	SEQ ID NO:119	NCR125	-	SEQ ID NO:588
SEQ ID NO:126	SEQ ID NO:120	NCR126	-	SEQ ID NO:589
SEQ ID NO:127	SEQ ID NO:121	NCR127	SEQ ID NO:519	SEQ ID NO:590
SEQ ID NO:128	SEQ ID NO:122	NCR128	SEQ ID NO:366	SEQ ID NO:591
SEQ ID NO:129	SEQ ID NO:123	NCR129	SEQ ID NO:388	SEQ ID NO:592
SEQ ID NO:130	SEQ ID NO:124	NCR130	SEQ ID NO:403	SEQ ID NO:593
SEQ ID NO:131	SEQ ID NO:125	NCR131	SEQ ID NO:476	SEQ ID NO:594
SEQ ID NO:132	SEQ ID NO:126	NCR132	-	SEQ ID NO:595
SEQ ID NO:133	SEQ ID NO:127	NCR133	SEQ ID NO:490	SEQ ID NO:596
SEQ ID NO:134	SEQ ID NO:128	NCR134	SEQ ID NO:547	SEQ ID NO:597
SEQ ID NO:135	SEQ ID NO:129	NCR135	SEQ ID NO:477	SEQ ID NO:598
SEQ ID NO:136	SEQ ID NO:130	NCR136	SEQ ID NO:367	SEQ ID NO:599
SEQ ID NO:137	SEQ ID NO:131	NCR137	SEQ ID NO:389	SEQ ID NO:600
SEQ ID NO:138	SEQ ID NO:132	NCR138	SEQ ID NO:390	SEQ ID NO:601
SEQ ID NO:139	SEQ ID NO:133	NCR139	SEQ ID NO:502	SEQ ID NO:602
SEQ ID NO:140	SEQ ID NO:134	NCR140	SEQ ID NO:563	SEQ ID NO:603
SEQ ID NO:141	SEQ ID NO:135	NCR141	SEQ ID NO:562	SEQ ID NO:604
SEQ ID NO:142	SEQ ID NO:136	NCR142	-	SEQ ID NO:605
SEQ ID NO:143	SEQ ID NO:137	NCR143	SEQ ID NO:404	SEQ ID NO:606
SEQ ID NO:144	SEQ ID NO:138	NCR144	SEQ ID NO:405	SEQ ID NO:607
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SEQ ID NO:146	SEQ ID NO:140	NCR146	SEQ ID NO:457	SEQ ID NO:609
SEQ ID NO:147	SEQ ID NO:141	NCR147	SEQ ID NO:406	SEQ ID NO:610
SEQ ID NO:148	SEQ ID NO:142	NCR148	-	SEQ ID NO:611
SEQ ID NO:149	SEQ ID NO:143	NCR149	-	SEQ ID NO:612
SEQ ID NO:150	SEQ ID NO:144	NCR150	SEQ ID NO:328	SEQ ID NO:613
SEQ ID NO:151	SEQ ID NO:145	NCR151	-	SEQ ID NO:614
SEQ ID NO:152	SEQ ID NO:146	NCR152	SEQ ID NO:407	SEQ ID NO:615
SEQ ID NO:153	SEQ ID NO:147	NCR153	-	SEQ ID NO:616
SEQ ID NO:154	SEQ ID NO:148	NCR154	SEQ ID NO:331	SEQ ID NO:617
SEQ ID NO:155	SEQ ID NO:149	NCR155	-	SEQ ID NO:618
SEQ ID NO:156	SEQ ID NO:150	NCR156	SEQ ID NO:428	SEQ ID NO:619
SEQ ID NO:157	SEQ ID NO:151	NCR157	SEQ ID NO:368	SEQ ID NO:620
SEQ ID NO:158	SEQ ID NO:152	NCR158	SEQ ID NO:527	SEQ ID NO:621

SEQ ID NO:159	SEQ ID NO:153	NCR159	SEQ ID NO:478	SEQ ID NO:622
SEQ ID NO:160	SEQ ID NO:154	NCR160	SEQ ID NO:408	SEQ ID NO:623
SEQ ID NO:161	SEQ ID NO:155	NCR161	SEQ ID NO:340	SEQ ID NO:624
SEQ ID NO:162	SEQ ID NO:156	NCR162	SEQ ID NO:429	SEQ ID NO:625
SEQ ID NO:163	SEQ ID NO:157	NCR163	SEQ ID NO:503	SEQ ID NO:626
SEQ ID NO:164	SEQ ID NO:158	NCR164	SEQ ID NO:444	SEQ ID NO:627
SEQ ID NO:165	SEQ ID NO:159	NCR165	SEQ ID NO:458	SEQ ID NO:628
SEQ ID NO:166	SEQ ID NO:160	NCR166	SEQ ID NO:496	SEQ ID NO:629
SEQ ID NO:167	SEQ ID NO:161	NCR167	SEQ ID NO:459	SEQ ID NO:630
SEQ ID NO:168	SEQ ID NO:162	NCR168	SEQ ID NO:479	SEQ ID NO:631
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SEQ ID NO:174	SEQ ID NO:168	NCR174	SEQ ID NO:491	SEQ ID NO:637
SEQ ID NO:175	SEQ ID NO:169	NCR175	SEQ ID NO:543	SEQ ID NO:638
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SEQ ID NO:187	SEQ ID NO:181	NCR187	SEQ ID NO:497	SEQ ID NO:650
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SEQ ID NO:191	-	NCR191	-	SEQ ID NO:654
SEQ ID NO:192	SEQ ID NO:184	NCR192	SEQ ID NO:332	SEQ ID NO:655
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SEQ ID NO:194	SEQ ID NO:186	NCR194	SEQ ID NO:391	SEQ ID NO:657
SEQ ID NO:195	SEQ ID NO:187	NCR195	SEQ ID NO:522	SEQ ID NO:658
SEQ ID NO:196	SEQ ID NO:188	NCR196	SEQ ID NO:493	SEQ ID NO:659
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SEQ ID NO:461	-	NCR465	-	SEQ ID NO:923
SEQ ID NO:462	-	NCR466	-	SEQ ID NO:924
SEQ ID NO:463	-	NCR467	-	SEQ ID NO:925

By the word “microorganisms” is meant organisms that are microscopic such as bacteria, fungi, archaea, protists or viruses.

By the term “bacteria”, it must be understood:

- Gram + bacteria such as Actinobacteria, Firmicutes or Tenericutes,
- Gram – bacteria such as Aquificae, Bacteroidetes/Chlorobi, Chlamydiae/Verrucomicrobia, Deinococcus-Thermus, Fusobacteria, Gemmatimonadetes, Nitrospirae, Proteobacteria, Spirochaetes, Synergistetes, and
- 5 - Others such as Acidobacteria, Chloroflexi, Chrysiogenetes, Cyanobacteria, Deferribacteres, Dictyoglomi, Fibrobacteres, Planctomycetes, Thermodesulfobacteria, Thermotogae.

By the expression “broad-spectrum” it must be understood that peptides of the invention are able to act as an antibiotic effective against a wide range of infectious microorganisms, in particular with activity against a wide range of disease-causing bacteria (e.g. Gram-
10 *Pseudomonas aeruginosa* and Gram+ *Staphylococcus aureus*).

This is in contrast to a narrow-spectrum antibiotic which is effective against only specific families of bacteria.

By the expression “fast antibiotic activity” is meant that peptides of the invention decreased
15 the bacterial count by 3 to 5 orders of magnitude (from 10^6 - 10^9 to 10^1 - 10^6) after the peptide was added to the bacterium culture for less than three hours at 10 μ M. See Tables V., VI. and IX. and example 6.

The broad spectrum activity and the fast antibiotic activity can be easily determined by a propidium iodide staining test (PI staining) or a colony forming unit (CFU) determination (see
20 example 1 and example 2, respectively).

Antibiotics have the capacity to kill (biocidal activity) or inhibit the growth (biostatic activity) of microorganisms.

Depending on the dose, peptides of the invention are able first to inhibit the growth of the bacteria or to directly kill the bacteria, i.e. lead to the eradication of the bacteria.

25 The time necessary to achieve this eradication is comprised from about 5 minutes to about 3 hours, preferably from about 10 minutes to about 1 hour, preferably from 15 minutes to about 1 hours, more preferably from about 20 minutes to 1 hour, more preferably from about 30 minutes to 1 hour, more preferably from about 40 minutes to 1 hour, more preferably from about 50 minutes to 1 hour, more preferably from about 55 minutes to 1 hour, more preferably from about
30 1 hour to about 3 hours, more preferably from about 1 hour to about 2 hours, in particular 1 hour.

Surprisingly, peptides of the invention exhibit no bactericidal activity, i.e. no activity of killing the bacterium, when they are locked in nodules and have surprisingly demonstrated their bactericidal activity once there have been isolated from nodules.

Therefore, one of the advantages of the invention is to provide peptides having a bactericidal activity on a large panel of bacteria (see example 5) and a fast action.

Another advantage of the invention is to provide peptides which are active not only in human but also in animals or plants.

In an advantageous embodiment, the present invention relates to the use of at least one peptide originated from *Medicago truncatula* nodules, comprising the SEQ IDs NO: 1 – 463 or at least one peptide having a sequence derived from said SEQ IDs NO: 1 – 463 by deletion of about 9 to about 44 contiguous amino acids, from the N-terminal part of the peptide, in particular peptides having the SEQ IDS NO: 464 to 925, for the preparation of a drug intended for the treatment of human or animal diseases induced by microorganisms, wherein said peptides have a broad-spectrum and fast antibiotic activity, in particular killing of the bacteria within 1 to 3 hours.

In an advantageous embodiment, the present invention relates to the use of at least one peptide originated from *Medicago truncatula* nodules, comprising the SEQ IDs NO: 1 – 463 or at least one peptide having a sequence derived from said SEQ IDs NO: 1 – 463 by deletion of about 9 to about 44 contiguous amino acids, from the N-terminal part of the peptide, in particular peptides having the SEQ IDS NO: 464 to 925, for the preparation of a drug intended for the treatment of plant diseases induced by microorganisms, wherein said peptides have a broad-spectrum and fast antibiotic activity, in particular killing of the bacteria within 1 to 3 hours.

In an advantageous embodiment, said peptides are administered on the plant surface for the prevention and/or the control of micro organisms inducing plant diseases.

Indeed, said peptides have stimulation properties of plant natural defences and/or fungicide properties.

Said peptides can thus be applied by different ways on the surface of the plants, in particular by spraying on the leaves or the and/or the stem.

In an advantageous embodiment, the present invention relates to the use of the peptides above defined, wherein said peptides derive from the peptides of SEQ ID NO: 1 to 925 above defined:

- 5 - by substitution, and/or suppression, and/or addition of one or several amino acids, of the above-mentioned peptides, and/or
- by modification of at least one -CO-NH- peptide linkage of the peptide chain of the above-mentioned peptides, particularly by introduction of a retro or retro-inverso type linkage, and/or
- by substitution of at least one amino acid of the peptide chain of the sequence or of the
10 above-mentioned peptide, with a non-proteinogenic amino acid,
 or are fragments of peptides of SEQ ID NO: 1 to 925, provided that the fast antibiotic activity is maintained,
 or peptides having more than 80%, preferably more than 85%, preferably more than 90% and more preferably more than 95% of homology with peptides of SEQ ID NO: 1 to 925,
15 provided that the fast antibiotic activity is maintained.

Thus, peptides having the SEQ ID NO:1 to 463 or SEQ ID NO: 464 to 925 can have an amino acid sequence wherein one or more amino acids are substituted or suppressed everywhere inside said sequence or wherein one or more proteinogenic amino acids, i.e. natural amino acids, are added to said sequence, provided that, in each of above cases the fast antibiotic activity of the
20 peptides would be conserved.

Peptides having the SEQ ID NO:1 to 463 or SEQ ID NO: 464 to 925 can also have an amide linkage (-CO-NH- peptide linkage) modified by introduction of a retro amide linkage (-NH- CO- peptide linkage) or a retro inverso amide linkage (-NH- CO- peptide linkage and an inverse absolute configuration of the amino acid) provided that, in each of above cases the
25 antibiotic activity of the peptides would be conserved.

By the expression “non-proteinogenic amino acids”, it must be understood either amino acids not found in proteins (like carnitine, GABA, or L-DOPA), or not coded for in the standard genetic code (like hydroxyproline and selenomethionine) provided that, in each of above cases the fast antibiotic activity of the peptides is conserved.

30 In an advantageous embodiment, the present invention relates to the use of peptides above defined, wherein said microorganisms are bacteria of Gram – or Gram + bacteria.

A list of Gram – or Gram + bacteria can be found in DSMZ catalogue:
(http://www.dsmz.de/microorganisms/bacteria_catalogue.php)

Examples of Gram – bacteria without being limited to them are the following:

Sinorhizobium meliloti, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Xanthomonas*...

5 Examples of Gram + bacteria without being limited to them are the following:

Clavibacter michigense, *Curtobacterium flaccumfaciens*, *Bacillus subtilis*, *Staphylococcus aureus*...

In an advantageous embodiment, the present invention relates to the use of peptides above defined, wherein said peptides further have an anti-viral and/or fungicidal activity.

10 Another advantage of the invention is to provide peptides having not only an antibacterial activity but also an antiviral activity or a fungicidal activity, for example on *Kluyveromyces lactis* or *Pichia pastoris*.

In a more advantageous embodiment, the present invention relates to the use of peptides above defined, wherein said peptides are cationic peptides ($pI > 8$) in association with one or more
15 anionic peptides ($pI < 6$) and/or one or more neutral peptides ($6 \leq pI \leq 8$).

The isoelectric point (pI), is the pH at which a peptide carries no net electrical charge.

pI can be determined with techniques well known by a man skilled in the art
(www.expasy.ch/tools/pi_tool.html)

20 Peptides are constituted with hydrophobic amino acids, hydrophilic amino acids, positively or negatively charged amino acids.

Peptides having a pI higher than 8 are cationic peptides.

Peptides having a pI lower than 6 are anionic peptides.

Peptides having a pI comprised between 6 and 8 are neutral peptides.

Therefore, according to the invention:

- 25 - cationic peptides can be associated with anionic peptides, or
- cationic peptides can be associated with neutral peptides, or
- cationic peptides can be associated with anionic and neutral peptides,

Surprisingly, it has been found by the invention that peptides having the better antibiotic activity are cationic peptides and that anionic or neutral peptides can stabilize cationic peptides
30 when associated with them, leading thus to an improved and fast antibiotic activity.

In a more advantageous embodiment, the present invention relates to the use of peptides above defined, wherein said cationic peptides are selected from the list consisting of SEQ ID NO:469; SEQ ID NO:474; SEQ ID NO:480; SEQ ID NO:483; SEQ ID NO:484; SEQ ID NO:485; SEQ ID NO:486; SEQ ID NO:489; SEQ ID NO:490; SEQ ID NO:492; SEQ ID NO:493; SEQ ID NO:494; SEQ ID NO:495; SEQ ID NO:496; SEQ ID NO:497; SEQ ID NO:498; SEQ ID NO:499; SEQ ID NO:500; SEQ ID NO:501; SEQ ID NO:502; SEQ ID NO:503; SEQ ID NO:504; SEQ ID NO:505; SEQ ID NO:506; SEQ ID NO:507; SEQ ID NO:508; SEQ ID NO:509; SEQ ID NO:510; SEQ ID NO:511; SEQ ID NO:512; SEQ ID NO:513; SEQ ID NO:515; SEQ ID NO:516; SEQ ID NO:518; SEQ ID NO:520; SEQ ID NO:523; SEQ ID NO:524; SEQ ID NO:530; SEQ ID NO:531; SEQ ID NO:532; SEQ ID NO:533; SEQ ID NO:534; SEQ ID NO:536; SEQ ID NO:538; SEQ ID NO:541; SEQ ID NO:543; SEQ ID NO:544; SEQ ID NO:546; SEQ ID NO:549; SEQ ID NO:554; SEQ ID NO:555; SEQ ID NO:556; SEQ ID NO:561; SEQ ID NO:563; SEQ ID NO:564; SEQ ID NO:565; SEQ ID NO:566; SEQ ID NO:567; SEQ ID NO:569; SEQ ID NO:573; SEQ ID NO:582; SEQ ID NO:583; SEQ ID NO:593; SEQ ID NO:596; SEQ ID NO:597; SEQ ID NO:598; SEQ ID NO:599; SEQ ID NO:600; SEQ ID NO:606; SEQ ID NO:610; SEQ ID NO:611; SEQ ID NO:613; SEQ ID NO:618; SEQ ID NO:620; SEQ ID NO:622; SEQ ID NO:623; SEQ ID NO:632; SEQ ID NO:633; SEQ ID NO:634; SEQ ID NO:635; SEQ ID NO:636; SEQ ID NO:639; SEQ ID NO:642; SEQ ID NO:644; SEQ ID NO:646; SEQ ID NO:647; SEQ ID NO:651; SEQ ID NO:652; SEQ ID NO:655; SEQ ID NO:658; SEQ ID NO:659; SEQ ID NO:662; SEQ ID NO:663; SEQ ID NO:670; SEQ ID NO:675; SEQ ID NO:681; SEQ ID NO:683; SEQ ID NO:684; SEQ ID NO:685; SEQ ID NO:690; SEQ ID NO:694; SEQ ID NO:700; SEQ ID NO:705; SEQ ID NO:706; SEQ ID NO:709; SEQ ID NO:713; SEQ ID NO:715; SEQ ID NO:720; SEQ ID NO:721; SEQ ID NO:725; SEQ ID NO:731; SEQ ID NO:737; SEQ ID NO:738; SEQ ID NO:739; SEQ ID NO:742; SEQ ID NO:743; SEQ ID NO:744; SEQ ID NO:746; SEQ ID NO:750; SEQ ID NO:753; SEQ ID NO:757; SEQ ID NO:758; SEQ ID NO:761; SEQ ID NO:766; SEQ ID NO:769; SEQ ID NO:772; SEQ ID NO:774; SEQ ID NO:775; SEQ ID NO:780; SEQ ID NO:781; SEQ ID NO:782; SEQ ID NO:783; SEQ ID NO:784; SEQ ID NO:787; SEQ ID NO:793; SEQ ID NO:794; SEQ ID NO:799; SEQ ID NO:800; SEQ ID NO:807; SEQ ID NO:812; SEQ ID NO:816; SEQ ID NO:817; SEQ ID NO:819; SEQ ID NO:820; SEQ ID NO:821; SEQ ID NO:824; SEQ ID

NO:825; SEQ ID NO:827; SEQ ID NO:828; SEQ ID NO:829; SEQ ID NO:830; SEQ ID NO:835; SEQ ID NO:836; SEQ ID NO:842; SEQ ID NO:846; SEQ ID NO:847; SEQ ID NO:848; SEQ ID NO:849; SEQ ID NO:850; SEQ ID NO:853; SEQ ID NO:858; SEQ ID NO:862; SEQ ID NO:863; SEQ ID NO:865; SEQ ID NO:866; SEQ ID NO:870; SEQ ID NO:872; SEQ ID NO:875; SEQ ID NO:877; SEQ ID NO:878; SEQ ID NO:879; SEQ ID NO:880; SEQ ID NO:881; SEQ ID NO:892; SEQ ID NO:894; SEQ ID NO:896; SEQ ID NO:897; SEQ ID NO:899; SEQ ID NO:909; SEQ ID NO:910; SEQ ID NO:913; SEQ ID NO:917; SEQ ID NO:919; SEQ ID NO:921 and SEQ ID NO:925.

Table II give the NCR correlation and the pI > 8 of the cationic peptides above defined:

TABLE II

SEQ ID NO:	PEPTIDE	pI
SEQ ID NO:469	NCR006	8,50
SEQ ID NO:474	NCR011	8,85
SEQ ID NO:480	NCR017	8,20
SEQ ID NO:483	NCR020	9,56
SEQ ID NO:484	NCR021	9,30
SEQ ID NO:485	NCR022	9,27
SEQ ID NO:486	NCR023	8,33
SEQ ID NO:489	NCR026	8,46
SEQ ID NO:490	NCR027	8,75
SEQ ID NO:492	NCR029	8,44
SEQ ID NO:493	NCR030	10,37
SEQ ID NO:494	NCR031	8,34
SEQ ID NO:495	NCR032	9,62
SEQ ID NO:496	NCR033	8,92
SEQ ID NO:497	NCR034	8,92
SEQ ID NO:498	NCR035	9,42
SEQ ID NO:499	NCR036	9,13
SEQ ID NO:500	NCR037	9,25
SEQ ID NO:501	NCR038	8,87
SEQ ID NO:502	NCR039	9,22
SEQ ID NO:503	NCR040	8,54
SEQ ID NO:504	NCR041	9,22
SEQ ID NO:505	NCR042	9,45
SEQ ID NO:506	NCR043	9,20
SEQ ID NO:507	NCR044	10,32
SEQ ID NO:508	NCR045	9,39
SEQ ID NO:509	NCR046	9,21
SEQ ID NO:510	NCR047	9,50

SEQ ID NO:511	NCR048	8,53
SEQ ID NO:512	NCR049	8,51
SEQ ID NO:513	NCR050	9,13
SEQ ID NO:515	NCR052	9,13
SEQ ID NO:516	NCR053	8,67
SEQ ID NO:518	NCR055	9,21
SEQ ID NO:520	NCR057	8,43
SEQ ID NO:523	NCR060	8,66
SEQ ID NO:524	NCR061	8,20
SEQ ID NO:530	NCR067	9,10
SEQ ID NO:531	NCR068	8,49
SEQ ID NO:532	NCR069	9,10
SEQ ID NO:533	NCR070	8,18
SEQ ID NO:534	NCR071	8,29
SEQ ID NO:536	NCR073	8,85
SEQ ID NO:538	NCR075	8,34
SEQ ID NO:541	NCR078	8,40
SEQ ID NO:543	NCR080	9,10
SEQ ID NO:544	NCR081	8,51
SEQ ID NO:546	NCR083	8,49
SEQ ID NO:549	NCR086	8,87
SEQ ID NO:554	NCR091	9,15
SEQ ID NO:555	NCR092	8,18
SEQ ID NO:556	NCR093	9,33
SEQ ID NO:561	NCR098	9,24
SEQ ID NO:563	NCR100	8,56
SEQ ID NO:564	NCR101	9,46
SEQ ID NO:565	NCR102	9,10
SEQ ID NO:566	NCR103	8,30
SEQ ID NO:567	NCR104	8,95
SEQ ID NO:569	NCR106	9,00
SEQ ID NO:573	NCR110	8,53
SEQ ID NO:582	NCR119	9,30
SEQ ID NO:583	NCR120	8,40
SEQ ID NO:593	NCR130	9,62
SEQ ID NO:596	NCR133	9,13
SEQ ID NO:597	NCR134	8,36
SEQ ID NO:598	NCR135	8,88
SEQ ID NO:599	NCR136	8,85
SEQ ID NO:600	NCR137	9,70
SEQ ID NO:606	NCR143	9,38
SEQ ID NO:610	NCR147	9,76
SEQ ID NO:611	NCR148	9,14
SEQ ID NO:613	NCR150	8,34
SEQ ID NO:618	NCR155	9,44
SEQ ID NO:620	NCR157	8,27
SEQ ID NO:622	NCR159	8,66

SEQ ID NO:623	NCR160	8,65
SEQ ID NO:632	NCR169	8,45
SEQ ID NO:633	NCR170	9,18
SEQ ID NO:634	NCR171	8,62
SEQ ID NO:635	NCR172	8,65
SEQ ID NO:636	NCR173	8,88
SEQ ID NO:639	NCR176	8,65
SEQ ID NO:642	NCR179	9,79
SEQ ID NO:644	NCR181	9,51
SEQ ID NO:646	NCR183	10,10
SEQ ID NO:647	NCR184	9,56
SEQ ID NO:651	NCR188	8,52
SEQ ID NO:652	NCR189	9,15
SEQ ID NO:655	NCR192	9,70
SEQ ID NO:658	NCR195	8,61
SEQ ID NO:659	NCR196	8,63
SEQ ID NO:662	NCR199	8,20
SEQ ID NO:663	NCR200	8,31
SEQ ID NO:670	NCR207	9,70
SEQ ID NO:675	NCR212	9,04
SEQ ID NO:681	NCR219	8,28
SEQ ID NO:683	NCR221	8,32
SEQ ID NO:684	NCR222	8,37
SEQ ID NO:685	NCR223	8,32
SEQ ID NO:690	NCR228	8,66
SEQ ID NO:694	NCR232	8,92
SEQ ID NO:700	NCR238	9,24
SEQ ID NO:705	NCR246	9,07
SEQ ID NO:706	NCR247	10,15
SEQ ID NO:709	NCR250	9,22
SEQ ID NO:713	NCR254	8,28
SEQ ID NO:715	NCR256	8,69
SEQ ID NO:720	NCR262	8,58
SEQ ID NO:721	NCR263	8,37
SEQ ID NO:725	NCR267	8,26
SEQ ID NO:731	NCR273	9,34
SEQ ID NO:737	NCR279	8,51
SEQ ID NO:738	NCR280	9,80
SEQ ID NO:739	NCR281	8,53
SEQ ID NO:742	NCR284	8,92
SEQ ID NO:743	NCR285	8,27
SEQ ID NO:744	NCR286	8,66
SEQ ID NO:746	NCR288	8,29
SEQ ID NO:750	NCR292	9,03
SEQ ID NO:753	NCR295	8,40
SEQ ID NO:757	NCR299	8,57
SEQ ID NO:758	NCR300	9,56

SEQ ID NO:761	NCR303	9,34
SEQ ID NO:766	NCR308	8,77
SEQ ID NO:769	NCR311	8,51
SEQ ID NO:772	NCR314	8,87
SEQ ID NO:774	NCR316	8,76
SEQ ID NO:775	NCR317	9,13
SEQ ID NO:780	NCR322	8,58
SEQ ID NO:781	NCR323	8,94
SEQ ID NO:782	NCR324	9,50
SEQ ID NO:783	NCR325	8,64
SEQ ID NO:784	NCR326	8,64
SEQ ID NO:787	NCR329	8,89
SEQ ID NO:793	NCR335	11,22
SEQ ID NO:794	NCR336	8,92
SEQ ID NO:799	NCR341	8,52
SEQ ID NO:800	NCR342	9,39
SEQ ID NO:807	NCR349	8,96
SEQ ID NO:812	NCR354	8,52
SEQ ID NO:816	NCR358	9,50
SEQ ID NO:817	NCR359	9,27
SEQ ID NO:819	NCR361	8,85
SEQ ID NO:820	NCR362	9,24
SEQ ID NO:821	NCR363	8,26
SEQ ID NO:824	NCR366	8,20
SEQ ID NO:825	NCR367	8,51
SEQ ID NO:827	NCR369	8,52
SEQ ID NO:828	NCR370	8,29
SEQ ID NO:829	NCR371	9,28
SEQ ID NO:830	NCR372	8,28
SEQ ID NO:835	NCR377	9,44
SEQ ID NO:836	NCR378	8,52
SEQ ID NO:842	NCR384	8,97
SEQ ID NO:846	NCR388	8,98
SEQ ID NO:847	NCR389	9,39
SEQ ID NO:848	NCR390	8,08
SEQ ID NO:849	NCR391	8,41
SEQ ID NO:850	NCR392	8,53
SEQ ID NO:853	NCR395	8,85
SEQ ID NO:858	NCR400	8,29
SEQ ID NO:862	NCR404	8,18
SEQ ID NO:863	NCR405	9,18
SEQ ID NO:865	NCR407	9,05
SEQ ID NO:866	NCR408	9,72
SEQ ID NO:870	NCR412	8,87
SEQ ID NO:872	NCR414	8,02
SEQ ID NO:875	NCR417	8,77
SEQ ID NO:877	NCR419	8,53

SEQ ID NO:878	NCR420	8,80
SEQ ID NO:879	NCR421	9,13
SEQ ID NO:880	NCR422	8,54
SEQ ID NO:881	NCR423	8,29
SEQ ID NO:892	NCR434	8,18
SEQ ID NO:894	NCR436	9,31
SEQ ID NO:896	NCR438	8,95
SEQ ID NO:897	NCR439	8,49
SEQ ID NO:899	NCR441	8,01
SEQ ID NO:909	NCR451	9,96
SEQ ID NO:910	NCR452	8,79
SEQ ID NO:913	NCR455	8,64
SEQ ID NO:917	NCR459	8,20
SEQ ID NO:919	NCR461	9,39
SEQ ID NO:921	NCR463	8,76
SEQ ID NO:925	NCR467	8,27

In a more advantageous embodiment, said cationic peptides above defined are selected from the list consisting of: SEQ ID NO:706 (NCR 247), SEQ ID NO:498 (NCR 035), SEQ ID NO:518 (NCR 055), SEQ ID NO:520 (NCR 57).

5 Examples 3-5 show that cationic peptides are potent antibiotic peptides and figure 1 shows that they have a fast antibiotic activity.

Indeed, at a concentration of 10 μ M in 3 hours, peptides of the invention can eradicate bacteria such as *Sinorhizobium meliloti* while tetracyclin and kanamycin which are well known antibiotics, cannot eradicate the sensitive bacteria in 3 hours at doses equal to or tenfold higher
10 than the invention peptide doses.

In a more advantageous embodiment, said anionic peptides are selected from the list consisting of SEQ ID NO:464; SEQ ID NO:465; SEQ ID NO:467; SEQ ID NO:468; SEQ ID NO:470; SEQ ID NO:471; SEQ ID NO:472; SEQ ID NO:476; SEQ ID NO:477; SEQ ID NO:478; SEQ ID
15 NO:479; SEQ ID NO:481; SEQ ID NO:482; SEQ ID NO:487; SEQ ID NO:488; SEQ ID NO:514; SEQ ID NO:521; SEQ ID NO:522; SEQ ID NO:526; SEQ ID NO:528; SEQ ID NO:529; SEQ ID NO:537; SEQ ID NO:539; SEQ ID NO:545; SEQ ID NO:550; SEQ ID NO:551; SEQ ID NO:552; SEQ ID NO:558; SEQ ID NO:562; SEQ ID NO:568; SEQ ID NO:571; SEQ ID NO:574; SEQ ID NO:575; SEQ ID NO:576; SEQ ID NO:577; SEQ ID
20 NO:580; SEQ ID NO:586; SEQ ID NO:588; SEQ ID NO:590; SEQ ID NO:592; SEQ ID NO:595; SEQ ID NO:602; SEQ ID NO:603; SEQ ID NO:604; SEQ ID NO:605; SEQ ID

NO:608; SEQ ID NO:614; SEQ ID NO:615; SEQ ID NO:616; SEQ ID NO:617; SEQ ID
 NO:619; SEQ ID NO:621; SEQ ID NO:625; SEQ ID NO:626; SEQ ID NO:627; SEQ ID
 NO:629; SEQ ID NO:630; SEQ ID NO:631; SEQ ID NO:640; SEQ ID NO:641; SEQ ID
 NO:643; SEQ ID NO:648; SEQ ID NO:650; SEQ ID NO:654; SEQ ID NO:656; SEQ ID
 5 NO:657; SEQ ID NO:666; SEQ ID NO:667; SEQ ID NO:672; SEQ ID NO:673; SEQ ID
 NO:674; SEQ ID NO:677; SEQ ID NO:678; SEQ ID NO:679; SEQ ID NO:680; SEQ ID
 NO:682; SEQ ID NO:686; SEQ ID NO:687; SEQ ID NO:688; SEQ ID NO:693; SEQ ID
 NO:696; SEQ ID NO:697; SEQ ID NO:702; SEQ ID NO:704; SEQ ID NO:711; SEQ ID
 NO:719; SEQ ID NO:722; SEQ ID NO:728; SEQ ID NO:729; SEQ ID NO:732; SEQ ID
 10 NO:733; SEQ ID NO:735; SEQ ID NO:736; SEQ ID NO:741; SEQ ID NO:745; SEQ ID
 NO:747; SEQ ID NO:748; SEQ ID NO:749; SEQ ID NO:752; SEQ ID NO:754; SEQ ID
 NO:759; SEQ ID NO:760; SEQ ID NO:762; SEQ ID NO:763; SEQ ID NO:767; SEQ ID
 NO:768; SEQ ID NO:770; SEQ ID NO:771; SEQ ID NO:773; SEQ ID NO:777; SEQ ID
 NO:778; SEQ ID NO:786; SEQ ID NO:792; SEQ ID NO:795; SEQ ID NO:796; SEQ ID
 15 NO:797; SEQ ID NO:803; SEQ ID NO:805; SEQ ID NO:808; SEQ ID NO:810; SEQ ID
 NO:811; SEQ ID NO:813; SEQ ID NO:814; SEQ ID NO:815; SEQ ID NO:818; SEQ ID
 NO:822; SEQ ID NO:823; SEQ ID NO:826; SEQ ID NO:831; SEQ ID NO:834; SEQ ID
 NO:837; SEQ ID NO:839; SEQ ID NO:840; SEQ ID NO:841; SEQ ID NO:845; SEQ ID
 NO:851; SEQ ID NO:852; SEQ ID NO:854; SEQ ID NO:855; SEQ ID NO:856; SEQ ID
 20 NO:857; SEQ ID NO:859; SEQ ID NO:860; SEQ ID NO:861; SEQ ID NO:868; SEQ ID
 NO:873; SEQ ID NO:876; SEQ ID NO:882; SEQ ID NO:883; SEQ ID NO:884; SEQ ID
 NO:887; SEQ ID NO:889; SEQ ID NO:891; SEQ ID NO:893; SEQ ID NO:895; SEQ ID
 NO:901; SEQ ID NO:902; SEQ ID NO:903; SEQ ID NO:904; SEQ ID NO:907; SEQ ID
 NO:908; SEQ ID NO:912; SEQ ID NO:915; SEQ ID NO:922 and SEQ ID NO:924;

25

Table III give the NCR correlation and the pI <6 of the anionic peptides above defined:

TABLE III

SEQ ID NO:	PEPTIDE	pI
SEQ ID NO:464	NCR001	5,01
SEQ ID NO:465	NCR002	4,29

SEQ ID NO:467	NCR004	5,66
SEQ ID NO:468	NCR005	5,38
SEQ ID NO:470	NCR007	4,97
SEQ ID NO:471	NCR008	5,15
SEQ ID NO:472	NCR009	4,36
SEQ ID NO:476	NCR013	5,61
SEQ ID NO:477	NCR014	4,78
SEQ ID NO:478	NCR015	5,39
SEQ ID NO:479	NCR016	4,71
SEQ ID NO:481	NCR018	4,68
SEQ ID NO:482	NCR019	4,45
SEQ ID NO:487	NCR024	4,03
SEQ ID NO:488	NCR025	4,59
SEQ ID NO:514	NCR051	4,14
SEQ ID NO:521	NCR058	4,53
SEQ ID NO:522	NCR059	4,52
SEQ ID NO:526	NCR063	4,52
SEQ ID NO:528	NCR065	5,43
SEQ ID NO:529	NCR066	3,21
SEQ ID NO:537	NCR074	4,51
SEQ ID NO:539	NCR076	4,86
SEQ ID NO:545	NCR082	4,63
SEQ ID NO:550	NCR087	4,13
SEQ ID NO:551	NCR088	4,23
SEQ ID NO:552	NCR089	5,68
SEQ ID NO:558	NCR095	3,62
SEQ ID NO:562	NCR099	5,84
SEQ ID NO:568	NCR105	5,39
SEQ ID NO:571	NCR108	4,70
SEQ ID NO:574	NCR111	5,40
SEQ ID NO:575	NCR112	4,71
SEQ ID NO:576	NCR113	5,00
SEQ ID NO:577	NCR114	4,40
SEQ ID NO:580	NCR117	4,52
SEQ ID NO:586	NCR123	3,87
SEQ ID NO:588	NCR125	5,29
SEQ ID NO:590	NCR127	4,45
SEQ ID NO:592	NCR129	4,44
SEQ ID NO:595	NCR132	4,55
SEQ ID NO:602	NCR139	4,38
SEQ ID NO:603	NCR140	4,39
SEQ ID NO:604	NCR141	4,36
SEQ ID NO:605	NCR142	3,82
SEQ ID NO:608	NCR145	4,89
SEQ ID NO:614	NCR151	3,21
SEQ ID NO:615	NCR152	5,00
SEQ ID NO:616	NCR153	3,96

SEQ ID NO:617	NCR154	4,39
SEQ ID NO:619	NCR156	4,23
SEQ ID NO:621	NCR158	3,50
SEQ ID NO:625	NCR162	3,71
SEQ ID NO:626	NCR163	4,70
SEQ ID NO:627	NCR164	3,90
SEQ ID NO:629	NCR166	4,94
SEQ ID NO:630	NCR167	3,71
SEQ ID NO:631	NCR168	3,61
SEQ ID NO:640	NCR177	4,56
SEQ ID NO:641	NCR178	5,38
SEQ ID NO:643	NCR180	4,63
SEQ ID NO:648	NCR185	4,90
SEQ ID NO:650	NCR187	4,07
SEQ ID NO:654	NCR191	3,84
SEQ ID NO:656	NCR193	4,78
SEQ ID NO:657	NCR194	5,79
SEQ ID NO:666	NCR203	4,64
SEQ ID NO:667	NCR204	4,66
SEQ ID NO:672	NCR209	4,50
SEQ ID NO:673	NCR210	4,44
SEQ ID NO:674	NCR211	5,45
SEQ ID NO:677	NCR214	3,81
SEQ ID NO:678	NCR215	3,97
SEQ ID NO:679	NCR216	4,03
SEQ ID NO:680	NCR217	5,48
SEQ ID NO:682	NCR220	5,30
SEQ ID NO:686	NCR224	4,65
SEQ ID NO:687	NCR225	4,86
SEQ ID NO:688	NCR226	3,51
SEQ ID NO:693	NCR231	5,00
SEQ ID NO:696	NCR234	4,26
SEQ ID NO:697	NCR235	4,53
SEQ ID NO:702	NCR242	4,51
SEQ ID NO:704	NCR245	4,66
SEQ ID NO:711	NCR252	4,45
SEQ ID NO:719	NCR261	5,54
SEQ ID NO:722	NCR264	5,65
SEQ ID NO:728	NCR270	5,36
SEQ ID NO:729	NCR271	5,13
SEQ ID NO:732	NCR274	4,14
SEQ ID NO:733	NCR275	4,60
SEQ ID NO:735	NCR277	4,13
SEQ ID NO:736	NCR278	3,96
SEQ ID NO:741	NCR283	4,94
SEQ ID NO:745	NCR287	3,41
SEQ ID NO:747	NCR289	4,30

SEQ ID NO:748	NCR290	4,78
SEQ ID NO:749	NCR291	4,50
SEQ ID NO:752	NCR294	4,72
SEQ ID NO:754	NCR296	4,13
SEQ ID NO:759	NCR301	4,36
SEQ ID NO:760	NCR302	4,34
SEQ ID NO:762	NCR304	5,66
SEQ ID NO:763	NCR305	4,88
SEQ ID NO:767	NCR309	5,37
SEQ ID NO:768	NCR310	4,35
SEQ ID NO:770	NCR312	4,84
SEQ ID NO:771	NCR313	4,95
SEQ ID NO:773	NCR315	3,98
SEQ ID NO:777	NCR319	4,96
SEQ ID NO:778	NCR320	4,80
SEQ ID NO:786	NCR328	5,62
SEQ ID NO:792	NCR334	5,62
SEQ ID NO:795	NCR337	5,02
SEQ ID NO:796	NCR338	4,52
SEQ ID NO:797	NCR339	4,65
SEQ ID NO:803	NCR345	4,57
SEQ ID NO:805	NCR347	3,65
SEQ ID NO:808	NCR350	4,72
SEQ ID NO:810	NCR352	4,63
SEQ ID NO:811	NCR353	3,73
SEQ ID NO:813	NCR355	3,57
SEQ ID NO:814	NCR356	4,26
SEQ ID NO:815	NCR357	4,86
SEQ ID NO:818	NCR360	4,92
SEQ ID NO:822	NCR364	5,57
SEQ ID NO:823	NCR365	5,03
SEQ ID NO:826	NCR368	4,13
SEQ ID NO:831	NCR373	4,83
SEQ ID NO:834	NCR376	4,49
SEQ ID NO:837	NCR379	5,41
SEQ ID NO:839	NCR381	4,72
SEQ ID NO:840	NCR382	5,41
SEQ ID NO:841	NCR383	3,89
SEQ ID NO:845	NCR387	4,43
SEQ ID NO:851	NCR393	5,88
SEQ ID NO:852	NCR394	4,57
SEQ ID NO:854	NCR396	3,61
SEQ ID NO:855	NCR397	3,44
SEQ ID NO:856	NCR398	3,69
SEQ ID NO:857	NCR399	3,58
SEQ ID NO:859	NCR401	4,02
SEQ ID NO:860	NCR402	3,94

SEQ ID NO:861	NCR403	4,14
SEQ ID NO:868	NCR410	4,81
SEQ ID NO:873	NCR415	4,56
SEQ ID NO:876	NCR418	4,93
SEQ ID NO:882	NCR424	4,13
SEQ ID NO:883	NCR425	4,12
SEQ ID NO:884	NCR426	4,74
SEQ ID NO:887	NCR429	3,92
SEQ ID NO:889	NCR431	5,46
SEQ ID NO:891	NCR433	4,20
SEQ ID NO:893	NCR435	4,56
SEQ ID NO:895	NCR437	4,93
SEQ ID NO:901	NCR443	5,76
SEQ ID NO:902	NCR444	4,65
SEQ ID NO:903	NCR445	4,31
SEQ ID NO:904	NCR446	5,49
SEQ ID NO:907	NCR449	4,83
SEQ ID NO:908	NCR450	3,68
SEQ ID NO:912	NCR454	4,53
SEQ ID NO:915	NCR457	4,14
SEQ ID NO:922	NCR464	3,82
SEQ ID NO:924	NCR466	4,70

In a more advantageous embodiment, said anionic peptides above defined are selected from the list consisting of: SEQ ID NO:686 (NCR 224), SEQ ID NO:697 (NCR 235), SEQ ID NO:514 (NCR 051), SEQ ID NO:464 (NCR 001).

5 Examples 3 and 4 show that anionic peptides are potential antibiotic peptides.

In a more advantageous embodiment, said neutral peptides are selected from the list consisting of:

SEQ ID NO:466; SEQ ID NO:473; SEQ ID NO:475; SEQ ID NO:491; SEQ ID NO:517;
 SEQ ID NO:519; SEQ ID NO:525; SEQ ID NO:527; SEQ ID NO:535; SEQ ID NO:540; SEQ ID
 10 NO:542; SEQ ID NO:547; SEQ ID NO:548; SEQ ID NO:553; SEQ ID NO:557; SEQ ID
 NO:559; SEQ ID NO:560; SEQ ID NO:570; SEQ ID NO:572; SEQ ID NO:578; SEQ ID
 NO:579; SEQ ID NO:581; SEQ ID NO:584; SEQ ID NO:585; SEQ ID NO:587; SEQ ID
 NO:589; SEQ ID NO:591; SEQ ID NO:594; SEQ ID NO:601; SEQ ID NO:607; SEQ ID
 NO:609; SEQ ID NO:612; SEQ ID NO:624; SEQ ID NO:628; SEQ ID NO:637; SEQ ID
 15 NO:638; SEQ ID NO:645; SEQ ID NO:649; SEQ ID NO:653; SEQ ID NO:660; SEQ ID
 NO:661; SEQ ID NO:664; SEQ ID NO:665; SEQ ID NO:668; SEQ ID NO:669; SEQ ID
 NO:671; SEQ ID NO:676; SEQ ID NO:689; SEQ ID NO:691; SEQ ID NO:692; SEQ ID

NO:695; SEQ ID NO:698; SEQ ID NO:699; SEQ ID NO:701; SEQ ID NO:703; SEQ ID NO:707; SEQ ID NO:708; SEQ ID NO:710; SEQ ID NO:712; SEQ ID NO:714; SEQ ID NO:716; SEQ ID NO:717; SEQ ID NO:718; SEQ ID NO:723; SEQ ID NO:724; SEQ ID NO:726; SEQ ID NO:727; SEQ ID NO:730; SEQ ID NO:734; SEQ ID NO:740; SEQ ID NO:751; SEQ ID NO:755; SEQ ID NO:756; SEQ ID NO:764; SEQ ID NO:765; SEQ ID NO:776; SEQ ID NO:779; SEQ ID NO:785; SEQ ID NO:788; SEQ ID NO:789; SEQ ID NO:790; SEQ ID NO:791; SEQ ID NO:798; SEQ ID NO:801; SEQ ID NO:802; SEQ ID NO:804; SEQ ID NO:806; SEQ ID NO:809; SEQ ID NO:832; SEQ ID NO:833; SEQ ID NO:838; SEQ ID NO:843; SEQ ID NO:844; SEQ ID NO:864; SEQ ID NO:867; SEQ ID NO:869; SEQ ID NO:871; SEQ ID NO:874; SEQ ID NO:885; SEQ ID NO:886; SEQ ID NO:888; SEQ ID NO:890; SEQ ID NO:898; SEQ ID NO:900; SEQ ID NO:905; SEQ ID NO:906; SEQ ID NO:911; SEQ ID NO:914; SEQ ID NO:916; SEQ ID NO:918; SEQ ID NO:920 and SEQ ID NO:923.

15

Table IV give the NCR correlation and the $6 \leq pI \leq 8$ of the neutral peptides above defined:

TABLE IV

SEQ ID NO:	PEPTIDE	pI
SEQ ID NO:466	NCR003	7,21
SEQ ID NO:473	NCR010	6,25
SEQ ID NO:475	NCR012	6,25
SEQ ID NO:491	NCR028	6,01
SEQ ID NO:517	NCR054	6,50
SEQ ID NO:519	NCR056	7,17
SEQ ID NO:525	NCR062	7,78
SEQ ID NO:527	NCR064	6,95
SEQ ID NO:535	NCR072	6,10
SEQ ID NO:540	NCR077	7,79
SEQ ID NO:542	NCR079	6,77
SEQ ID NO:547	NCR084	6,71
SEQ ID NO:548	NCR085	6,03
SEQ ID NO:553	NCR090	6,66
SEQ ID NO:557	NCR094	6,21
SEQ ID NO:559	NCR096	6,01
SEQ ID NO:560	NCR097	7,86

SEQ ID NO:570	NCR107	6,34
SEQ ID NO:572	NCR109	7,88
SEQ ID NO:578	NCR115	7,53
SEQ ID NO:579	NCR116	6,05
SEQ ID NO:581	NCR118	7,89
SEQ ID NO:584	NCR121	6,71
SEQ ID NO:585	NCR122	6,42
SEQ ID NO:587	NCR124	6,92
SEQ ID NO:589	NCR126	6,56
SEQ ID NO:591	NCR128	7,01
SEQ ID NO:594	NCR131	6,97
SEQ ID NO:601	NCR138	6,13
SEQ ID NO:607	NCR144	6,92
SEQ ID NO:609	NCR146	7,86
SEQ ID NO:612	NCR149	7,80
SEQ ID NO:624	NCR161	7,96
SEQ ID NO:628	NCR165	7,85
SEQ ID NO:637	NCR174	6,02
SEQ ID NO:638	NCR175	6,97
SEQ ID NO:645	NCR182	6,11
SEQ ID NO:649	NCR186	6,23
SEQ ID NO:653	NCR190	6,87
SEQ ID NO:660	NCR197	7,73
SEQ ID NO:661	NCR198	6,13
SEQ ID NO:664	NCR201	6,74
SEQ ID NO:665	NCR202	7,11
SEQ ID NO:668	NCR205	7,63
SEQ ID NO:669	NCR206	6,25
SEQ ID NO:671	NCR208	7,76
SEQ ID NO:676	NCR213	6,25
SEQ ID NO:689	NCR227	6,36
SEQ ID NO:691	NCR229	6,55
SEQ ID NO:692	NCR230	6,11
SEQ ID NO:695	NCR233	7,96
SEQ ID NO:698	NCR236	7,72
SEQ ID NO:699	NCR237	6,71
SEQ ID NO:701	NCR239	6,94
SEQ ID NO:703	NCR243	6,88
SEQ ID NO:707	NCR248	7,62
SEQ ID NO:708	NCR249	6,03
SEQ ID NO:710	NCR251	6,27
SEQ ID NO:712	NCR253	6,88
SEQ ID NO:714	NCR255	7,20
SEQ ID NO:716	NCR258	6,15
SEQ ID NO:717	NCR259	7,65
SEQ ID NO:718	NCR260	6,89
SEQ ID NO:723	NCR265	6,71

SEQ ID NO:724	NCR266	7,79
SEQ ID NO:726	NCR268	7,10
SEQ ID NO:727	NCR269	6,71
SEQ ID NO:730	NCR272	6,12
SEQ ID NO:734	NCR276	7,05
SEQ ID NO:740	NCR282	7,77
SEQ ID NO:751	NCR293	6,87
SEQ ID NO:755	NCR297	7,77
SEQ ID NO:756	NCR298	7,75
SEQ ID NO:764	NCR306	6,04
SEQ ID NO:765	NCR307	6,26
SEQ ID NO:776	NCR318	7,89
SEQ ID NO:779	NCR321	7,86
SEQ ID NO:785	NCR327	6,88
SEQ ID NO:788	NCR330	6,09
SEQ ID NO:789	NCR331	7,96
SEQ ID NO:790	NCR332	7,86
SEQ ID NO:791	NCR333	6,40
SEQ ID NO:798	NCR340	7,88
SEQ ID NO:801	NCR343	6,35
SEQ ID NO:802	NCR344	7,79
SEQ ID NO:804	NCR346	7,75
SEQ ID NO:806	NCR348	7,75
SEQ ID NO:809	NCR351	7,76
SEQ ID NO:832	NCR374	7,77
SEQ ID NO:833	NCR375	7,72
SEQ ID NO:838	NCR380	6,29
SEQ ID NO:843	NCR385	7,76
SEQ ID NO:844	NCR386	6,88
SEQ ID NO:864	NCR406	6,31
SEQ ID NO:867	NCR409	7,61
SEQ ID NO:869	NCR411	7,14
SEQ ID NO:871	NCR413	6,88
SEQ ID NO:874	NCR416	7,64
SEQ ID NO:885	NCR427	7,61
SEQ ID NO:886	NCR428	7,70
SEQ ID NO:888	NCR430	7,73
SEQ ID NO:890	NCR432	7,75
SEQ ID NO:898	NCR440	6,23
SEQ ID NO:900	NCR442	7,15
SEQ ID NO:905	NCR447	6,26
SEQ ID NO:906	NCR448	7,79
SEQ ID NO:911	NCR453	7,78
SEQ ID NO:914	NCR456	7,71
SEQ ID NO:916	NCR458	7,64
SEQ ID NO:918	NCR460	6,54
SEQ ID NO:920	NCR462	7,64

SEQ ID NO:923 NCR465 7,61

In a more advantageous embodiment, said neutral peptides above defined are selected from the list consisting of: SEQ ID NO:547 (NCR 084), SEQ ID NO:691 (NCR 229).

5 In a more advantageous embodiment, the present invention relates to the use of peptides as defined above, wherein said peptides are active against both Gram – and Gram + bacteria.

Antibiotic such as polymyxin B are active against Gram – bacteria limiting thus their use.

Peptides of the invention are active against Gram – and Gram + as demonstrated by example 5 (in the rest of the specification, polymyxin or polymyxin B represent the same compound).

10 Further, as can be seen from example 7 and example 8, peptides of the invention have no adverse effects on mammalian cells and on plants, respectively.

In a more advantageous embodiment, said peptides above defined are active at a concentration range from about 1 to about 100 µg/ml, preferably from about 1 to 50µg/ml, in particular from about 10 to about 50 µg/ml.

15 Below 1 µg/ml, the concentration is too low to produce an effect.

Above 100µg/ml, the concentration is too high and can lead to adverse effects.

As can be shown by example 3, peptides of the invention are active at low doses such as 50µg/ml and example 4B shows that peptides of the invention present a higher activity than the one of polymyxin.

20 In a more advantageous embodiment, peptides of the invention are active at doses of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml

In a preferred embodiment, peptides of the invention are active at doses of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/ml.

25 In a more advantageous embodiment, peptides of the invention are insensitive or weakly sensitive to physiological salt (2 mM) of divalent cations such as Mg^{++} or Ca^{++} . The sensitivity towards Mg^{++} or Ca^{++} could be compensated by addition of EDTA at equimolar concentration.

In a more advantageous embodiment, the present invention relates to the use of peptides above defined, wherein the number of colony forming units of bacteria dropped 3 to 9 orders of magnitude, in particular 5 to 7 orders, in 3 hours of contact with said peptides.

Example 5 shows that peptides of the invention are able to diminish the content of the bacteria, starting from an initial concentration of bacteria comprised from 10^6 to 10^9 , by a factor 1000 to one billion to reach a final concentration in bacteria comprised from 0 to 10^6 .

5 In another aspect, the present invention relates to the use of at least one gene coding for a peptide originated from *Medicago truncatula* nodules, comprising the SEQ IDs NO: 1 – 463 or at least one gene coding for a peptide having a sequence derived from said SEQ IDs NO: 1 – 463 by deletion of about 9 to about 44 contiguous amino acids, from the N-terminal part of the peptide, in particular a gene coding for a peptide having the SEQ IDS NO: 464 to 925, for the preparation of transgenic plants that do not express said peptide in their genome or in a part of the said plant
10 such as leaves or stem.

In another aspect, the invention relates to a pharmaceutical composition comprising at least two peptides of SEQ IDs NO: 1 – 463, as above defined, or peptides of SEQ ID 464 to 925, as above defined, in association with a pharmaceutical acceptable vehicle, provided that said peptides have a purity being equal or more than about 90%, in particular more than about 95%.

15 Peptides of the pharmaceutical composition can be isolated from *Medicago truncatula* nodules or synthesized by chemical or biological way with a purity higher or equal to about 90%, in particular more than about 95%.

Thus when the peptides of the invention are isolated from *Medicago truncatula* nodules, they have not the same environment as in the nodules, and therefore have different functions
20 from that of the peptides contained in the nodules.

In an advantageous embodiment, the above defined pharmaceutical composition, comprises one or more cationic peptides ($pI > 8$) in association with one or more anionic peptides ($pI < 6$) and/or one or more neutral peptides ($6 \leq pI \leq 8$).

25 In an advantageous embodiment, said cationic peptides comprised in the pharmaceutical composition above defined, are selected from the list consisting of : SEQ ID NO:469; SEQ ID NO:474; SEQ ID NO:480; SEQ ID NO:483; SEQ ID NO:484; SEQ ID NO:485; SEQ ID NO:486; SEQ ID NO:489; SEQ ID NO:490; SEQ ID NO:492; SEQ ID NO:493; SEQ ID NO:494; SEQ ID NO:495; SEQ ID NO:496; SEQ ID NO:497; SEQ ID NO:498; SEQ ID
30 NO:499; SEQ ID NO:500; SEQ ID NO:501; SEQ ID NO:502; SEQ ID NO:503; SEQ ID NO:504; SEQ ID NO:505; SEQ ID NO:506; SEQ ID NO:507; SEQ ID NO:508; SEQ ID

NO:509; SEQ ID NO:510; SEQ ID NO:511; SEQ ID NO:512; SEQ ID NO:513; SEQ ID
NO:515; SEQ ID NO:516; SEQ ID NO:518; SEQ ID NO:520; SEQ ID NO:523; SEQ ID
NO:524; SEQ ID NO:530; SEQ ID NO:531; SEQ ID NO:532; SEQ ID NO:533; SEQ ID
NO:534; SEQ ID NO:536; SEQ ID NO:538; SEQ ID NO:541; SEQ ID NO:543; SEQ ID
5 NO:544; SEQ ID NO:546; SEQ ID NO:549; SEQ ID NO:554; SEQ ID NO:555; SEQ ID
NO:556; SEQ ID NO:561; SEQ ID NO:563; SEQ ID NO:564; SEQ ID NO:565; SEQ ID
NO:566; SEQ ID NO:567; SEQ ID NO:569; SEQ ID NO:573; SEQ ID NO:582; SEQ ID
NO:583; SEQ ID NO:593; SEQ ID NO:596; SEQ ID NO:597; SEQ ID NO:598; SEQ ID
NO:599; SEQ ID NO:600; SEQ ID NO:606; SEQ ID NO:610; SEQ ID NO:611; SEQ ID
10 NO:613; SEQ ID NO:618; SEQ ID NO:620; SEQ ID NO:622; SEQ ID NO:623; SEQ ID
NO:632; SEQ ID NO:633; SEQ ID NO:634; SEQ ID NO:635; SEQ ID NO:636; SEQ ID
NO:639; SEQ ID NO:642; SEQ ID NO:644; SEQ ID NO:646; SEQ ID NO:647; SEQ ID
NO:651; SEQ ID NO:652; SEQ ID NO:655; SEQ ID NO:658; SEQ ID NO:659; SEQ ID
NO:662; SEQ ID NO:663; SEQ ID NO:670; SEQ ID NO:675; SEQ ID NO:681; SEQ ID
15 NO:683; SEQ ID NO:684; SEQ ID NO:685; SEQ ID NO:690; SEQ ID NO:694; SEQ ID
NO:700; SEQ ID NO:705; SEQ ID NO:706; SEQ ID NO:709; SEQ ID NO:713; SEQ ID
NO:715; SEQ ID NO:720; SEQ ID NO:721; SEQ ID NO:725; SEQ ID NO:731; SEQ ID
NO:737; SEQ ID NO:738; SEQ ID NO:739; SEQ ID NO:742; SEQ ID NO:743; SEQ ID
NO:744; SEQ ID NO:746; SEQ ID NO:750; SEQ ID NO:753; SEQ ID NO:757; SEQ ID
20 NO:758; SEQ ID NO:761; SEQ ID NO:766; SEQ ID NO:769; SEQ ID NO:772; SEQ ID
NO:774; SEQ ID NO:775; SEQ ID NO:780; SEQ ID NO:781; SEQ ID NO:782; SEQ ID
NO:783; SEQ ID NO:784; SEQ ID NO:787; SEQ ID NO:793; SEQ ID NO:794; SEQ ID
NO:799; SEQ ID NO:800; SEQ ID NO:807; SEQ ID NO:812; SEQ ID NO:816; SEQ ID
NO:817; SEQ ID NO:819; SEQ ID NO:820; SEQ ID NO:821; SEQ ID NO:824; SEQ ID
25 NO:825; SEQ ID NO:827; SEQ ID NO:828; SEQ ID NO:829; SEQ ID NO:830; SEQ ID
NO:835; SEQ ID NO:836; SEQ ID NO:842; SEQ ID NO:846; SEQ ID NO:847; SEQ ID
NO:848; SEQ ID NO:849; SEQ ID NO:850; SEQ ID NO:853; SEQ ID NO:858; SEQ ID
NO:862; SEQ ID NO:863; SEQ ID NO:865; SEQ ID NO:866; SEQ ID NO:870; SEQ ID
NO:872; SEQ ID NO:875; SEQ ID NO:877; SEQ ID NO:878; SEQ ID NO:879; SEQ ID
30 NO:880; SEQ ID NO:881; SEQ ID NO:892; SEQ ID NO:894; SEQ ID NO:896; SEQ ID

NO:897; SEQ ID NO:899; SEQ ID NO:909; SEQ ID NO:910; SEQ ID NO:913; SEQ ID NO:917; SEQ ID NO:919; SEQ ID NO:921 and SEQ ID NO:925.

For the correlation with NCR peptides, see table II above.

5 In an advantageous embodiment, said cationic peptides comprised in the pharmaceutical composition above defined, are selected from the list consisting of: SEQ ID NO:706 (NCR 247), SEQ ID NO:498 (NCR 035), SEQ ID NO:518 (NCR 055), SEQ ID NO:520 (NCR 57).

In an advantageous embodiment, said anionic peptides comprised in the pharmaceutical composition above defined, are selected from the list consisting of:

10 SEQ ID NO:464; SEQ ID NO:465; SEQ ID NO:467; SEQ ID NO:468; SEQ ID NO:470; SEQ ID NO:471; SEQ ID NO:472; SEQ ID NO:476; SEQ ID NO:477; SEQ ID NO:478; SEQ ID NO:479; SEQ ID NO:481; SEQ ID NO:482; SEQ ID NO:487; SEQ ID NO:488; SEQ ID NO:514; SEQ ID NO:521; SEQ ID NO:522; SEQ ID NO:526; SEQ ID NO:528; SEQ ID NO:529; SEQ ID NO:537; SEQ ID NO:539; SEQ ID NO:545; SEQ ID NO:550; SEQ ID
15 NO:551; SEQ ID NO:552; SEQ ID NO:558; SEQ ID NO:562; SEQ ID NO:568; SEQ ID NO:571; SEQ ID NO:574; SEQ ID NO:575; SEQ ID NO:576; SEQ ID NO:577; SEQ ID NO:580; SEQ ID NO:586; SEQ ID NO:588; SEQ ID NO:590; SEQ ID NO:592; SEQ ID NO:595; SEQ ID NO:602; SEQ ID NO:603; SEQ ID NO:604; SEQ ID NO:605; SEQ ID NO:608; SEQ ID NO:614; SEQ ID NO:615; SEQ ID NO:616; SEQ ID NO:617; SEQ ID
20 NO:619; SEQ ID NO:621; SEQ ID NO:625; SEQ ID NO:626; SEQ ID NO:627; SEQ ID NO:629; SEQ ID NO:630; SEQ ID NO:631; SEQ ID NO:640; SEQ ID NO:641; SEQ ID NO:643; SEQ ID NO:648; SEQ ID NO:650; SEQ ID NO:654; SEQ ID NO:656; SEQ ID NO:657; SEQ ID NO:666; SEQ ID NO:667; SEQ ID NO:672; SEQ ID NO:673; SEQ ID NO:674; SEQ ID NO:677; SEQ ID NO:678; SEQ ID NO:679; SEQ ID NO:680; SEQ ID
25 NO:682; SEQ ID NO:686; SEQ ID NO:687; SEQ ID NO:688; SEQ ID NO:693; SEQ ID NO:696; SEQ ID NO:697; SEQ ID NO:702; SEQ ID NO:704; SEQ ID NO:711; SEQ ID NO:719; SEQ ID NO:722; SEQ ID NO:728; SEQ ID NO:729; SEQ ID NO:732; SEQ ID NO:733; SEQ ID NO:735; SEQ ID NO:736; SEQ ID NO:741; SEQ ID NO:745; SEQ ID NO:747; SEQ ID NO:748; SEQ ID NO:749; SEQ ID NO:752; SEQ ID NO:754; SEQ ID
30 NO:759; SEQ ID NO:760; SEQ ID NO:762; SEQ ID NO:763; SEQ ID NO:767; SEQ ID NO:768; SEQ ID NO:770; SEQ ID NO:771; SEQ ID NO:773; SEQ ID NO:777; SEQ ID

NO:778; SEQ ID NO:786; SEQ ID NO:792; SEQ ID NO:795; SEQ ID NO:796; SEQ ID
NO:797; SEQ ID NO:803; SEQ ID NO:805; SEQ ID NO:808; SEQ ID NO:810; SEQ ID
NO:811; SEQ ID NO:813; SEQ ID NO:814; SEQ ID NO:815; SEQ ID NO:818; SEQ ID
NO:822; SEQ ID NO:823; SEQ ID NO:826; SEQ ID NO:831; SEQ ID NO:834; SEQ ID
5 NO:837; SEQ ID NO:839; SEQ ID NO:840; SEQ ID NO:841; SEQ ID NO:845; SEQ ID
NO:851; SEQ ID NO:852; SEQ ID NO:854; SEQ ID NO:855; SEQ ID NO:856; SEQ ID
NO:857; SEQ ID NO:859; SEQ ID NO:860; SEQ ID NO:861; SEQ ID NO:868; SEQ ID
NO:873; SEQ ID NO:876; SEQ ID NO:882; SEQ ID NO:883; SEQ ID NO:884; SEQ ID
NO:887; SEQ ID NO:889; SEQ ID NO:891; SEQ ID NO:893; SEQ ID NO:895; SEQ ID
10 NO:901; SEQ ID NO:902; SEQ ID NO:903; SEQ ID NO:904; SEQ ID NO:907; SEQ ID
NO:908; SEQ ID NO:912; SEQ ID NO:915; SEQ ID NO:922 and SEQ ID NO:924;

For the correlation with NCR peptides, see table III above.

In an advantageous embodiment, said anionic peptides comprised in the pharmaceutical
15 composition above defined, are selected from the list consisting of: SEQ ID NO:686 (NCR 224),
SEQ ID NO:697 (NCR 235), SEQ ID NO:514 (NCR 051), SEQ ID NO:464 (NCR 001).

In an advantageous embodiment, said neutral peptides comprised in the pharmaceutical
composition above defined, are selected from the list consisting of:

SEQ ID NO:466; SEQ ID NO:473; SEQ ID NO:475; SEQ ID NO:491; SEQ ID NO:517;
20 SEQ ID NO:519; SEQ ID NO:525; SEQ ID NO:527; SEQ ID NO:535; SEQ ID NO:540; SEQ ID
NO:542; SEQ ID NO:547; SEQ ID NO:548; SEQ ID NO:553; SEQ ID NO:557; SEQ ID
NO:559; SEQ ID NO:560; SEQ ID NO:570; SEQ ID NO:572; SEQ ID NO:578; SEQ ID
NO:579; SEQ ID NO:581; SEQ ID NO:584; SEQ ID NO:585; SEQ ID NO:587; SEQ ID
NO:589; SEQ ID NO:591; SEQ ID NO:594; SEQ ID NO:601; SEQ ID NO:607; SEQ ID
25 NO:609; SEQ ID NO:612; SEQ ID NO:624; SEQ ID NO:628; SEQ ID NO:637; SEQ ID
NO:638; SEQ ID NO:645; SEQ ID NO:649; SEQ ID NO:653; SEQ ID NO:660; SEQ ID
NO:661; SEQ ID NO:664; SEQ ID NO:665; SEQ ID NO:668; SEQ ID NO:669; SEQ ID
NO:671; SEQ ID NO:676; SEQ ID NO:689; SEQ ID NO:691; SEQ ID NO:692; SEQ ID
NO:695; SEQ ID NO:698; SEQ ID NO:699; SEQ ID NO:701; SEQ ID NO:703; SEQ ID
30 NO:707; SEQ ID NO:708; SEQ ID NO:710; SEQ ID NO:712; SEQ ID NO:714; SEQ ID
NO:716; SEQ ID NO:717; SEQ ID NO:718; SEQ ID NO:723; SEQ ID NO:724; SEQ ID

NO:726; SEQ ID NO:727; SEQ ID NO:730; SEQ ID NO:734; SEQ ID NO:740; SEQ ID
 NO:751; SEQ ID NO:755; SEQ ID NO:756; SEQ ID NO:764; SEQ ID NO:765; SEQ ID
 NO:776; SEQ ID NO:779; SEQ ID NO:785; SEQ ID NO:788; SEQ ID NO:789; SEQ ID
 NO:790; SEQ ID NO:791; SEQ ID NO:798; SEQ ID NO:801; SEQ ID NO:802; SEQ ID
 5 NO:804; SEQ ID NO:806; SEQ ID NO:809; SEQ ID NO:832; SEQ ID NO:833; SEQ ID
 NO:838; SEQ ID NO:843; SEQ ID NO:844; SEQ ID NO:864; SEQ ID NO:867; SEQ ID
 NO:869; SEQ ID NO:871; SEQ ID NO:874; SEQ ID NO:885; SEQ ID NO:886; SEQ ID
 NO:888; SEQ ID NO:890; SEQ ID NO:898; SEQ ID NO:900; SEQ ID NO:905; SEQ ID
 NO:906; SEQ ID NO:911; SEQ ID NO:914; SEQ ID NO:916; SEQ ID NO:918; SEQ ID
 10 NO:920 and SEQ ID NO:923.

For the correlation with NCR peptides, see table IV above.

In an advantageous embodiment, said neutral peptides comprised in the pharmaceutical
 composition above defined, are selected from the list consisting of: SEQ ID NO:547 (NCR 084),
 15 SEQ ID NO:691 (NCR 229).

DESCRIPTION OF THE FIGURES

Figure 1 presents the CFU obtained after treatment during 3 hours of bacterium
Sinorhizobium meliloti (starting CFU was : 10^7 colony/ml) with:

- 20 A: control without NCR peptides or antibiotics
- B: tetracyclin (Tc) at 10 μ M,
- C: tetracyclin at 100 μ M,
- D: kanamycin (Km) at 10 μ M,
- E: kanamycin at 100 μ M,
- 25 F: NCR 055 at 10 μ M,
- F: NCR 035 at 10 μ M,
- F: NCR 247 at 10 μ M,
- G: polymyxin-B at 10 μ M.

Figures 2A and 2B presents the results obtained after treatment of *Sinorhizobium meliloti* AK631 bacteria with 50 µg/ml of various NCRs in microtiter plates.

Figure 2A: PI staining:

x-axis from left to right: buffer (control), polymyxin B (50 µg/ml, positive control),
5 bacteria heat killed (control), NCR035, NCR051, NCR055, NCR057, NCR084, NCR0224,
NCR229, NCR235, NCR247.

y-axis: percentage of PI staining.

Figure 2B: CFU determination:

x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control),
10 bacteria heat killed (control), NCR035, NCR051, NCR055, NCR057, NCR084, NCR0224,
NCR229, NCR235, NCR247.

y-axis: percentage of relative CFU obtained.

Figures 3A to 3E present the results obtained for the relative PI fluorescence of NCR
treated *S. meliloti* cells with various concentrations of NCR in comparison to polymyxin B and
15 heat treatments.

Figure 3A: PI staining for NCR035:

x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control),
bacteria heat killed (control), NCR035 (1µg/ml), NCR035 (2µg/ml), NCR035 (5µg/ml), NCR035
(10µg/ml), NCR035 (20µg/ml), NCR035 (50µg/ml).

20 y-axis: percentage of maximum PI staining obtained with heat killed bacteria.

Figure 3B: PI staining for NCR055:

x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control),
bacteria heat killed (control), NCR055 (1µg/ml), NCR055 (2µg/ml), NCR055 (5µg/ml), NCR055
(10µg/ml), NCR055 (20µg/ml), NCR055 (50µg/ml).

25 y-axis: percentage of maximum PI staining obtained with heat killed bacteria.

Figure 3C: PI staining for NCR057:

x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control),
bacteria heat killed (control), NCR057 (1µg/ml), NCR057 (2µg/ml), NCR057 (5µg/ml), NCR057
(10µg/ml), NCR057 (20µg/ml), NCR057 (50µg/ml).

30 y-axis: percentage of maximum PI staining obtained with heat killed bacteria.

Figure 3D: PI staining for NCR0247:

x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control), bacteria heat killed (control), NCR0247 (1µg/ml), NCR0247 (2µg/ml), NCR0247 (5µg/ml), NCR0247 (10µg/ml), NCR0247 (20µg/ml), NCR0247 (50µg/ml).

y-axis: percentage of maximum PI staining obtained with heat killed bacteria.

5 Figure 3E: PI staining for NCR224:

x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control), bacteria heat killed (control), NCR224 (1µg/ml), NCR224 (2µg/ml), NCR224 (5µg/ml), NCR224 (10µg/ml), NCR224 (20µg/ml), NCR224 (50µg/ml).

y-axis: percentage of maximum PI staining obtained with heat killed bacteria.

10 Figures 4A to 4E presents the CFUs of *S. meliloti* after treatment with various NCRs at 20 and 50 µg/ml concentrations for 1 hr.

Figure 4A:

x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control), bacteria heat killed (control), NCR035 (20µg/ml), NCR035 (50µg/ml).

15 y-axis: percentage of relative CFU.

Figure 4B:

x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control), bacteria heat killed (control), NCR055 (20µg/ml), NCR055 (50µg/ml).

y-axis: percentage of relative CFU.

20 Figure 4C:

x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control), bacteria heat killed (control), NCR057 (20µg/ml), NCR057 (50µg/ml).

y-axis: percentage of relative CFU.

Figure 4D:

25 x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control), bacteria heat killed (control), NCR224 (20µg/ml), NCR224 (50µg/ml).

y-axis: percentage of relative CFU.

Figure 4E:

30 x-axis: from left to right: buffer (control), polymyxin B (50 µg/ml, positive control), bacteria heat killed (control), NCR247 (20µg/ml), NCR247 (50µg/ml).

y-axis: percentage of relative CFU.

Figure 5 presents the comparison between Polymyxin B and NCR 035 at different doses.

x-axis: from left to right: NCR035 peptide (black histograms) or polymyxin (dark grey histograms) concentrations: 0, 1, 2, 5, 10, 20, 50 µg/ml

5 y-axis: percentage of relative CFU.

Figure 6 and 7 presents the effect of salts of divalent cations (Mg^{++} and Ca^{++}) on the activity of NCR peptides.

Figure 6: Ca cation

10 x-axis : from left to right: untreated cells, heat killed, NCR247S + 0.1mM $CaCl_2$, NCR247S + 0.5mM $CaCl_2$, NCR247S + 1mM $CaCl_2$, NCR247S + 2mM $CaCl_2$, NCR247S + 5mM $CaCl_2$,

y-axis: percentage of maximum PI staining obtained with heat killed bacteria.

NCRxxxS correspond to NCRxxx

Figure 7: Mg cation

15 x-axis : from left to right: untreated cells, heat killed, NCR247S + 0.1mM $MgCl_2$, NCR247S + 0.5mM $MgCl_2$, NCR247S + 1mM $MgCl_2$, NCR247S + 2mM $MgCl_2$, NCR247S + 5mM $MgCl_2$,

y-axis: percentage of maximum PI staining obtained with heat killed bacteria.

NCRxxxS correspond to NCRxxx

20 Figure 8 presents the effects of the peptides on human cell cultures (Caski cells).

This figure shows the PI staining after one hour of incubation with the peptides.

x-axis : PI staining positive cells (%)

y-axis: from left to right histogram: Caski (control), Caski (PI), NCR055 (PI), NCR084 (PI), NCR235 (PI), NCR035 (PI), NCR051 (PI), NCR057 (PI), NCR224 (PI), NCR247 (PI).

25 Figure 9A to 9D presents the effect of NCR peptides (final peptide concentration: 50 µM) on *Arabidopsis* germination and seedling growth.

Figure 9A: treatment with NCR 057 or NCR 087 or NCR 224 peptides.

Figure 9B: treatment with NCR 035 or NCR 051 or NCR 055 peptides.

Figure 9C: treatment with NCR 229 or NCR 235 or NCR 247 peptides.

30 Figure 9D: treatment with water or polymyxin B (50 µg/ml = 42µM).

Figure 10 presents the effect of NCR 247 peptide on a bacterial strain (*Pseudomonas aeruginosa*) resistant to conventional antibiotics at 20 and 50 µg/ml. At 50 µg/ml, the *Pseudomonas aeruginosa* is completely eradicated by NCR 247.

x-axis: from left to right: control, NCR 247 at 20 µg/ml and NCR at 50 µg/ml.

y-axis : CFU

Figures 11A to 11 F present the antifungal effect of NCR 247 peptide on *Fusarium graminearum* (causing fusariose in plants) after 24h or 48h of incubation.

Figures 11 A to 11C show the development of spores onto solid minimal medium (example 9).

Figure 11D to 11F show the hyphae development of *Fusarium graminearum*.

Figures 11A and 11D: control

Figures 11B and 11E: Medicago sativa defensin (MsDef)

Figures 11C and 11F: NCR 247 showing that NCR 247 inhibits the spores and hyphae development at 50 µg/ml.

Results are the same at 24h and 48h.

Figure 12A to 12E present the antifungal effect of NCR 247 on *Aspergillus flavus* at various concentration after 24h of incubation.

Figure 12A : Control showing the growth of the fungi.

Figure 12B : Effect of Amphotericin B (AMB antifungal agent) at 125 µg/ml.

Figure 12C : Effect of NCR 247 at 25 µg/ml

Figure 12D : Effect of NRC 247 at 50 µg/ml

Figure 12 E : Effect of NCR 247 at 100 µg/ml.

Figure 13A to 13D present the antifungal effect of NCR 247 on *Aspergillus flavus* at various concentration after 48h of incubation.

Figure 13A : Control showing the growth of the fungi.

Figure 13B : Effect of NCR 247 at 25 µg/ml

Figure 13C : Effect of NRC 247 at 50 µg/ml

Figure 13D : Effect of NCR 247 at 100 µg/ml.

Figure 14A to 14C present the antifungal effect of NCR 035 on *Aspergillus flavus* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see figure 12A)

5 Figure 14A : Effect of NCR 035 at 25 µg/ml

Figure 14B : Effect of NRC 035 at 50 µg/ml

Figure 14C : Effect of NCR 035 at 100 µg/ml.

10 Figure 15 presents the antifungal effect of NCR 035 on *Aspergillus flavus* at 100 µg/ml after 48h of incubation.

Control showing the growth of the fungi (see figure 12A)

Figure 15 : Effect of NCR 035.

Results are the same with 25 µg/ml and 50 µg/ml for 48h of incubation for this peptide.

15 Figure 16A to 16B present the antifungal effect of NCR 055 on *Aspergillus flavus* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see figure 12A)

Figure 16A : Effect of NCR 055 at 50 µg/ml

Figure 16B : Effect of NRC 055at 100 µg/ml

20 Figure 17A to 17B present the antifungal effect of NCR 055 on *Aspergillus flavus* at various concentration after 48h of incubation.

Control showing the growth of the fungi (see figure 12A)

Figure 17A : Effect of NCR 055 at 50 µg/ml

25 Figure 17B : Effect of NRC 055 at 100 µg/ml

Figure 18A to 18C present the antifungal effect of NCR 247 on *Aspergillus niger* at various concentration after 24h of incubation.

Figure 18A: Control showing the growth of the fungi at 24h

30 Figure 18B : Effect of NCR 247 at 50 µg/ml

Figure 18C : Effect of NRC 247at 100 µg/ml

Figure 19A to 19D present the antifungal effect of NCR 035 on *Aspergillus niger* at various concentration after 24h or 48h of incubation.

Control showing the growth of the fungi at 24h (see figure 18A)

Figure 19A: Control showing the growth of the fungi at 48h

5 Figure 19B : Effect of NCR 035 at 50 µg/ml at 24h

Figure 19C : Effect of NRC 035 at 100 µg/ml at 24h

Figure 19D : Effect of NRC 035 at 100 µg/ml at 48h

10 Figure 20A to 20C present the antifungal effect of NCR 055 on *Aspergillus niger* at various concentration after 24h or 48h of incubation.

Control showing the growth of the fungi at 24 and 48 h (see figure 18A and 19A)

Figure 20A : Effect of NCR 055 at 50 µg/ml at 24h

Figure 20B : Effect of NRC 055 at 100 µg/ml at 24h

Figure 20C : Effect of NRC 055 at 100 µg/ml at 48h

15 The result is the same for NCR 055 at 50 µg/ml at 48h as at 24h

Figure 21A to 21C present the antifungal effect of NCR 247 on *Candida albicans* at various concentration after 24h of incubation.

Figure 21A : Control showing the growth of the fungi.

20 Figure 21B : Effect of Amphotericin B (AMB antifungal agent) at 125 µg/ml.

Figure 21C : Effect of NCR 247 at 100 µg/ml

Figure 22A to 22C present the antifungal effect of NCR 035 on *Candida albicans* at various concentration after 24h of incubation.

25 Control showing the growth of the fungi (see figure 21A)

Figure 22A : Effect of NCR 035 at 25 µg/ml

Figure 22B : Effect of NRC 035 at 50 µg/ml

Figure 22C: Effect of NCR 035 at 100 µg/ml.

30 Figure 23A to 23B present the antifungal effect of NCR 055 on *Candida albicans* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see figure 21A)

Figure 23A : Effect of NRC 055 at 50 µg/ml

Figure 23B: Effect of NCR 055 at 100 µg/ml.

5 Figure 24A to 24C present the antifungal effect of NCR 035 on *Candida albicans* at various concentration after 48h of incubation.

Figure 24A : Control showing the growth of the fungi.

Figure 24B : Effect of NRC 035 at 25 µg/ml

Figure 24C: Effect of NCR 035 at 100 µg/ml.

10 Figure 25A to 25E present the antifungal effect of NCR 247 on *Candida crusei* at various concentration after 24h of incubation.

Figure 25A : Control showing the growth of the fungi.

Figure 25B : Effect of Amphotericin B (AMB antifungal agent) at 125 µg/ml.

15 Figure 25C : Effect of NCR 247 at 25 µg/ml

Figure 25D : Effect of NCR 247 at 50 µg/ml

Figure 25E : Effect of NCR 247 at 100 µg/ml

20 Figure 26A to 26C present the antifungal effect of NCR 035 on *Candida crusei* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see Figure 25A)

Figure 26A : Effect of NCR 035 at 25 µg/ml

Figure 26B : Effect of NCR 035 at 50 µg/ml

Figure 26C : Effect of NCR 035 at 100 µg/ml

25 Figure 27A to 27B present the antifungal effect of NCR 055 on *Candida crusei* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see Figure 25A)

Figure 27A : Effect of NCR 055 at 50 µg/ml

30 Figure 27B : Effect of NCR 055 at 100 µg/ml

Figure 28A to 28B present the antifungal effect of NCR 035 on *Candida crusei* at various concentration after 48h of incubation.

Figure 28A : Control showing the growth of the fungi

Figure 28B : Effect of NCR 035 at 25 µg/ml

5

Figure 29A to 29E present the antifungal effect of NCR 247 on *Candida parapsilosis* at various concentration after 24h of incubation.

Figure 29A : Control showing the growth of the fungi.

Figure 29B : Effect of Amphotericin B (AMB antifungal agent) at 125 µg/ml.

10

Figure 29C : Effect of NCR 247 at 25 µg/ml

Figure 29D : Effect of NCR 247 at 50 µg/ml

Figure 29E : Effect of NCR 247 at 100 µg/ml

15

Figure 30A to 30C present the antifungal effect of NCR 035 on *Candida parapsilosis* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see Figure 29A)

Figure 30A : Effect of NCR 035 at 25 µg/ml

Figure 30B : Effect of NCR 035 at 50 µg/ml

Figure 30C : Effect of NCR 035 at 100 µg/ml

20

Figure 31A to 31B present the antifungal effect of NCR 055 on *Candida parapsilosis* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see Figure 29A)

Figure 31A : Effect of NCR 055 at 50 µg/ml

25

Figure 31B : Effect of NCR 055 at 100 µg/ml

Figure 32A to 32E present the antifungal effect of NCR 247 on *Rhizopus stolonifer* var. *stolonifer* at various concentration after 24h of incubation.

Figure 32A : Control showing the growth of the fungi.

30

Figure 32B : Effect of Amphotericin B (AMB antifungal agent) at 125 µg/ml.

Figure 32C : Effect of NCR 247 at 25 µg/ml

Figure 32D : Effect of NCR 247 at 50 µg/ml

Figure 32E : Effect of NCR 247 at 100 µg/ml

Figure 33A to 33C present the antifungal effect of NCR 035 on *Rhizopus stolonifer* var. *stolonifer* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see Figure 32A)

Figure 33A : Effect of NCR 035 at 25 µg/ml

Figure 33B : Effect of NCR 035 at 50 µg/ml

Figure 33C : Effect of NCR 035 at 100 µg/ml

Figure 34A to 34B present the antifungal effect of NCR 055 on *Rhizopus stolonifer* var. *stolonifer* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see Figure 32A)

Figure 34A : Effect of NCR 055 at 50 µg/ml

Figure 34B : Effect of NCR 055 at 100 µg/ml

Figure 35A to 35D present the antifungal effect of NCR 247 on *Fusarium graminearum* at various concentration after 24h of incubation.

Figure 35A : Control showing the growth of the fungi.

Figure 35B : Effect of Amphotericin B (AMB antifungal agent) at 125 µg/ml.

Figure 35C : Effect of NCR 247 at 25 µg/ml

Figure 35D : Effect of NCR 247 at 100 µg/ml

Figure 36A to 36C present the antifungal effect of NCR 035 on *Fusarium graminearum* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see Figure 35A)

Figure 36A : Effect of NCR 035 at 25 µg/ml

Figure 36B : Effect of NCR 035 at 50 µg/ml

Figure 36C : Effect of NCR 035 at 100 µg/ml

Figure 37A to 37B present the antifungal effect of NCR 055 on *Fusarium graminearum* at various concentration after 24h of incubation.

Control showing the growth of the fungi (see Figure 35A)

Figure 37A : Effect of NCR 055 at 50 µg/ml

5 Figure 37B : Effect of NCR 055 at 100 µg/ml

Figure 38A to 38B present the antifungal effect of NCR 035 on *Fusarium graminearum* at various concentration after 48h of incubation.

Control showing the growth of the fungi (see Figure 35A)

10 Figure 38A : Control showing the growth of the fungi.

Figure 38B : Effect of NCR 035 at 100 µg/ml

EXAMPLES:

Example 1: PI staining

15 Propidium Iodide (PI) is able to intercalate into the DNA structure and emits fluorescence only in DNA bound form. PI is able to enter only into cells that lost the membrane integrity therefore it is commonly used for identifying dead cells by detecting fluorescence emission intensity. Intact membranes are impervious for PI, therefore PI is excluded from alive cells, and no fluorescence is observed.

20 PI staining was measured in a BMG Labtech Fluostar Optima fluorimeter (excitation: 520 nm, emission: 610 nm). Reactions were initiated by addition of NCR peptides to the bacteria. Kinetic measurements were performed, in most cases a plateau of fluorescence intensity was reached within minutes.

Control reactions:

- 25 - Buffer only: to measure background fluorescence and to determine the PI staining of the untreated cells (negative control).
- Polymyxin B (positive control, 50 µg/ml final concentration): a cyclic peptide antibiotic with attached fatty acid was used as positive control for PI uptake.

This is a bacteriostatic and bactericidal antibiotic mainly effective against Gram-negative
30 bacteria (e.g. *Pseudomonas aeruginosa*).

- Heat treatment (positive control): boiling the bacteria for 10 minutes resulted in maximal PI fluorescence.

Example 2: CFU (colony forming unit) determination: To determine the bactericidal effect, peptide-treated bacteria were plated at different dilutions and CFUs were counted, inhibitory concentration was determined.

Control reactions:

- Buffer only: to determine the CFUs of the untreated cells (negative control).
- Polymyxin B (positive control, 50 µg/ml final concentration) was used as positive control for cell killing.
- Heat treatment (positive control): boiling the bacteria for 10 mins killed the bacteria.

Example 3: Treatment of *Sinorhizobium meliloti* or other bacteria with 50 µg/ml NCRs in microtiter plates for 3 hours.

Growth of bacteria:

From a fresh plate, a single colony was inoculated in 2 ml liquid medium and grown overnight at 30°C with shaking.

Next morning a 100-fold dilution in liquid medium was carried out and the culture was grown up to OD₆₀₀=0.25 and diluted again to OD₆₀₀=0.05 and then grown until OD₆₀₀=0.2.

Bacteria were harvested by centrifugation at 13000 rpm for 1 min and resuspended in 10 mM K-P buffer, pH7 at OD₆₀₀=0.1 .

Treatment setup:

80 µl log phase bacteria (OD=0.1, approximately 2-3x10⁶ cells) in 10 mM K phosphate buffer (pH=7.0) was mixed with 10 µl peptide in buffer (50 µg/ml final concentration) and 10 µl PI in buffer (5 µg/ml final concentration).

Total volume: 100 µl.

Results:

Figure 2A presents the results for PI staining:

NCR35, NCR55 and to a lesser extent NCR57 and NCR247 provoked PI uptake.

The effects of NCR35 and NCR55 were comparable to heat killed bacteria or bacteria treated with polymyxin B.

Figure 2B presents the results obtained for CFU determination:

Out of the $2-3 \times 10^6$ bacteria, none formed colony after treatment with 50 µg of NCR35, NCR55 and NCR247 peptides as in the case of heat killed or polymyxin B treated bacteria.

NCR57 and NCR224 resulted in reduced growth capacity.

5 **Example 4: Treatment of *Sinorhizobium meliloti* with different concentrations of peptides**

PI staining:

Peptides (NCRs) were added at 1, 2, 5, 10, 20 and 50 µg/ml concentrations to log phase bacteria. Polymyxin B was added at 50µg/ml.

10 NCR35, NCR55 and NCR247 resulted in significant PI fluorescence at 10 or 20 µg/ml while NCR 57 was more active at higher concentrations (20 and 50 µg/ml).

Results:

Figures 3A to 3E present the results obtained for the relative PI fluorescence of NCR treated *S. meliloti* cells with various concentrations of NCR in comparison to polymyxin B and heat
15 treatments.

NCR 035 shows a high activity from 2 µg/ml in contrast to polymyxin.

CFU determination:

A) Table V to IX and figures 4A to 4E present the CFUs of *S. meliloti*, after treatment with NCRs at 20 and 50 µg/ml concentrations for 1 hr.

20 **Results with NCR 035:**

TABLE V

	CFU%	CFU(colony/ml)
buffer	100	9900000
polymyxin B (50µg/ml)	0	0
heat killed	0	0
20 µg/ml NCR 035	0	21000
50 µg/ml NCR 035	0	0

Results with NCR 055:**TABLE VI**

	CFU%	CFU(colony/ml)
buffer	100	4100000
polymyxin B (50µg/ml)	0	0
heat killed	0	0
20 µg/ml NCR055	0	1800
50 µg/ml NCR055	0	0

Results with NCR 057:**TABLE VII**

	CFU%	CFU(colony/ml)
buffer	100	6600000
polymyxin B (50µg/ml)	0	0
heat killed	0	0
20 µg/ml NCR057	148	9800000
50 µg/ml NCR057	68	4500000

Results with NCR 224:**TABLE VIII**

	CFU%	CFU(colony/ml)
buffer	100	40800000
polymyxin B (50µg/ml)	0	0
heat killed	0	0
20 µg/ml NCR224	130	52900000
50 µg/ml NCR224	48	19600000

Results with NCR 247:**TABLE IX**

	CFU%	CFU(colony/ml)
buffer	100	3100000
polymyxin B (50µg/ml)	0	0
heat killed	0	0
20 µg/ml NCR247	0	0
50 µg/ml NCR247	0	0

NCR35, 55, 57 and 247 are able to eradicate the bacteria in less than an hour from the low dose of 20 µg/ml.

B) Comparison between peptides (NCR035) or polymyxin B

Peptides (NCR035) or polymyxin B were added at 1, 2, 5, 10, 20 and 50 µg/ml concentrations to log phase bacteria.

Figure 5 present the results comparison between Polymyxin B and NCR 035 at different doses.

Example 5: Broad spectrum activity treatment of *Sinorhizobium meliloti* and other bacteria with 50 µg/ml NCRs in microtiter plates

Method as in example 3

TABLE X

BACTERIA	NONE	Polymyxin B	NCR 035	NCR 055	NCR 057	NCR 247
Gram negatives						
<i>Sinorhizobium meliloti</i> (AK631)	PI CFU: 10 ⁷	PI:70 CFU:10 ⁵	PI:90 CFU:0	PI:80 CFU:0	PI:35 CFU:10 ⁶	PI:70 CFU:0
<i>Pseudomonas aeruginosa</i>	PI CFU: 10 ⁷	PI:50 CFU:0	- -	PI:10 CFU:10 ⁵	- -	PI:15 CFU:0
<i>Pseudomonas syringae</i> pv	PI CFU: 10 ⁷	PI:95 CFU:0	PI:100 CFU:10 ⁷	PI:85 CFU:0	PI:50 CFU:10 ⁶	PI:60 CFU:0

<i>tomato DC3000</i>						
<i>Xanthomonas</i> <i>2880</i>	PI CFU: 10^7	PI:100 CFU:0	PI:100 CFU: <u>10^2</u>	PI:100 CFU: 10^4	PI:65 CFU: 10^6	PI:48 CFU:0
Gram positives						
<i>Clavibacter</i> <i>michigense</i>	PI CFU: 10^9	PI:60 CFU:0	PI:110 CFU: <u>10^4</u>	PI:100 CFU:0	PI:70 CFU: 10^6	PI:50 CFU:0
<i>Curtobacterium</i> , <i>flaccumfaciens</i>	PI CFU: 10^9	PI:60 CFU:0	PI:78 CFU: 10^5	PI:80 CFU:0	PI:60 CFU:0	PI:27 CFU:0
<i>Bacillus subtilis</i>	PI CFU: 10^6	PI:60 CFU: 10^6	PI:100 CFU: <u>10^3</u>	PI:100 CFU:0	PI:- CFU: 10^6	PI:18 CFU: 10^6
<i>Staphylococcus</i> <i>aureus</i>	PI CFU: 10^6	PI:75 CFU: 10^6	PI:100 CFU: 10^6	PI:100 CFU: <u>10^4</u>	PI:47 CFU: 10^6	PI:30 CFU: 10^5

PI does not need to be equal to 100% to lead to an eradication of bacteria (CFU = 0).

NCR 035, 055 and 247 are the most active and possess the broader spectrum of activity.

5 Interestingly, NCR 055 presents a better killing activity than polymyxin B on bacteria such as resistant bacteria, for example *Staphylococcus aureus*.

Also, NCR 247 present a potent killing activity on antibiotic resistant bacteria such as *Pseudomonas aeruginosa* as also shown in figure 10.

Example 6: Fast antibiotic activity.

10 NCR peptides (10 μ M) and 10^7 bacteria (*Sinorhizobium meliloti*) were incubated in microtiter plates and after 3 hours the number of surviving bacteria was counted in a dilution series on the basis of the colony forming units. .

Similarly, tetracycline or kanamycin (both at 10 or 100 μ M) or Polymyxin B at 10 μ M have also been tested and a negative control containing no peptides or antibiotics has been carried out.

15 Tetracycline or kanamycin at concentrations tenfold higher than NCR 035, 055 or 247 was unable to kill bacteria during 3 hours showing thus the fast antibiotic activity of peptides of the invention.

Results are presented in figure 1.

Example 7: toxicity of NCR peptides in mammalian cell culture

Testing the cytotoxicity of the peptides for eukaryotic cells

The effects of the peptides on human cell cultures were studied by several approaches.

- 5 1. Cell viability was evaluated by measuring respiratory activity using colorimetric assays (MTT or XTT metabolism assay) on MRC5 normal fibroblast, HT1080 fibrosarcoma, U2OS osteosarcoma and MCF7 breast carcinoma cells. The cell viability of the peptide treated and untreated control cultures were comparable or identical.
2. The effect of the peptides on eukaryotic cell membrane was studied using PI staining and
 10 FACS on CaSki cervical carcinoma cells. No membrane permeability change was detected with any of the tested peptides.

These results suggest that the tested plant peptides have no cytotoxicity on human cell cultures.

Results are presented in Figure 8.

MTT: Methylthiazolyldiphenyl-tetrazolium bromide

- 15 XTT: 2,3-Bis(2-methoxy-4-nitro-5-sulfohenyl)-2H-tetrazolium-5-carboxanilide inner salt

PI: propidium iodide

FACS: fluorescence activated cell sorting

Example 8: effect of NCR peptides on plants growth

- 20 **Protocol:** Effect of the peptides on *Arabidopsis* germination and seedling growth (A. Allen et al, Planta (2008) 227:331–339).

- The synthesized peptides were diluted to 100µM concentration in 10 mM Tris-HCl pH 7.4 and filter sterilized. This dilution was mixed with equal volume of 2x root assay media (per liter: 2.15g Murashige and Skoog basal salts, 1g MES, 2% sucrose, 2x Gamborg's vitamins)
 25 (final peptide conc. 50 µM) in a 96-well assay plate. A single, sterilized *Arabidopsis* (ecotype: wassilewskiya) seed was placed in each well and grown with gentle shaking (100 rpm) at 22°C under a 16h photoperiod for 7 days. The germinated seedlings were transferred on 1x root assay medium containing 1% agar and photographed after 4 days of incubation. Polymyxin B (50µg/ml =42 µM) was used as an AMP (antimicrobial peptide) control in the assay.

- 30 **Results:** Polymyxin B affects the plant growth, in particular it inhibits growth whereas NCR 035, 051, 055, 057, 087, 224, 229, 235, 247 peptides have no effect on the growth.

Results are presented in Figure 9.

Example 9: effect of salts of divalent cations (Mg^{++} and Ca^{++}) on the activity of NCR peptides.

5

TABLE XI

Plate	A1	A2	A3	A4	A5	A6	A7	A8
	Negative control.	Positive control.	NCR247	NCR247	NCR247	NCR247	NCR247	NCR247
Bacteria <i>S. meliloti</i> (AK631)	70 μ l	70 μ l	70 μ l	70 μ l	70 μ l	70 μ l	70 μ l	70 μ l
Buffer 10mM K-P pH:7	20 μ l	20 μ l	10 μ l	Ø	Ø	Ø	Ø	Ø
Peptide (500 μ g/ml)	Ø	Ø	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
CaCl ₂	Ø	Ø	Ø	10 μ l 1mM	10 μ l 5mM	10 μ l 10mM	10 μ l 20mM	10 μ l 50mM
PI 10X	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l

Plate	B1	B2	B3	B4	B5	B6	B7	B8
	Negative control	Positive control	NCR247	NCR247	NCR247	NCR247	NCR247	NCR247
Bacteria <i>S. meliloti</i> (AK631)	70 μ l	70 μ l	70 μ l	70 μ l	70 μ l	70 μ l	70 μ l	70 μ l
Buffer 10mM K-P pH.:7	20 μ l	20 μ l	10 μ l	Ø	Ø	Ø	Ø	Ø
Peptide (500 μ g/ml)	Ø	Ø	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
MgCl ₂	Ø	Ø	Ø	10 μ l 1mM	10 μ l 5mM	10 μ l 10mM	10 μ l 20mM	10 μ l 50mM
PI 10X	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l

Mg^{++} or Ca^{++} was added at final concentrations from 0.1 mM to 5 mM to bacteria (*S. meliloti* (AK631), in a buffer (10mM K-P pH:7), containing or not NCR 247 peptide (NCR247) and the PI staining was determined.

10 Heat killed cells: 95°C – 10 minutes (in buffer)

The results are presented in figure 6 and 7.

At a final concentration comprised between 0.1 mM and 2mM, Mg^{++} or Ca^{++} do not change the activity of NCR peptides.

At a final concentration of 5mM, Mg and Ca to a lesser extent, exert negative effects on activity of NCR peptides that can be compensated by the addition of EDTA 5 mM (or by increasing the concentration of peptides).

Example 10: Anti-fungal activity of NCR peptides

Protocol:

The fungal strains were maintained on malt extract medium (0.5% malt extract, 0.5% yeast extract, 0.5% glucose, 1.0% KH_2PO_4 and 1.5% agar).

The antifungal activity of NCR peptides was measured in an in vitro assay using 96-well microtiter plates. Fifty microliters of each protein dilution were added to each well of the microtiter plate containing 50 μ l of spore suspension prepared in low-salt fungal medium at a concentration of 10^4 spores/ml. The photographs were taken after incubation for 24 and 48 h at 25°C and the optical density of the cultures were measured at 590nm in the same time.

The germination-inhibitory effect of NCRs was also examined on solid minimal medium in the case of *Fusarium graminearum*. 50 μ l of spore suspension prepared in low-salt medium was incubated with 50 μ l NCR247 at the indicated concentrations for 20h at 25°C than it was plated onto solid minimal medium.

The low-salt medium contained: $K_2 HPO_4$ (5 mM), $MgSO_4$ (100 μ M), $FeSO_4$ (10 μ M), $CoCl_2$ (0.2 μ M), $CuSO_4$ (0.2 μ M), $Na_2 MoO_4$ (4 μ M), $H_3 BO_3$ (1 μ M), KI (0.2 μ M), $ZnSO_4$ (1 μ M), $MnSO_4$ (0.2 μ M), glucose (20 g/l), asparagine (2 g/l), methionine (40 mg/l), myo-inositol (4 mg/l), biotin (0.4 mg/l), thiamine-HCl (2 mg/l), and pyridoxine-HCl (0.4 mg/l).

The minimal medium contained: 1% glucose, 0.5% $(NH_4)_2SO_4$, 0.1% KH_2PO_4 , 0.05% $MgSO_4$ and was solidified with 1.5% agar.

Results:

The results are presented in the table below (table XII and XIII) and on figures 10 to 12:

TABLE XII

24h	NCR247			NCR035			NCR055		AMB
	100 µg/ml	50 µg/ml	25 µg/ml	100 µg/ml	50 µg/ml	25 µg/ml	100 µg/ml	50 µg/ml	125 µg/ml
<i>Aspergillus flavus</i>	+++	+++	+++	++	+	-	++	+	+++
<i>Aspergillus niger</i>	+++	+++	+++	+	-	-	++	++	+++
<i>Candida albicans</i>	+++	+++	+++	++	++	+	+++	+++	+++
<i>Candida crusei</i>	+++	+++	+++	++	++	+	+++	+++	+++
<i>Candida parapsilosis</i>	+++	+++	+++	++	++	+	+++	+++	+++
<i>Rhizopus stolonifer</i> var. <i>stolonifer</i>	+++	+++	+++	++	+	-	+++	++	+++
<i>Fusarium graminearum</i>	++	++	++	++	+	-	++	-	++

Fungal (hyphal) growth after 48hours of incubation

AMB: Amfotericin B (antifungal agent).

5 +++: high sensitivity, no hyphal growth

++: medium sensitivity

+: low sensitivity

-: no effect

10

15

TABLE XIII

48h	NCR247			NCR035			NCR055		AMB
	100 µg/ml	50 µg/ml	25 µg/ml	100 µg/ml	50 µg/ml	25 µg/ml	100 µg/ml	50 µg/ml	125 µg/ml
<i>Aspergillus flavus</i>	++	++	+	-	-	-	+	-	+++
<i>Aspergillus niger</i>	+++	+++	+++	-	-	-	++	+	+++
<i>Candida albicans</i>	+++	+++	+++	+	+	-	+++	+++	+++
<i>Candida crusei</i>	+++	+++	+++	++	++	-	+++	+++	+++
<i>Candida parapsilosis</i>	+++	+++	+++	++	++	+	+++	+++	+++
<i>Rhizopus stolonifer</i> var. <i>stolonifer</i>	+++	+++	+++	++	+	-	+++	+	+++
<i>Fusarium graminearum</i>	++	++	++	+	+	-	++	-	++

Fungal (hyphal) growth after 48hours of incubation

5 AMB: Amfotericin B.

+++ : high sensitivity, no hyphal growth

++ : medium sensitivity

+: low sensitivity

- : no effect

10 Results in light grey boxes: increased inhibition versus 24h of incubation (table XII)

Results in dark grey boxes: decreased inhibition versus 24h of incubation (table XII).

CLAIMS

1. Use of at least one peptide originated from *Medicago truncatula* nodules, comprising the
5 SEQ IDs NO: 1 – 463 or at least one peptide having a sequence derived from said SEQ
IDs NO: 1 – 463 by deletion of about 9 to about 44 contiguous amino acids, from the N-
terminal part of the peptide, in particular peptides having the SEQ IDS NO: 464 to 925,
for the preparation of a drug intended for the treatment of diseases induced by
microorganisms, wherein said peptides have a broad-spectrum and fast antibiotic activity,
10 in particular killing of the bacteria within 1 to 3 hours.
2. Use according to claim 1, wherein said peptides derive from the peptides of SEQ ID NO:
1 to 925 according to claim 1:
 - by substitution, and/or suppression, and/or addition of one or several amino acids, of the
15 above-mentioned peptides, and/or
 - by modification of at least one -CO-NH- peptide linkage of the peptide chain of the
above-mentioned peptides, particularly by introduction of a retro or retro-inverso type
linkage, and/or
 - by substitution of at least one amino acid of the peptide chain of the sequence or of the
20 above-mentioned peptide, with a non-proteinogenic amino acid,
or said peptides correspond to fragment of SEQ ID NO: 1 to 925,
in particular, peptides or fragments of peptides having more than 80%, preferably more
than 85%, preferably more than 90% and more preferably more than 95% of homology,
provided that the fast antibiotic activity is maintained.
- 25 3. Use according to claims 1 or 2, wherein said microorganisms are bacteria of Gram – or
Gram + bacteria.
4. Use according to anyone of claims 1 to 3, wherein said peptides further have an anti-viral
30 and/or fungicide activity.

5. Use according to anyone of claims 1 to 4, wherein said peptides are cationic peptides ($pI > 8$) in association with one or more anionic peptides ($pI < 6$) and/or one or more neutral peptides ($6 \leq pI \leq 8$).

5 6. Use according to claim 5, wherein said cationic peptides are selected from the list consisting of

SEQ ID NO:469; SEQ ID NO:474; SEQ ID NO:480; SEQ ID NO:483; SEQ ID NO:484;
SEQ ID NO:485; SEQ ID NO:486; SEQ ID NO:489; SEQ ID NO:490; SEQ ID NO:492;
SEQ ID NO:493; SEQ ID NO:494; SEQ ID NO:495; SEQ ID NO:496; SEQ ID NO:497;
10 SEQ ID NO:498; SEQ ID NO:499; SEQ ID NO:500; SEQ ID NO:501; SEQ ID NO:502;
SEQ ID NO:503; SEQ ID NO:504; SEQ ID NO:505; SEQ ID NO:506; SEQ ID NO:507;
SEQ ID NO:508; SEQ ID NO:509; SEQ ID NO:510; SEQ ID NO:511; SEQ ID NO:512;
SEQ ID NO:513; SEQ ID NO:515; SEQ ID NO:516; SEQ ID NO:518; SEQ ID NO:520;
SEQ ID NO:523; SEQ ID NO:524; SEQ ID NO:530; SEQ ID NO:531; SEQ ID NO:532;
15 SEQ ID NO:533; SEQ ID NO:534; SEQ ID NO:536; SEQ ID NO:538; SEQ ID NO:541;
SEQ ID NO:543; SEQ ID NO:544; SEQ ID NO:546; SEQ ID NO:549; SEQ ID NO:554;
SEQ ID NO:555; SEQ ID NO:556; SEQ ID NO:561; SEQ ID NO:563; SEQ ID NO:564;
SEQ ID NO:565; SEQ ID NO:566; SEQ ID NO:567; SEQ ID NO:569; SEQ ID NO:573;
SEQ ID NO:582; SEQ ID NO:583; SEQ ID NO:593; SEQ ID NO:596; SEQ ID NO:597;
20 SEQ ID NO:598; SEQ ID NO:599; SEQ ID NO:600; SEQ ID NO:606; SEQ ID NO:610;
SEQ ID NO:611; SEQ ID NO:613; SEQ ID NO:618; SEQ ID NO:620; SEQ ID NO:622;
SEQ ID NO:623; SEQ ID NO:632; SEQ ID NO:633; SEQ ID NO:634; SEQ ID NO:635;
SEQ ID NO:636; SEQ ID NO:639; SEQ ID NO:642; SEQ ID NO:644; SEQ ID NO:646;
SEQ ID NO:647; SEQ ID NO:651; SEQ ID NO:652; SEQ ID NO:655; SEQ ID NO:658;
25 SEQ ID NO:659; SEQ ID NO:662; SEQ ID NO:663; SEQ ID NO:670; SEQ ID NO:675;
SEQ ID NO:681; SEQ ID NO:683; SEQ ID NO:684; SEQ ID NO:685; SEQ ID NO:690;
SEQ ID NO:694; SEQ ID NO:700; SEQ ID NO:705; SEQ ID NO:706; SEQ ID NO:709;
SEQ ID NO:713; SEQ ID NO:715; SEQ ID NO:720; SEQ ID NO:721; SEQ ID NO:725;
SEQ ID NO:731; SEQ ID NO:737; SEQ ID NO:738; SEQ ID NO:739; SEQ ID NO:742;
30 SEQ ID NO:743; SEQ ID NO:744; SEQ ID NO:746; SEQ ID NO:750; SEQ ID NO:753;
SEQ ID NO:757; SEQ ID NO:758; SEQ ID NO:761; SEQ ID NO:766; SEQ ID NO:769;

SEQ ID NO:772; SEQ ID NO:774; SEQ ID NO:775; SEQ ID NO:780; SEQ ID NO:781;
SEQ ID NO:782; SEQ ID NO:783; SEQ ID NO:784; SEQ ID NO:787; SEQ ID NO:793;
SEQ ID NO:794; SEQ ID NO:799; SEQ ID NO:800; SEQ ID NO:807; SEQ ID NO:812;
SEQ ID NO:816; SEQ ID NO:817; SEQ ID NO:819; SEQ ID NO:820; SEQ ID NO:821;
5 SEQ ID NO:824; SEQ ID NO:825; SEQ ID NO:827; SEQ ID NO:828; SEQ ID NO:829;
SEQ ID NO:830; SEQ ID NO:835; SEQ ID NO:836; SEQ ID NO:842; SEQ ID NO:846;
SEQ ID NO:847; SEQ ID NO:848; SEQ ID NO:849; SEQ ID NO:850; SEQ ID NO:853;
SEQ ID NO:858; SEQ ID NO:862; SEQ ID NO:863; SEQ ID NO:865; SEQ ID NO:866;
SEQ ID NO:870; SEQ ID NO:872; SEQ ID NO:875; SEQ ID NO:877; SEQ ID NO:878;
10 SEQ ID NO:879; SEQ ID NO:880; SEQ ID NO:881; SEQ ID NO:892; SEQ ID NO:894;
SEQ ID NO:896; SEQ ID NO:897; SEQ ID NO:899; SEQ ID NO:909; SEQ ID NO:910;
SEQ ID NO:913; SEQ ID NO:917; SEQ ID NO:919; SEQ ID NO:921 and SEQ ID NO:925.

7. Use according to claim 6, wherein said cationic peptides above defined are selected from
15 the list consisting of: SEQ ID NO:706 (NCR 247), SEQ ID NO:498 (NCR 035), SEQ ID
NO:518 (NCR 055), SEQ ID NO:520 (NCR 57).

8. Use according to anyone of claims 1 to 7, wherein anionic peptides are selected from the
list consisting of

SEQ ID NO:464; SEQ ID NO:465; SEQ ID NO:467; SEQ ID NO:468; SEQ ID NO:470;
20 SEQ ID NO:471; SEQ ID NO:472; SEQ ID NO:476; SEQ ID NO:477; SEQ ID NO:478;
SEQ ID NO:479; SEQ ID NO:481; SEQ ID NO:482; SEQ ID NO:487; SEQ ID NO:488;
SEQ ID NO:514; SEQ ID NO:521; SEQ ID NO:522; SEQ ID NO:526; SEQ ID NO:528;
SEQ ID NO:529; SEQ ID NO:537; SEQ ID NO:539; SEQ ID NO:545; SEQ ID NO:550;
SEQ ID NO:551; SEQ ID NO:552; SEQ ID NO:558; SEQ ID NO:562; SEQ ID NO:568;
25 SEQ ID NO:571; SEQ ID NO:574; SEQ ID NO:575; SEQ ID NO:576; SEQ ID NO:577;
SEQ ID NO:580; SEQ ID NO:586; SEQ ID NO:588; SEQ ID NO:590; SEQ ID NO:592;
SEQ ID NO:595; SEQ ID NO:602; SEQ ID NO:603; SEQ ID NO:604; SEQ ID NO:605;
SEQ ID NO:608; SEQ ID NO:614; SEQ ID NO:615; SEQ ID NO:616; SEQ ID NO:617;
SEQ ID NO:619; SEQ ID NO:621; SEQ ID NO:625; SEQ ID NO:626; SEQ ID NO:627;
30 SEQ ID NO:629; SEQ ID NO:630; SEQ ID NO:631; SEQ ID NO:640; SEQ ID NO:641;
SEQ ID NO:643; SEQ ID NO:648; SEQ ID NO:650; SEQ ID NO:654; SEQ ID NO:656;

SEQ ID NO:657; SEQ ID NO:666; SEQ ID NO:667; SEQ ID NO:672; SEQ ID NO:673;
 SEQ ID NO:674; SEQ ID NO:677; SEQ ID NO:678; SEQ ID NO:679; SEQ ID NO:680;
 SEQ ID NO:682; SEQ ID NO:686; SEQ ID NO:687; SEQ ID NO:688; SEQ ID NO:693;
 SEQ ID NO:696; SEQ ID NO:697; SEQ ID NO:702; SEQ ID NO:704; SEQ ID NO:711;
 5 SEQ ID NO:719; SEQ ID NO:722; SEQ ID NO:728; SEQ ID NO:729; SEQ ID NO:732;
 SEQ ID NO:733; SEQ ID NO:735; SEQ ID NO:736; SEQ ID NO:741; SEQ ID NO:745;
 SEQ ID NO:747; SEQ ID NO:748; SEQ ID NO:749; SEQ ID NO:752; SEQ ID NO:754;
 SEQ ID NO:759; SEQ ID NO:760; SEQ ID NO:762; SEQ ID NO:763; SEQ ID NO:767;
 SEQ ID NO:768; SEQ ID NO:770; SEQ ID NO:771; SEQ ID NO:773; SEQ ID NO:777;
 10 SEQ ID NO:778; SEQ ID NO:786; SEQ ID NO:792; SEQ ID NO:795; SEQ ID NO:796;
 SEQ ID NO:797; SEQ ID NO:803; SEQ ID NO:805; SEQ ID NO:808; SEQ ID NO:810;
 SEQ ID NO:811; SEQ ID NO:813; SEQ ID NO:814; SEQ ID NO:815; SEQ ID NO:818;
 SEQ ID NO:822; SEQ ID NO:823; SEQ ID NO:826; SEQ ID NO:831; SEQ ID NO:834;
 SEQ ID NO:837; SEQ ID NO:839; SEQ ID NO:840; SEQ ID NO:841; SEQ ID NO:845;
 15 SEQ ID NO:851; SEQ ID NO:852; SEQ ID NO:854; SEQ ID NO:855; SEQ ID NO:856;
 SEQ ID NO:857; SEQ ID NO:859; SEQ ID NO:860; SEQ ID NO:861; SEQ ID NO:868;
 SEQ ID NO:873; SEQ ID NO:876; SEQ ID NO:882; SEQ ID NO:883; SEQ ID NO:884;
 SEQ ID NO:887; SEQ ID NO:889; SEQ ID NO:891; SEQ ID NO:893; SEQ ID NO:895;
 SEQ ID NO:901; SEQ ID NO:902; SEQ ID NO:903; SEQ ID NO:904; SEQ ID NO:907;
 20 SEQ ID NO:908; SEQ ID NO:912; SEQ ID NO:915; SEQ ID NO:922 and SEQ ID NO:924;

9. Use according to claim 8, wherein said anionic peptides are selected from the list
 consisting of: SEQ ID NO:686 (NCR 224), SEQ ID NO:697 (NCR 235), SEQ ID NO:514
 (NCR 051), SEQ ID NO:464 (NCR 001).

25

10. Use according to anyone of claims 1 to 9, wherein neutral peptides are selected from the
 list consisting of

SEQ ID NO:466; SEQ ID NO:473; SEQ ID NO:475; SEQ ID NO:491; SEQ ID NO:517;
 30 SEQ ID NO:519; SEQ ID NO:525; SEQ ID NO:527; SEQ ID NO:535; SEQ ID NO:540;
 SEQ ID NO:542; SEQ ID NO:547; SEQ ID NO:548; SEQ ID NO:553; SEQ ID NO:557;

SEQ ID NO:559; SEQ ID NO:560; SEQ ID NO:570; SEQ ID NO:572; SEQ ID NO:578;
SEQ ID NO:579; SEQ ID NO:581; SEQ ID NO:584; SEQ ID NO:585; SEQ ID NO:587;
SEQ ID NO:589; SEQ ID NO:591; SEQ ID NO:594; SEQ ID NO:601; SEQ ID NO:607;
SEQ ID NO:609; SEQ ID NO:612; SEQ ID NO:624; SEQ ID NO:628; SEQ ID NO:637;
5 SEQ ID NO:638; SEQ ID NO:645; SEQ ID NO:649; SEQ ID NO:653; SEQ ID NO:660;
SEQ ID NO:661; SEQ ID NO:664; SEQ ID NO:665; SEQ ID NO:668; SEQ ID NO:669;
SEQ ID NO:671; SEQ ID NO:676; SEQ ID NO:689; SEQ ID NO:691; SEQ ID NO:692;
SEQ ID NO:695; SEQ ID NO:698; SEQ ID NO:699; SEQ ID NO:701; SEQ ID NO:703;
SEQ ID NO:707; SEQ ID NO:708; SEQ ID NO:710; SEQ ID NO:712; SEQ ID NO:714;
10 SEQ ID NO:716; SEQ ID NO:717; SEQ ID NO:718; SEQ ID NO:723; SEQ ID NO:724;
SEQ ID NO:726; SEQ ID NO:727; SEQ ID NO:730; SEQ ID NO:734; SEQ ID NO:740;
SEQ ID NO:751; SEQ ID NO:755; SEQ ID NO:756; SEQ ID NO:764; SEQ ID NO:765;
SEQ ID NO:776; SEQ ID NO:779; SEQ ID NO:785; SEQ ID NO:788; SEQ ID NO:789;
SEQ ID NO:790; SEQ ID NO:791; SEQ ID NO:798; SEQ ID NO:801; SEQ ID NO:802;
15 SEQ ID NO:804; SEQ ID NO:806; SEQ ID NO:809; SEQ ID NO:832; SEQ ID NO:833;
SEQ ID NO:838; SEQ ID NO:843; SEQ ID NO:844; SEQ ID NO:864; SEQ ID NO:867;
SEQ ID NO:869; SEQ ID NO:871; SEQ ID NO:874; SEQ ID NO:885; SEQ ID NO:886;
SEQ ID NO:888; SEQ ID NO:890; SEQ ID NO:898; SEQ ID NO:900; SEQ ID NO:905;
SEQ ID NO:906; SEQ ID NO:911; SEQ ID NO:914; SEQ ID NO:916; SEQ ID NO:918;
20 SEQ ID NO:920 and SEQ ID NO:923.

11. Use according to claim 10, wherein said neutral peptides are selected from the list
consisting of: SEQ ID NO:547 (NCR 084), SEQ ID NO:691 (NCR 229).
25
12. Use according to anyone of claims 1 to 11, wherein said peptides are active against both
Gram – and Gram + bacteria.
13. Use according to anyone of claims 1 to 12, wherein said peptides are active at a
30 concentration range from about 1 to about 100 µg/ml, preferably from about 1 to
50µg/ml, in particular from about 10 to about 50 µg/ml.

14. Use according to claim 13, wherein the number of colony forming units of bacteria dropped 3 to 9 orders of magnitude, in particular 5 to 7 orders, in 3 hours contacting with said peptides.

5

15. Pharmaceutical composition comprising at least two peptides of SEQ IDs NO: 1 – 463, as defined in claim 1, or peptides of SEQ ID 464 to 925, as defined in claim 1, in association with a pharmaceutical acceptable vehicle, provided that said peptides have a purity being equal or more than about 90%, in particular more than about 95%.

10

16. Pharmaceutical composition according to claim 15, comprising one or more cationic peptides ($pI > 8$) in association with one or more anionic peptides ($pI < 6$) and/or one or more neutral peptides ($6 \leq pI \leq 8$).

15 17. Pharmaceutical composition according to claim 16, wherein said cationic peptides are selected from the list consisting of:

SEQ ID NO:469; SEQ ID NO:474; SEQ ID NO:480; SEQ ID NO:483; SEQ ID NO:484;
SEQ ID NO:485; SEQ ID NO:486; SEQ ID NO:489; SEQ ID NO:490; SEQ ID NO:492;
SEQ ID NO:493; SEQ ID NO:494; SEQ ID NO:495; SEQ ID NO:496; SEQ ID NO:497;
20 SEQ ID NO:498; SEQ ID NO:499; SEQ ID NO:500; SEQ ID NO:501; SEQ ID NO:502;
SEQ ID NO:503; SEQ ID NO:504; SEQ ID NO:505; SEQ ID NO:506; SEQ ID NO:507;
SEQ ID NO:508; SEQ ID NO:509; SEQ ID NO:510; SEQ ID NO:511; SEQ ID NO:512;
SEQ ID NO:513; SEQ ID NO:515; SEQ ID NO:516; SEQ ID NO:518; SEQ ID NO:520;
SEQ ID NO:523; SEQ ID NO:524; SEQ ID NO:530; SEQ ID NO:531; SEQ ID NO:532;
25 SEQ ID NO:533; SEQ ID NO:534; SEQ ID NO:536; SEQ ID NO:538; SEQ ID NO:541;
SEQ ID NO:543; SEQ ID NO:544; SEQ ID NO:546; SEQ ID NO:549; SEQ ID NO:554;
SEQ ID NO:555; SEQ ID NO:556; SEQ ID NO:561; SEQ ID NO:563; SEQ ID NO:564;
SEQ ID NO:565; SEQ ID NO:566; SEQ ID NO:567; SEQ ID NO:569; SEQ ID NO:573;
SEQ ID NO:582; SEQ ID NO:583; SEQ ID NO:593; SEQ ID NO:596; SEQ ID NO:597;
30 SEQ ID NO:598; SEQ ID NO:599; SEQ ID NO:600; SEQ ID NO:606; SEQ ID NO:610;
SEQ ID NO:611; SEQ ID NO:613; SEQ ID NO:618; SEQ ID NO:620; SEQ ID NO:622;

SEQ ID NO:623; SEQ ID NO:632; SEQ ID NO:633; SEQ ID NO:634; SEQ ID NO:635;
SEQ ID NO:636; SEQ ID NO:639; SEQ ID NO:642; SEQ ID NO:644; SEQ ID NO:646;
SEQ ID NO:647; SEQ ID NO:651; SEQ ID NO:652; SEQ ID NO:655; SEQ ID NO:658;
SEQ ID NO:659; SEQ ID NO:662; SEQ ID NO:663; SEQ ID NO:670; SEQ ID NO:675;
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SEQ ID NO:694; SEQ ID NO:700; SEQ ID NO:705; SEQ ID NO:706; SEQ ID NO:709;
SEQ ID NO:713; SEQ ID NO:715; SEQ ID NO:720; SEQ ID NO:721; SEQ ID NO:725;
SEQ ID NO:731; SEQ ID NO:737; SEQ ID NO:738; SEQ ID NO:739; SEQ ID NO:742;
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SEQ ID NO:772; SEQ ID NO:774; SEQ ID NO:775; SEQ ID NO:780; SEQ ID NO:781;
SEQ ID NO:782; SEQ ID NO:783; SEQ ID NO:784; SEQ ID NO:787; SEQ ID NO:793;
SEQ ID NO:794; SEQ ID NO:799; SEQ ID NO:800; SEQ ID NO:807; SEQ ID NO:812;
SEQ ID NO:816; SEQ ID NO:817; SEQ ID NO:819; SEQ ID NO:820; SEQ ID NO:821;
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SEQ ID NO:870; SEQ ID NO:872; SEQ ID NO:875; SEQ ID NO:877; SEQ ID NO:878;
20 SEQ ID NO:879; SEQ ID NO:880; SEQ ID NO:881; SEQ ID NO:892; SEQ ID NO:894;
SEQ ID NO:896; SEQ ID NO:897; SEQ ID NO:899; SEQ ID NO:909; SEQ ID NO:910;
SEQ ID NO:913; SEQ ID NO:917; SEQ ID NO:919; SEQ ID NO:921 and SEQ ID NO:925.

18. Pharmaceutical composition according to claim 17, wherein said cationic peptides are
25 selected from the list consisting of: SEQ ID NO:706 (NCR 247), SEQ ID NO:498 (NCR
035SEQ ID NO:518 (NCR 055), SEQ ID NO:520 (NCR 57).

19. Pharmaceutical composition according to anyone of claims 15 to 18, wherein anionic
peptides are selected from the list consisting of:
30 SEQ ID NO:464; SEQ ID NO:465; SEQ ID NO:467; SEQ ID NO:468; SEQ ID NO:470;
SEQ ID NO:471; SEQ ID NO:472; SEQ ID NO:476; SEQ ID NO:477; SEQ ID NO:478;

SEQ ID NO:479; SEQ ID NO:481; SEQ ID NO:482; SEQ ID NO:487; SEQ ID NO:488;
SEQ ID NO:514; SEQ ID NO:521; SEQ ID NO:522; SEQ ID NO:526; SEQ ID NO:528;
SEQ ID NO:529; SEQ ID NO:537; SEQ ID NO:539; SEQ ID NO:545; SEQ ID NO:550;
SEQ ID NO:551; SEQ ID NO:552; SEQ ID NO:558; SEQ ID NO:562; SEQ ID NO:568;
5 SEQ ID NO:571; SEQ ID NO:574; SEQ ID NO:575; SEQ ID NO:576; SEQ ID NO:577;
SEQ ID NO:580; SEQ ID NO:586; SEQ ID NO:588; SEQ ID NO:590; SEQ ID NO:592;
SEQ ID NO:595; SEQ ID NO:602; SEQ ID NO:603; SEQ ID NO:604; SEQ ID NO:605;
SEQ ID NO:608; SEQ ID NO:614; SEQ ID NO:615; SEQ ID NO:616; SEQ ID NO:617;
SEQ ID NO:619; SEQ ID NO:621; SEQ ID NO:625; SEQ ID NO:626; SEQ ID NO:627;
10 SEQ ID NO:629; SEQ ID NO:630; SEQ ID NO:631; SEQ ID NO:640; SEQ ID NO:641;
SEQ ID NO:643; SEQ ID NO:648; SEQ ID NO:650; SEQ ID NO:654; SEQ ID NO:656;
SEQ ID NO:657; SEQ ID NO:666; SEQ ID NO:667; SEQ ID NO:672; SEQ ID NO:673;
SEQ ID NO:674; SEQ ID NO:677; SEQ ID NO:678; SEQ ID NO:679; SEQ ID NO:680;
SEQ ID NO:682; SEQ ID NO:686; SEQ ID NO:687; SEQ ID NO:688; SEQ ID NO:693;
15 SEQ ID NO:696; SEQ ID NO:697; SEQ ID NO:702; SEQ ID NO:704; SEQ ID NO:711;
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SEQ ID NO:733; SEQ ID NO:735; SEQ ID NO:736; SEQ ID NO:741; SEQ ID NO:745;
SEQ ID NO:747; SEQ ID NO:748; SEQ ID NO:749; SEQ ID NO:752; SEQ ID NO:754;
SEQ ID NO:759; SEQ ID NO:760; SEQ ID NO:762; SEQ ID NO:763; SEQ ID NO:767;
20 SEQ ID NO:768; SEQ ID NO:770; SEQ ID NO:771; SEQ ID NO:773; SEQ ID NO:777;
SEQ ID NO:778; SEQ ID NO:786; SEQ ID NO:792; SEQ ID NO:795; SEQ ID NO:796;
SEQ ID NO:797; SEQ ID NO:803; SEQ ID NO:805; SEQ ID NO:808; SEQ ID NO:810;
SEQ ID NO:811; SEQ ID NO:813; SEQ ID NO:814; SEQ ID NO:815; SEQ ID NO:818;
SEQ ID NO:822; SEQ ID NO:823; SEQ ID NO:826; SEQ ID NO:831; SEQ ID NO:834;
25 SEQ ID NO:837; SEQ ID NO:839; SEQ ID NO:840; SEQ ID NO:841; SEQ ID NO:845;
SEQ ID NO:851; SEQ ID NO:852; SEQ ID NO:854; SEQ ID NO:855; SEQ ID NO:856;
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SEQ ID NO:887; SEQ ID NO:889; SEQ ID NO:891; SEQ ID NO:893; SEQ ID NO:895;
30 SEQ ID NO:901; SEQ ID NO:902; SEQ ID NO:903; SEQ ID NO:904; SEQ ID NO:907;

SEQ ID NO:908; SEQ ID NO:912; SEQ ID NO:915; SEQ ID NO:922 and SEQ ID NO:924;

20. Pharmaceutical composition according to claim 19, wherein said anionic peptides are
5 selected from the list consisting of: SEQ ID NO:686 (NCR 224), SEQ ID NO:697 (NCR 235), SEQ ID NO:514 (NCR 051), SEQ ID NO:464 (NCR 001).

21. Pharmaceutical composition according to claims 15 to 20, wherein neutral peptides are
selected from the list consisting of:

10 SEQ ID NO:466; SEQ ID NO:473; SEQ ID NO:475; SEQ ID NO:491; SEQ ID NO:517;
SEQ ID NO:519; SEQ ID NO:525; SEQ ID NO:527; SEQ ID NO:535; SEQ ID NO:540;
SEQ ID NO:542; SEQ ID NO:547; SEQ ID NO:548; SEQ ID NO:553; SEQ ID NO:557;
SEQ ID NO:559; SEQ ID NO:560; SEQ ID NO:570; SEQ ID NO:572; SEQ ID NO:578;
SEQ ID NO:579; SEQ ID NO:581; SEQ ID NO:584; SEQ ID NO:585; SEQ ID NO:587;
15 SEQ ID NO:589; SEQ ID NO:591; SEQ ID NO:594; SEQ ID NO:601; SEQ ID NO:607;
SEQ ID NO:609; SEQ ID NO:612; SEQ ID NO:624; SEQ ID NO:628; SEQ ID NO:637;
SEQ ID NO:638; SEQ ID NO:645; SEQ ID NO:649; SEQ ID NO:653; SEQ ID NO:660;
SEQ ID NO:661; SEQ ID NO:664; SEQ ID NO:665; SEQ ID NO:668; SEQ ID NO:669;
SEQ ID NO:671; SEQ ID NO:676; SEQ ID NO:689; SEQ ID NO:691; SEQ ID NO:692;
20 SEQ ID NO:695; SEQ ID NO:698; SEQ ID NO:699; SEQ ID NO:701; SEQ ID NO:703;
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SEQ ID NO:716; SEQ ID NO:717; SEQ ID NO:718; SEQ ID NO:723; SEQ ID NO:724;
SEQ ID NO:726; SEQ ID NO:727; SEQ ID NO:730; SEQ ID NO:734; SEQ ID NO:740;
SEQ ID NO:751; SEQ ID NO:755; SEQ ID NO:756; SEQ ID NO:764; SEQ ID NO:765;
25 SEQ ID NO:776; SEQ ID NO:779; SEQ ID NO:785; SEQ ID NO:788; SEQ ID NO:789;
SEQ ID NO:790; SEQ ID NO:791; SEQ ID NO:798; SEQ ID NO:801; SEQ ID NO:802;
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SEQ ID NO:838; SEQ ID NO:843; SEQ ID NO:844; SEQ ID NO:864; SEQ ID NO:867;
SEQ ID NO:869; SEQ ID NO:871; SEQ ID NO:874; SEQ ID NO:885; SEQ ID NO:886;
30 SEQ ID NO:888; SEQ ID NO:890; SEQ ID NO:898; SEQ ID NO:900; SEQ ID NO:905;

SEQ ID NO:906; SEQ ID NO:911; SEQ ID NO:914; SEQ ID NO:916; SEQ ID NO:918; SEQ ID NO:920 and SEQ ID NO:923.

- 5 22. Pharmaceutical composition according to claim 21, wherein said neutral peptides are selected from the list consisting of: SEQ ID NO:547 (NCR 084), SEQ ID NO:691 (NCR 229).

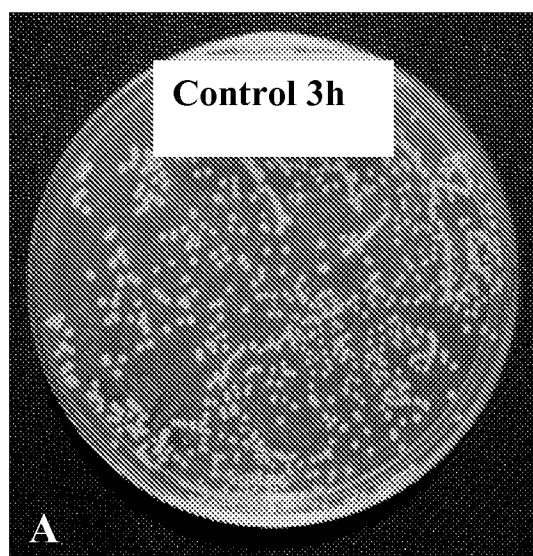


FIGURE 1 A

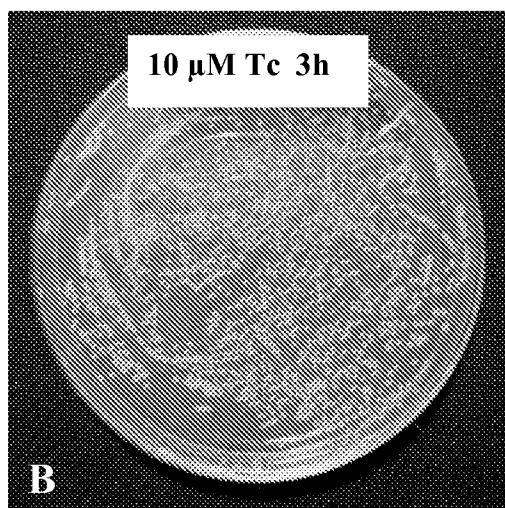


FIGURE 1B

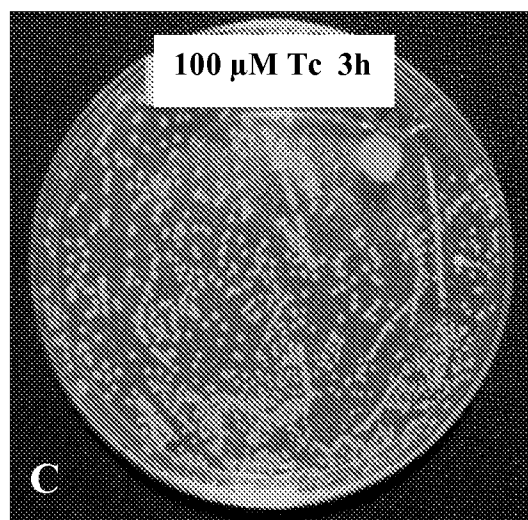


FIGURE 1C

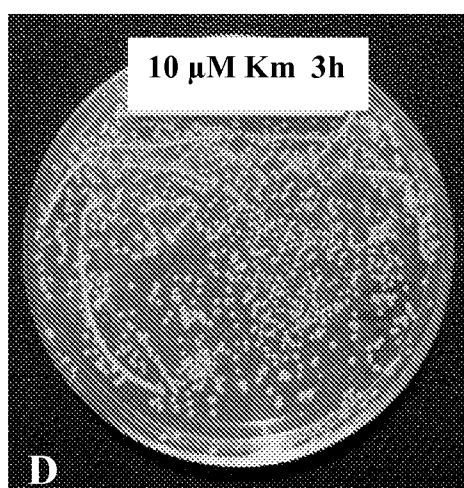


FIGURE 1D

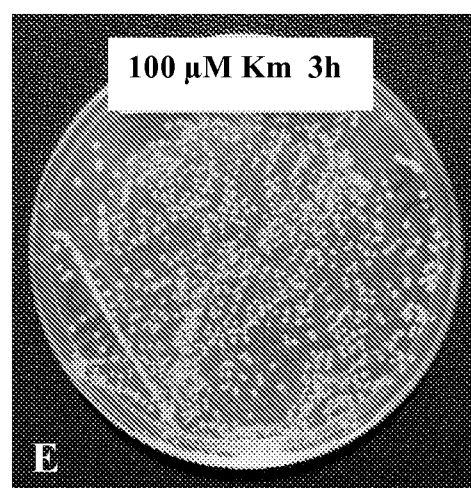


FIGURE 1E

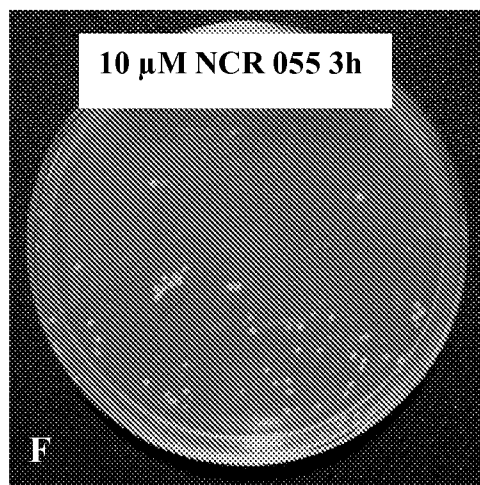


FIGURE 1F

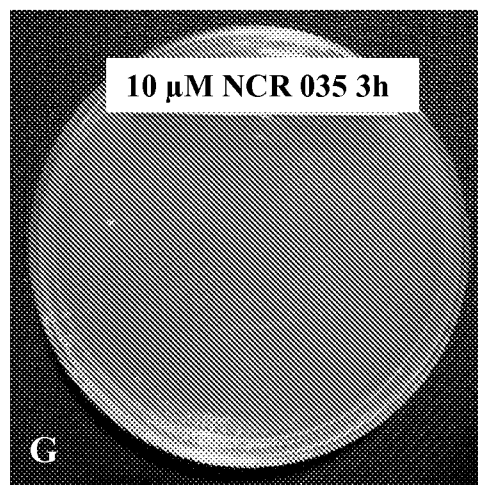


FIGURE 1G

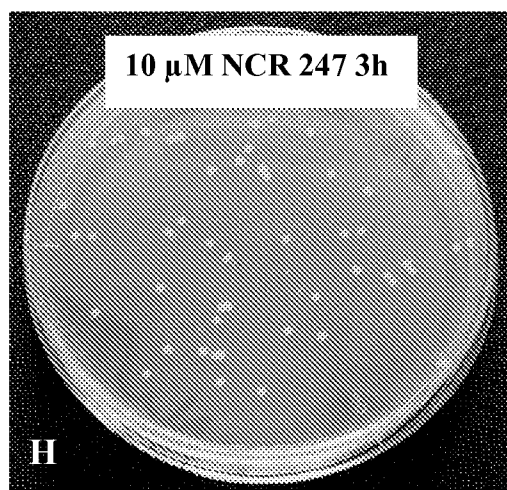


FIGURE 1H

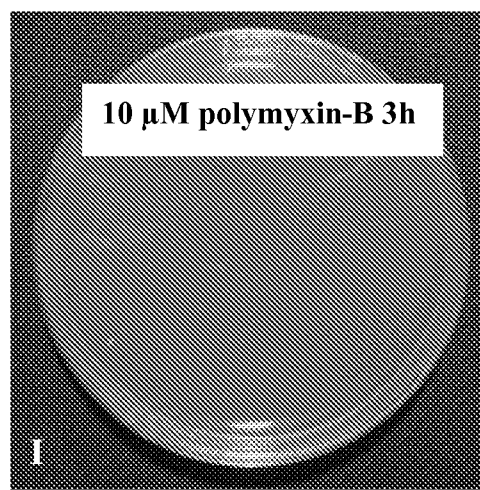


FIGURE 1I

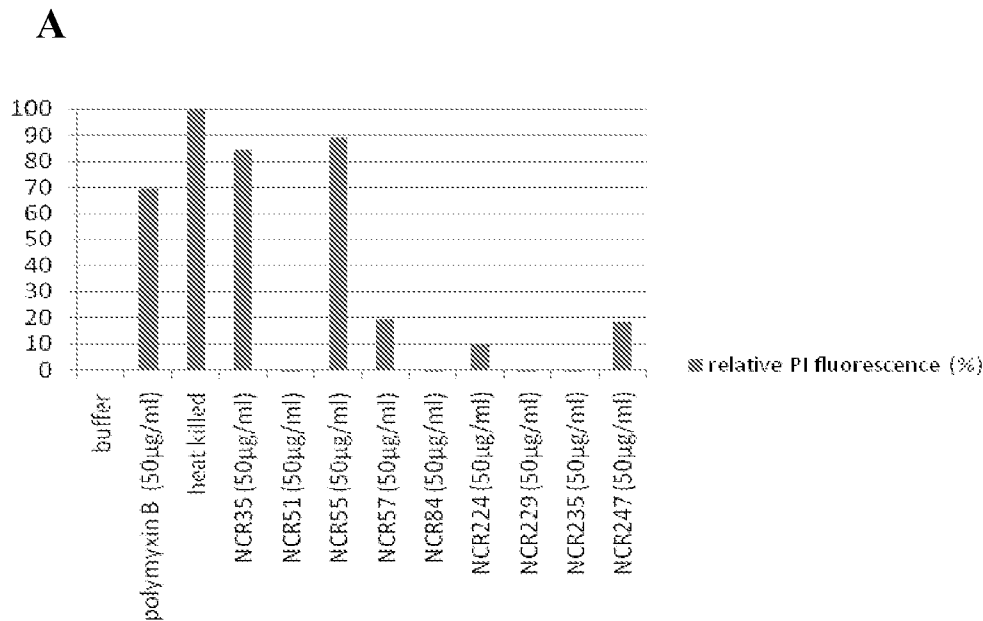


FIGURE 2A

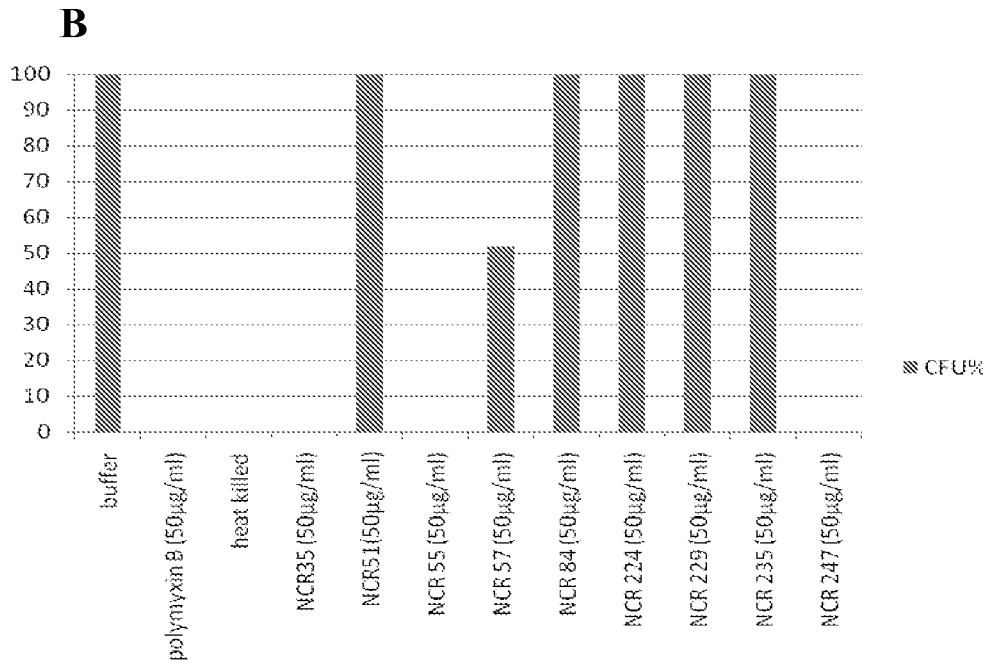


FIGURE 2B

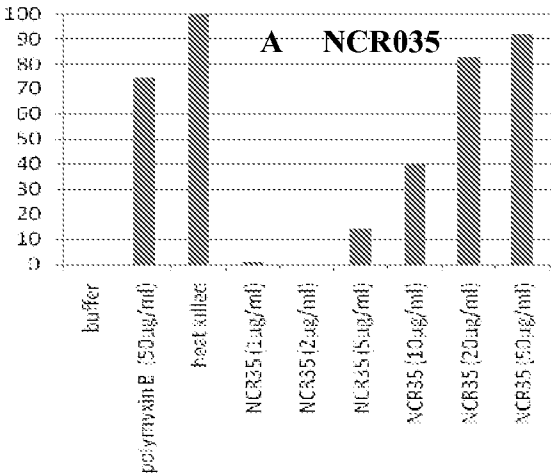


FIGURE 3A

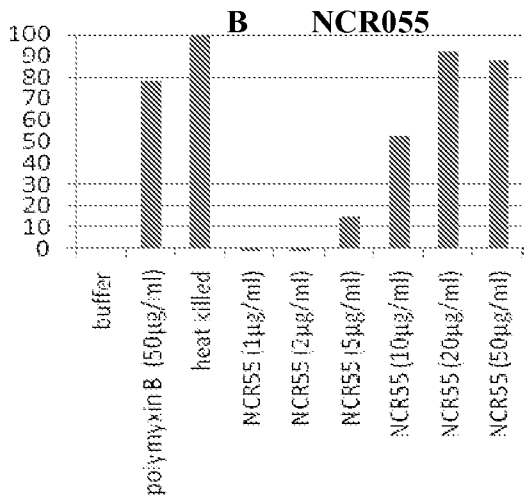


FIGURE 3B

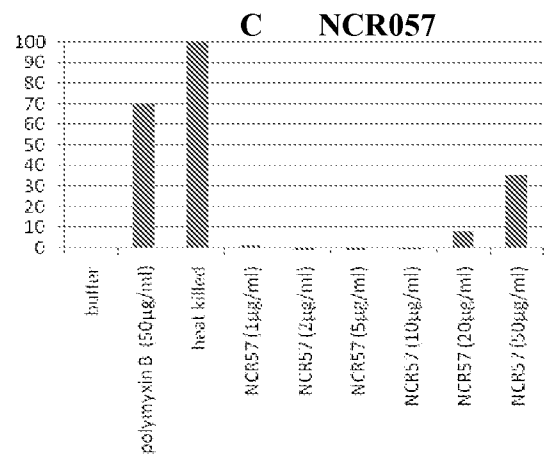


FIGURE 3C

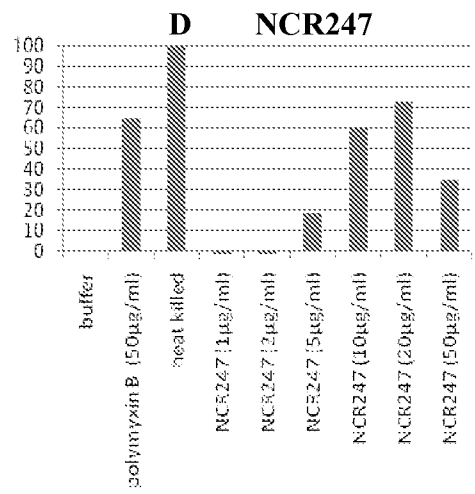


FIGURE 3D

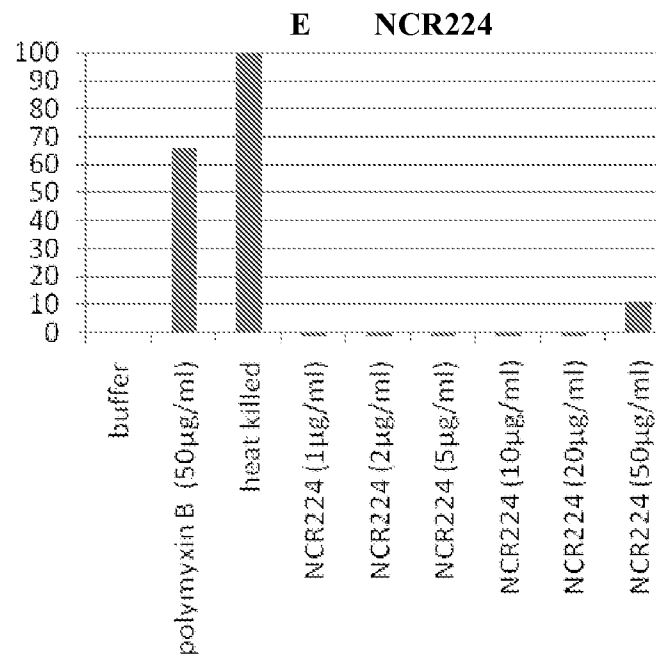


FIGURE 3E

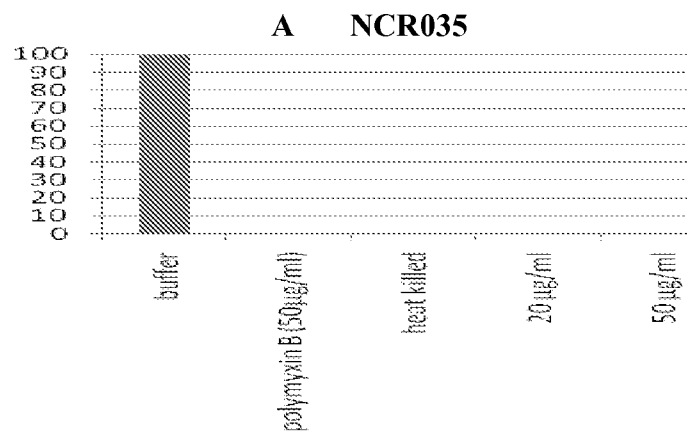


FIGURE 4A

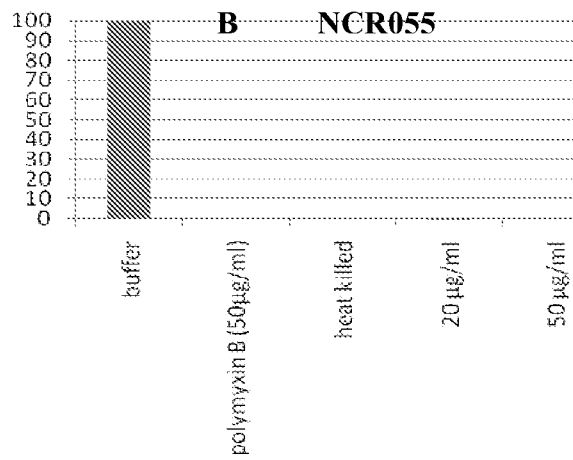


FIGURE 4B

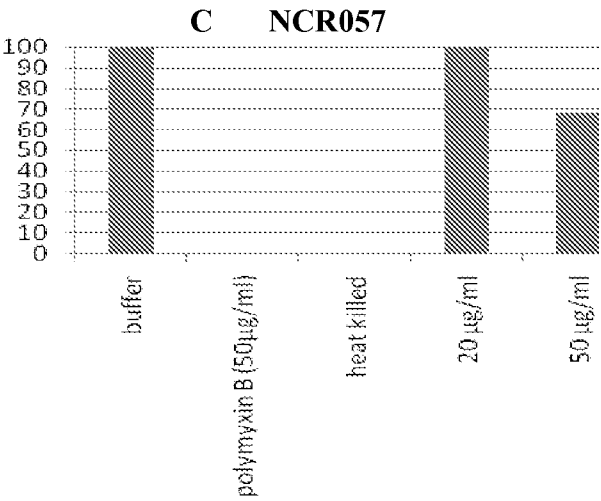


FIGURE 4C

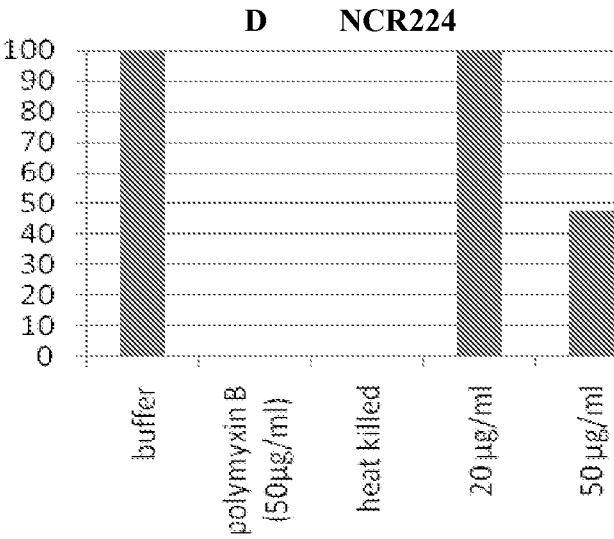


FIGURE 4D

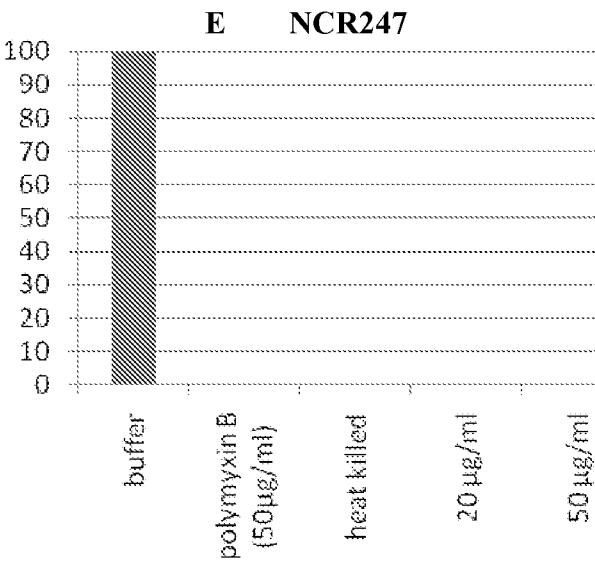
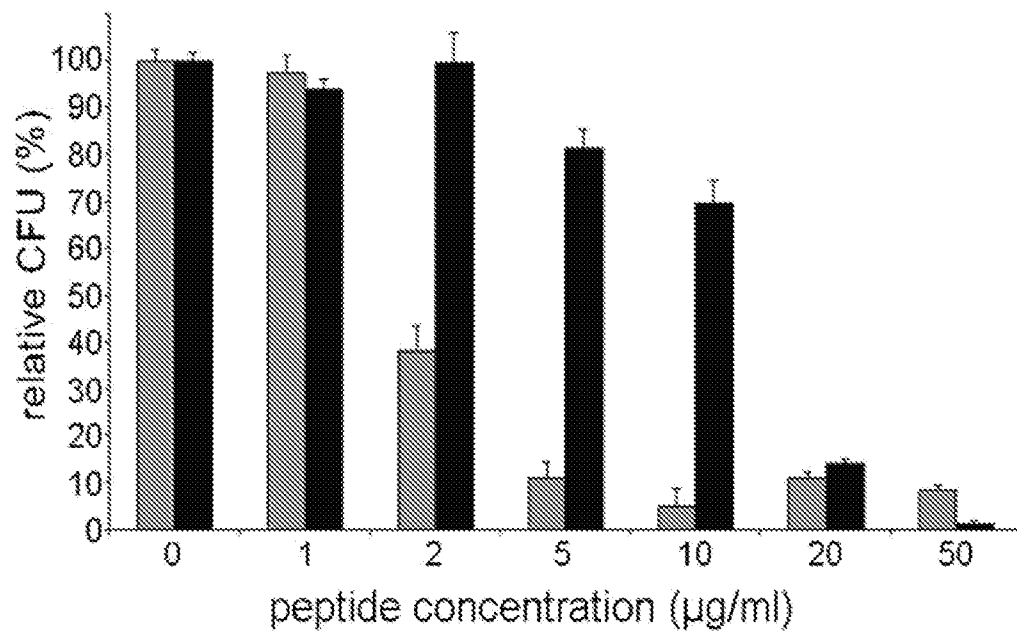


FIGURE 4E

11 /44

**FIGURE 5**

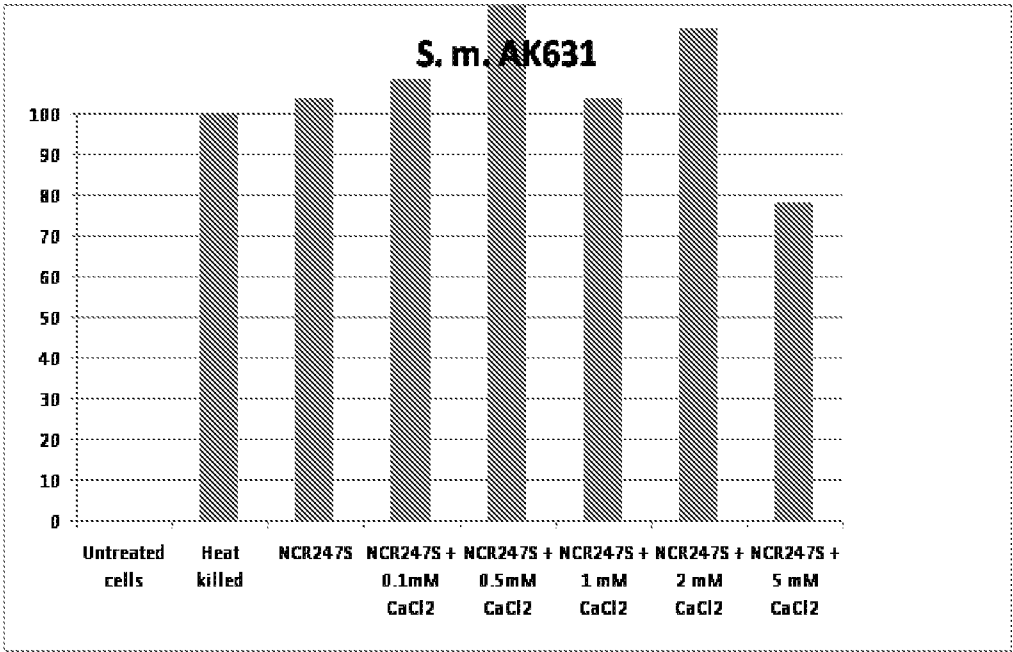


FIGURE 6

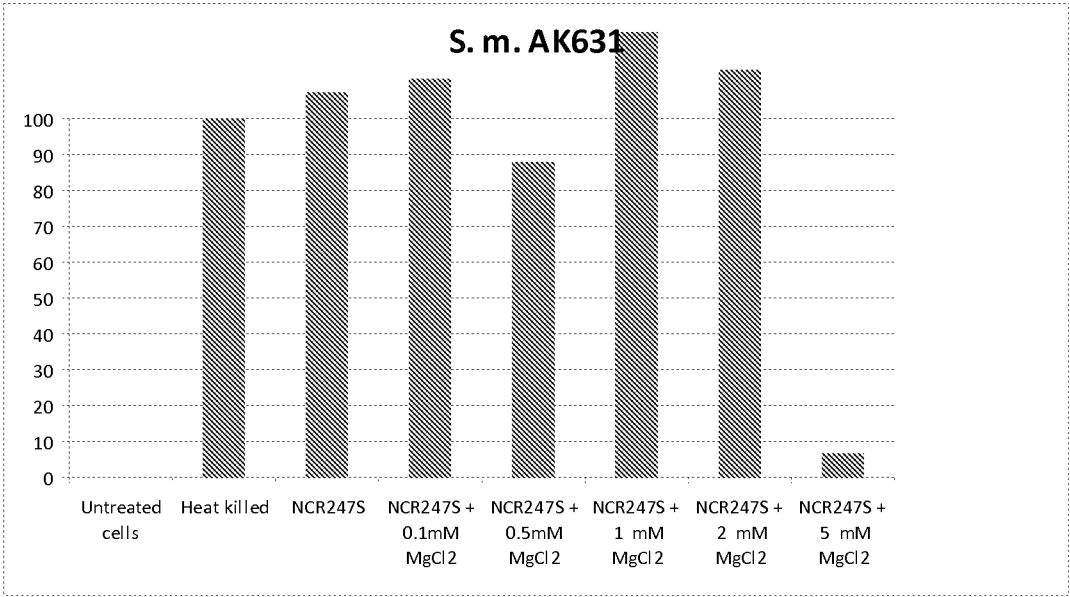


FIGURE 7

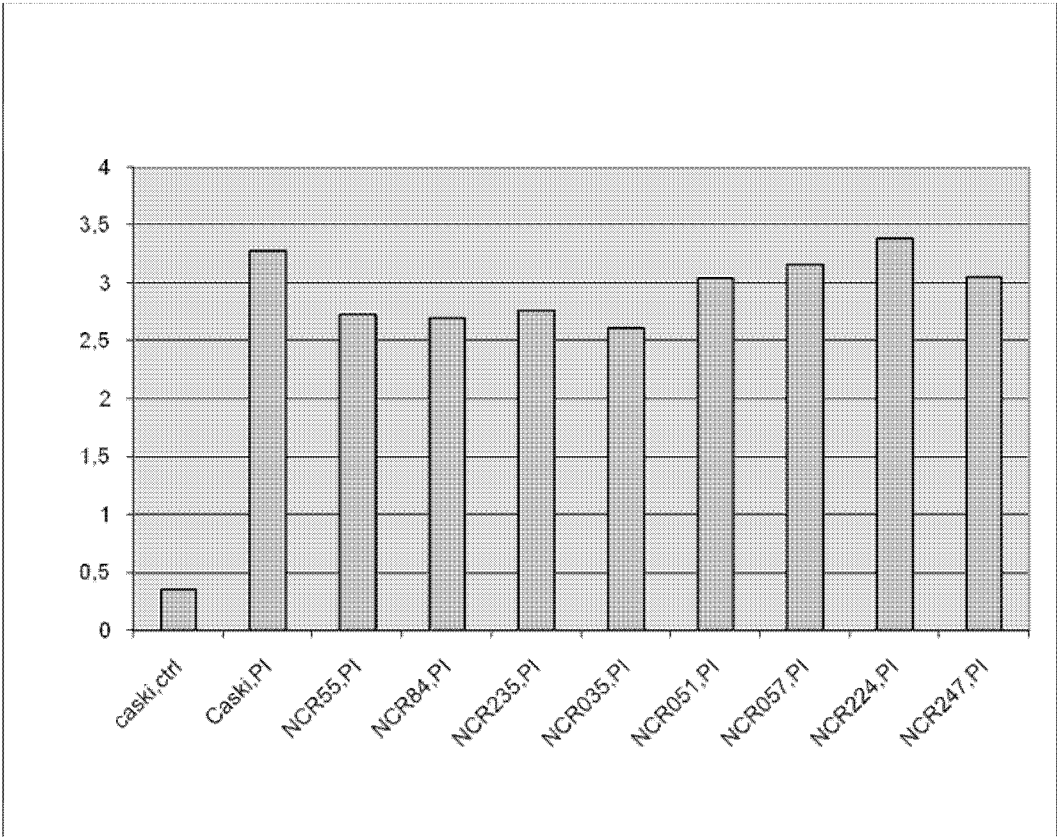


FIGURE 8

FIGURE 9A

FIGURE 9B

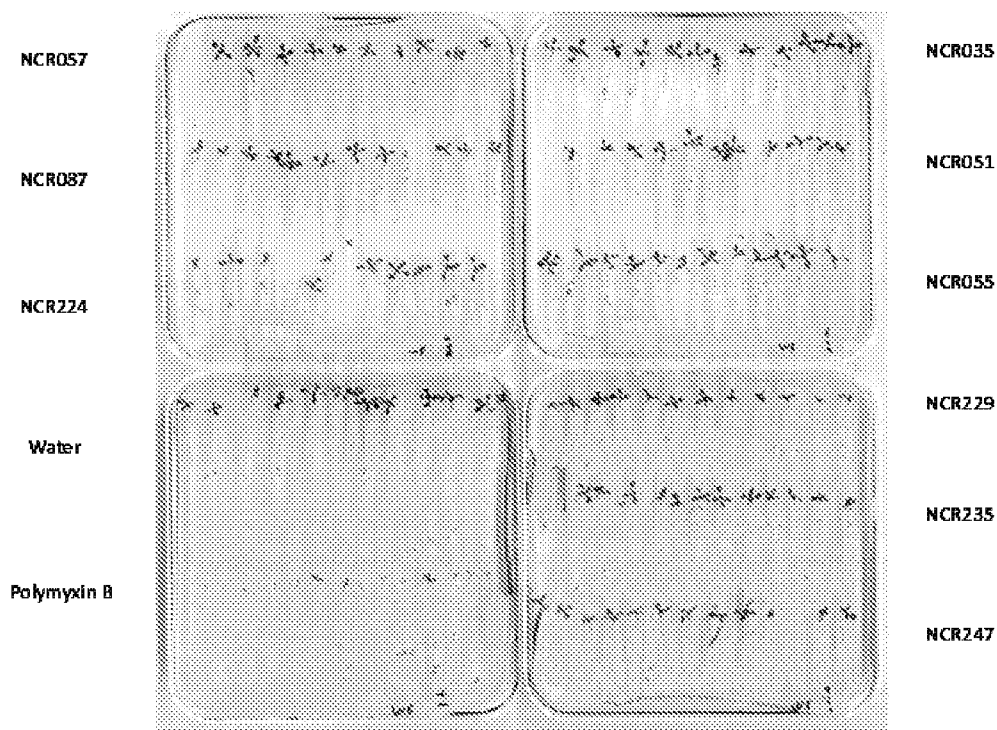
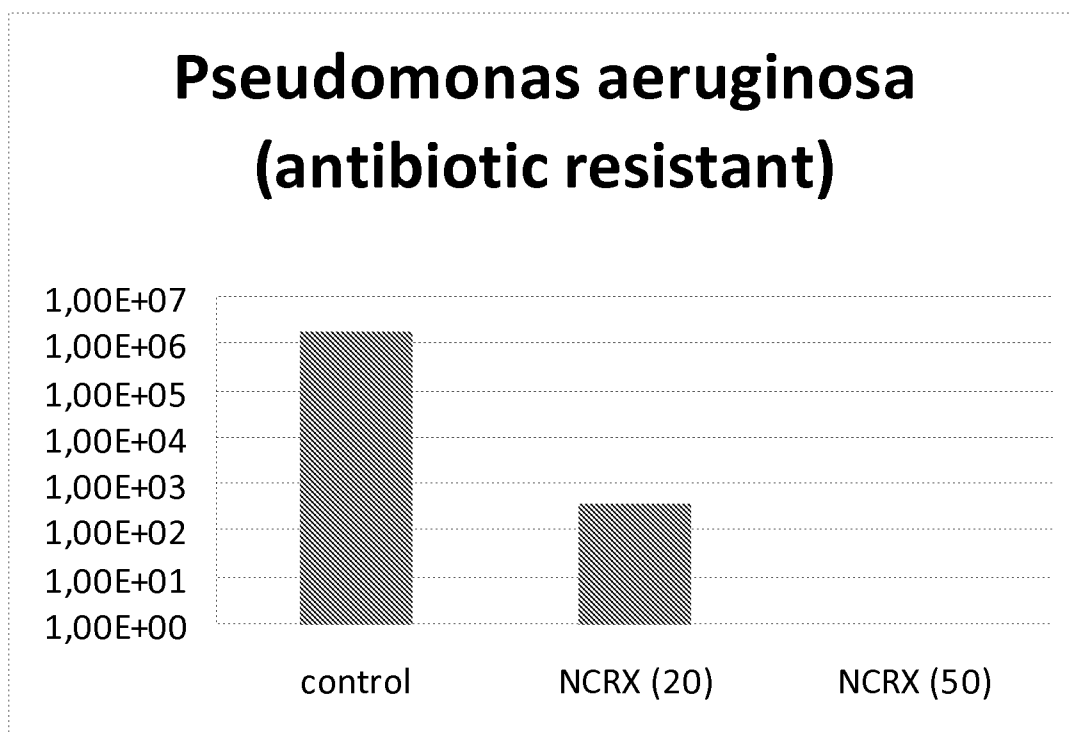


FIGURE 9D

FIGURE 9C

**FIGURE 10**

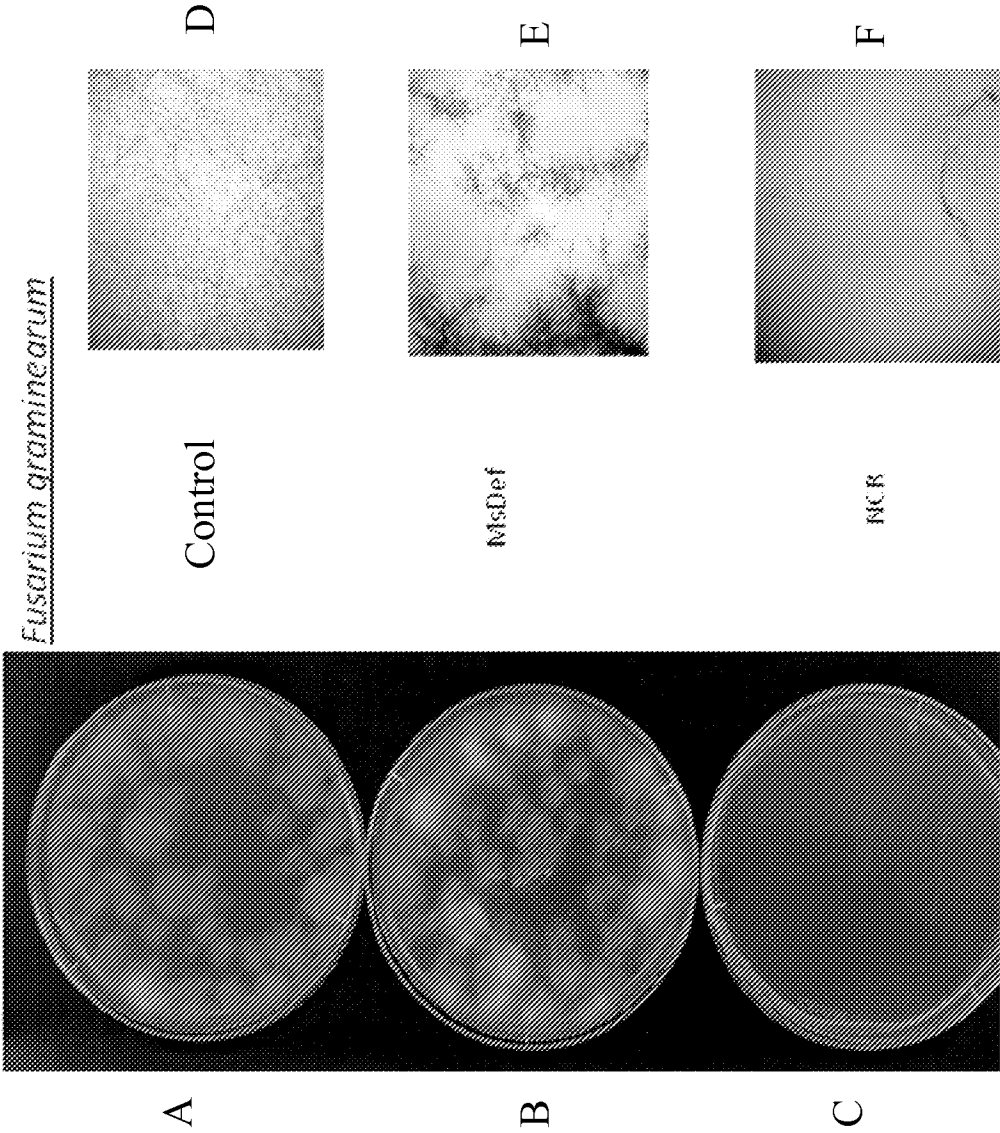


FIGURE 11

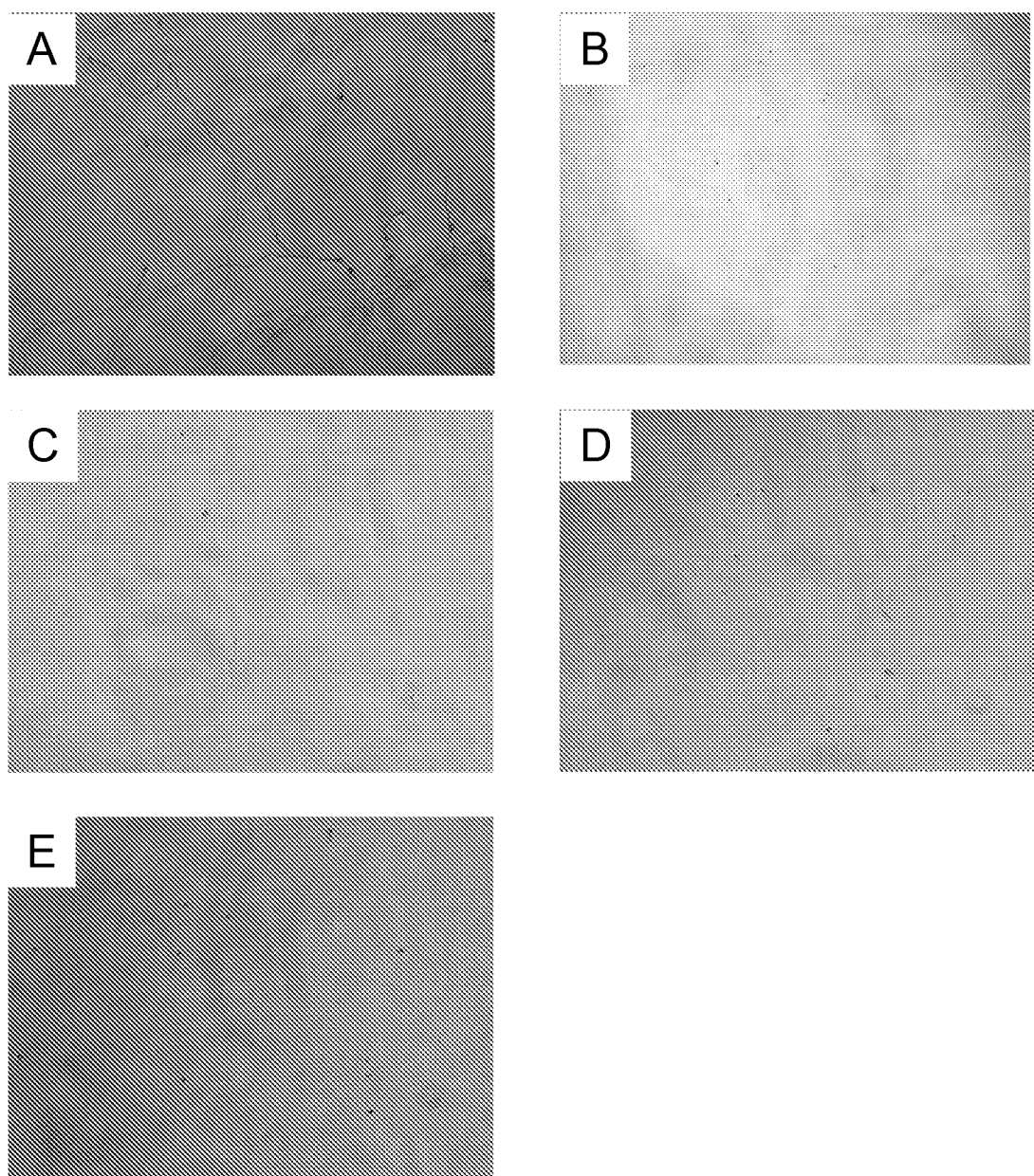


Figure 12

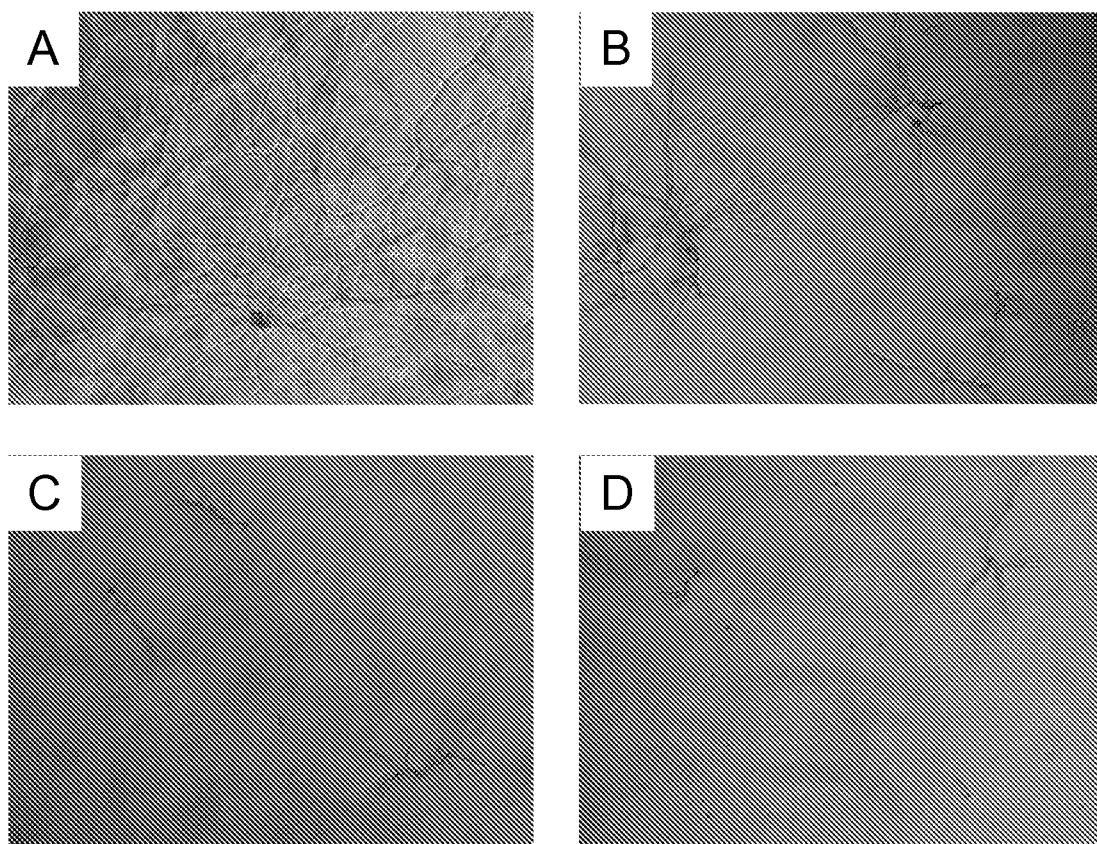


Figure 13

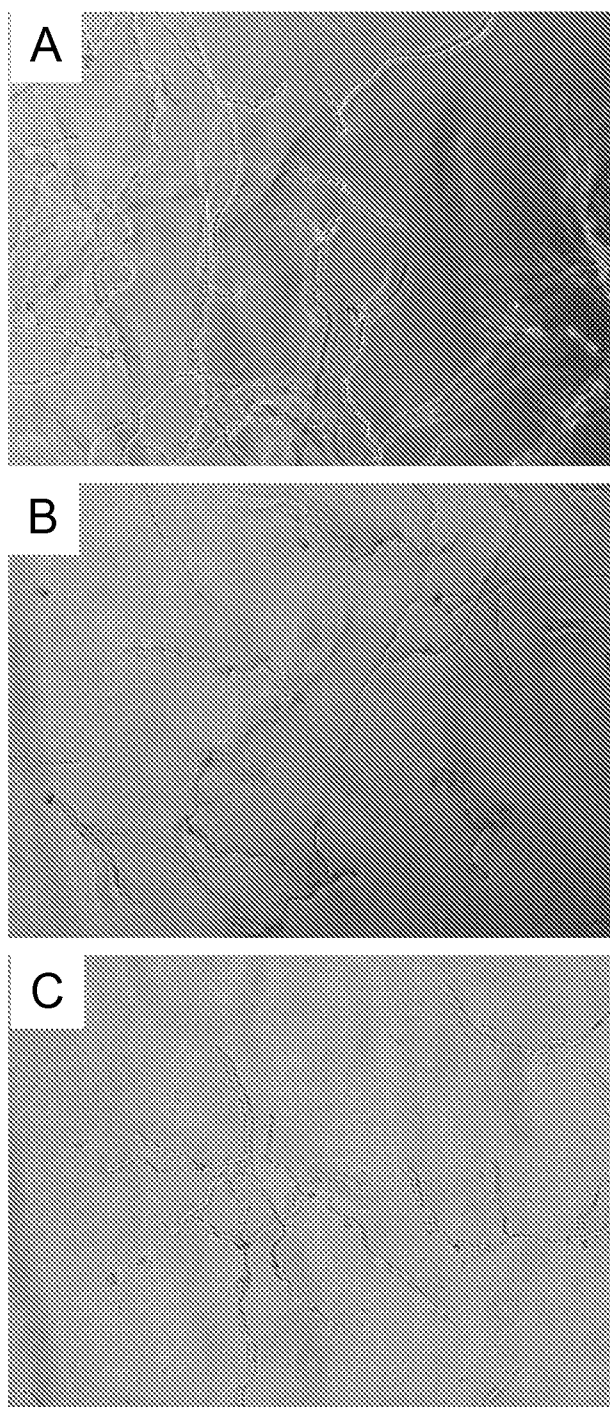


Figure 14

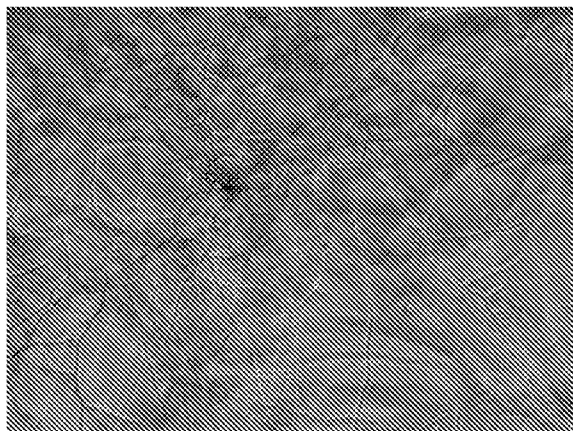


Figure 15

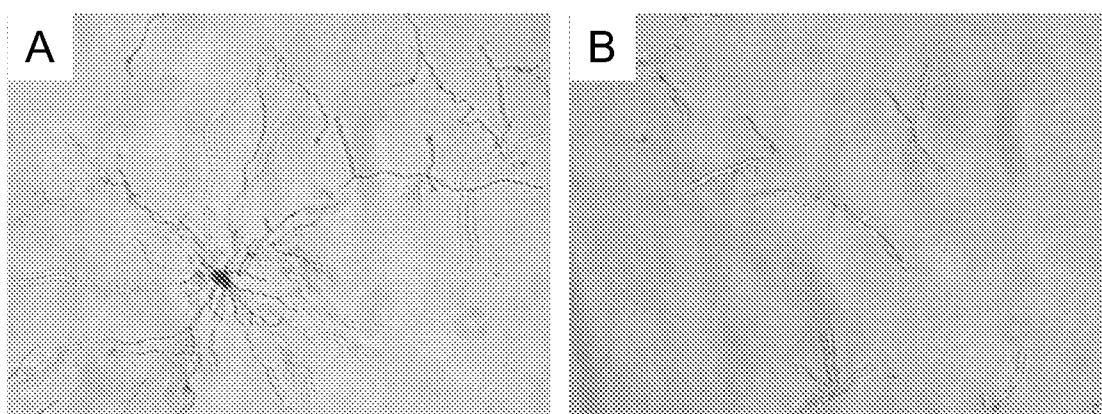


Figure 16

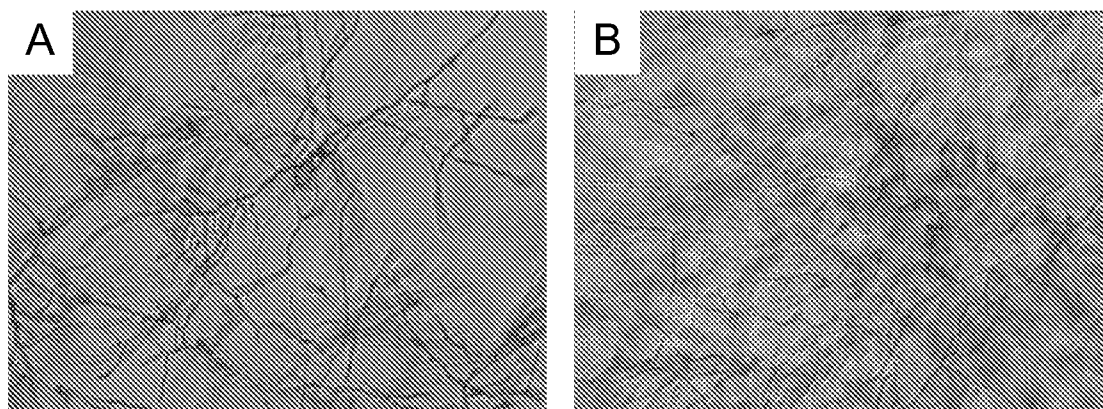


Figure 17

24 /44

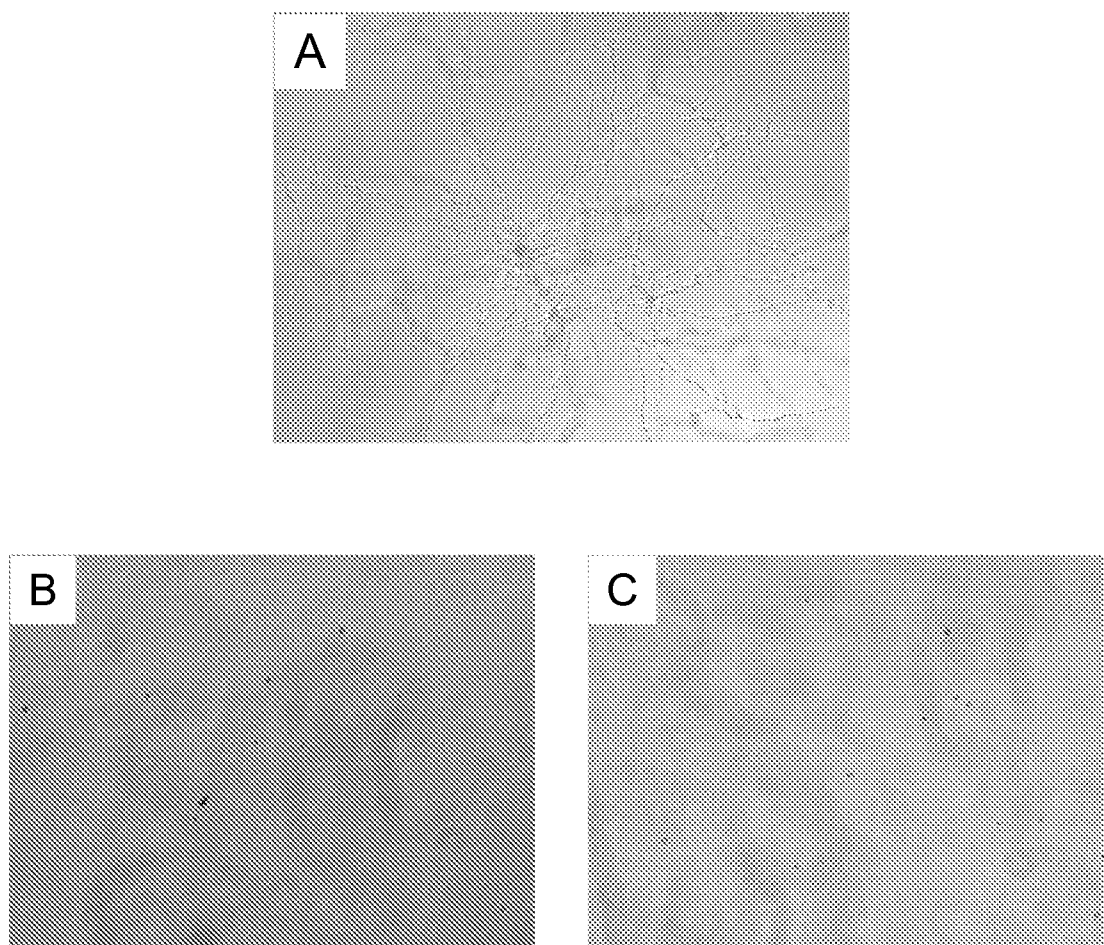


Figure 18

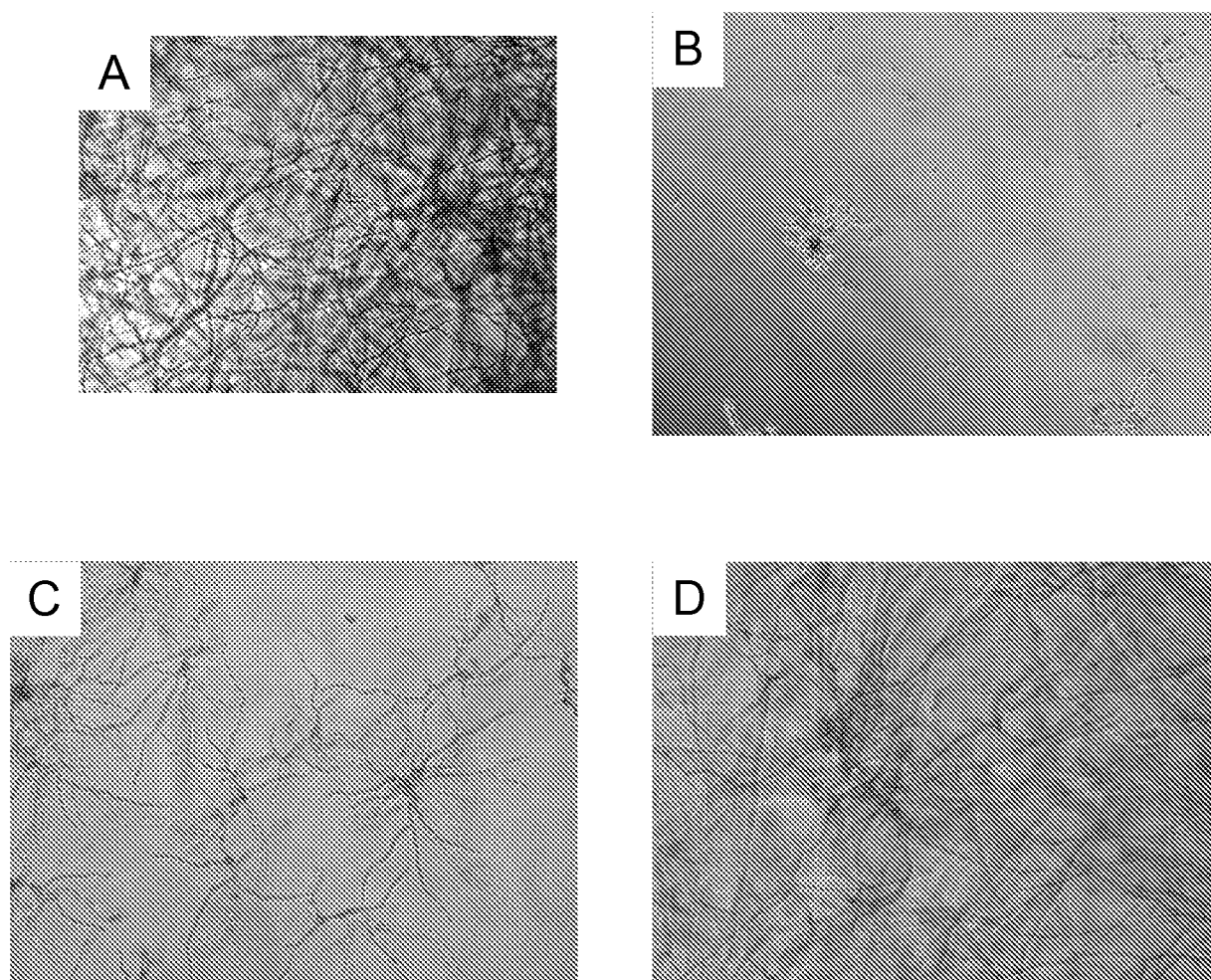


Figure 19

26 /44

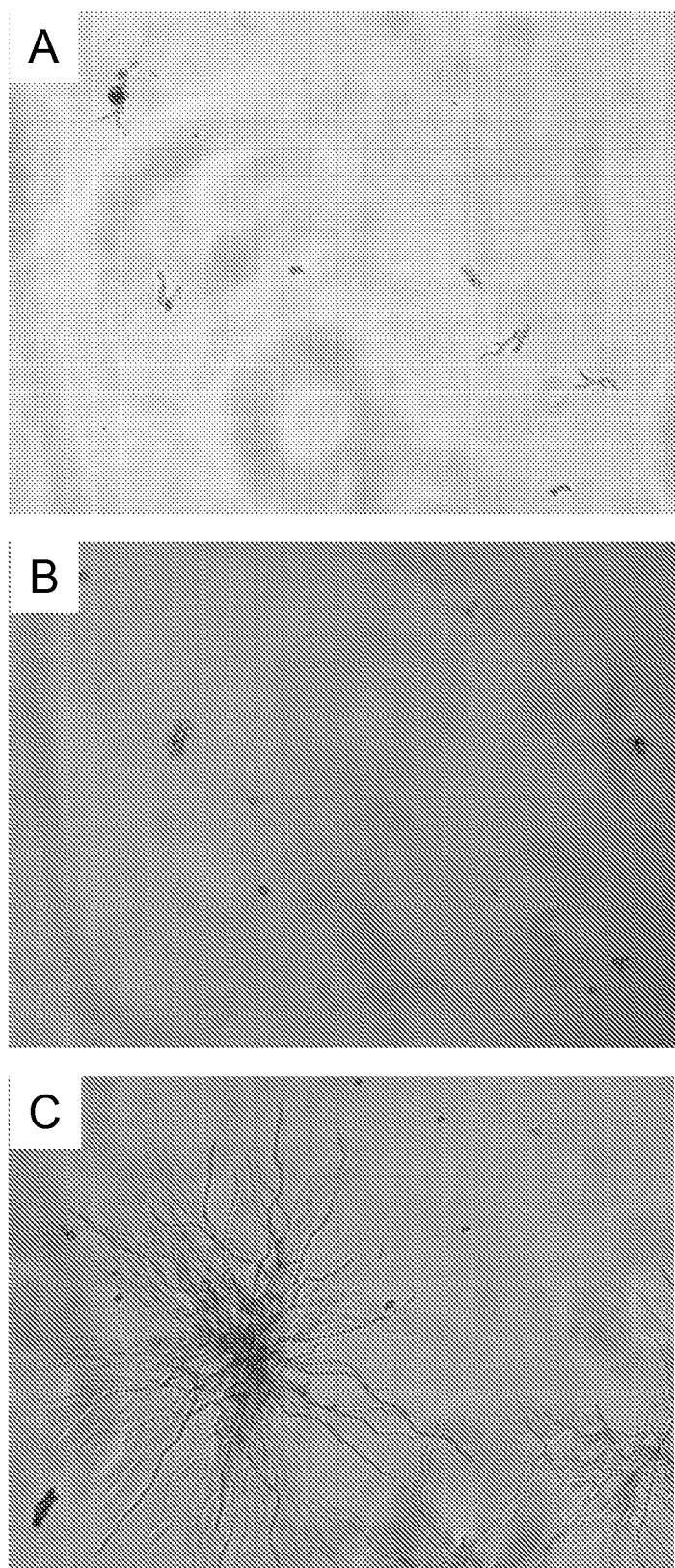


Figure 20

27 /44

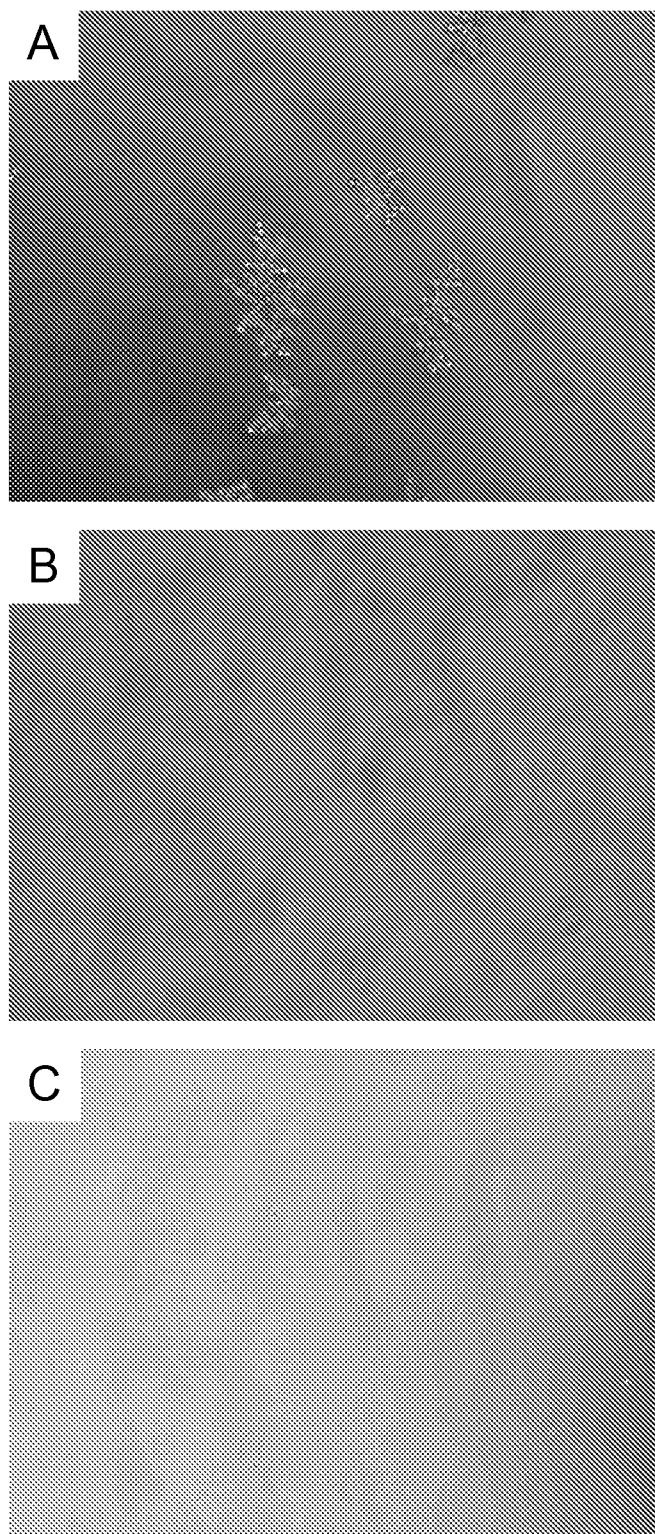


Figure 21

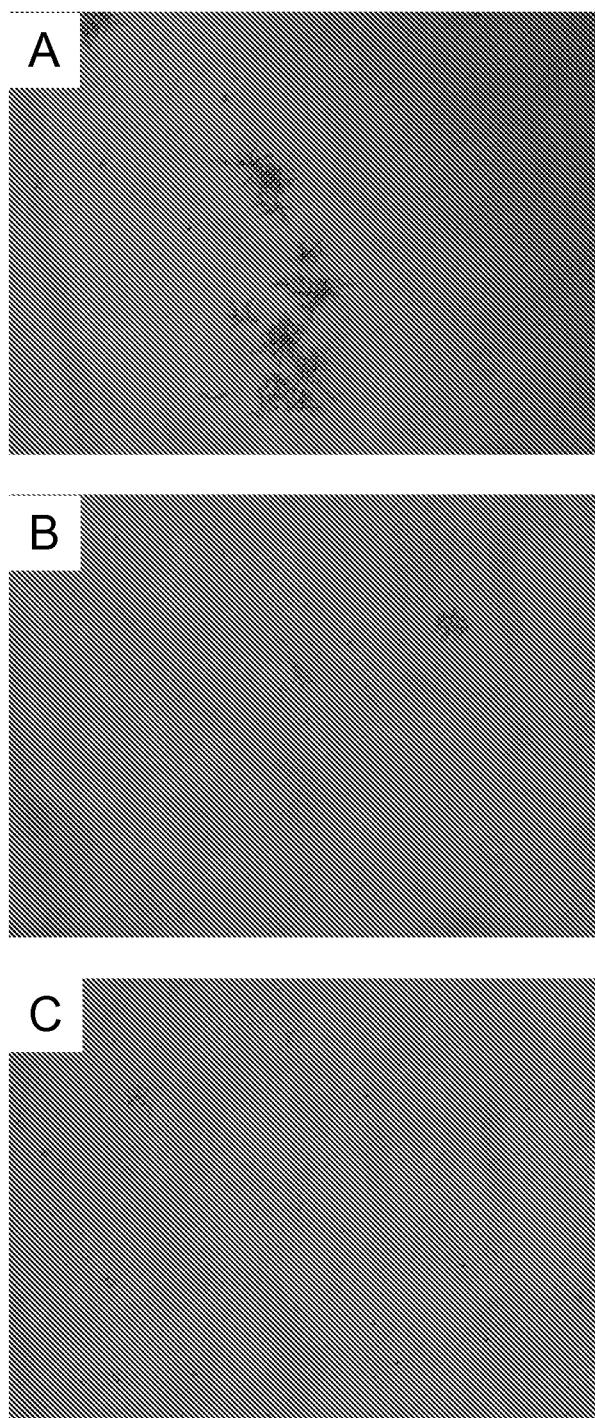


Figure 22

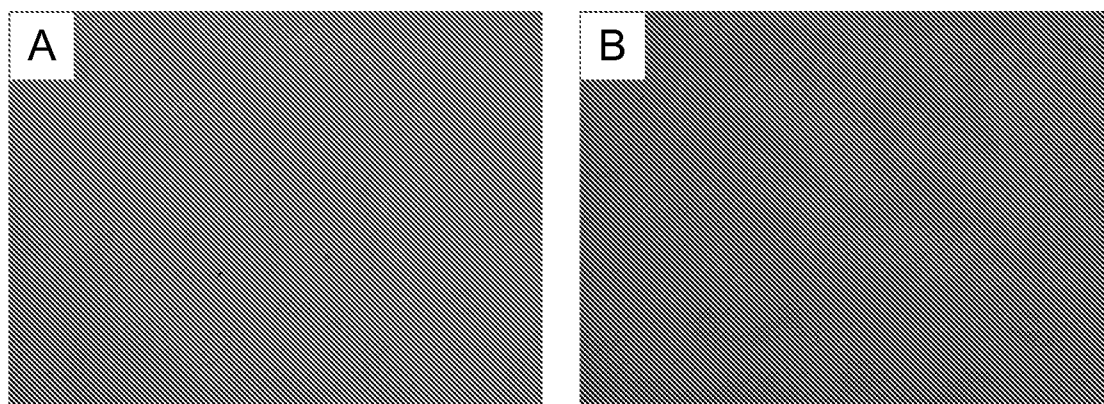


Figure 23

30 /44

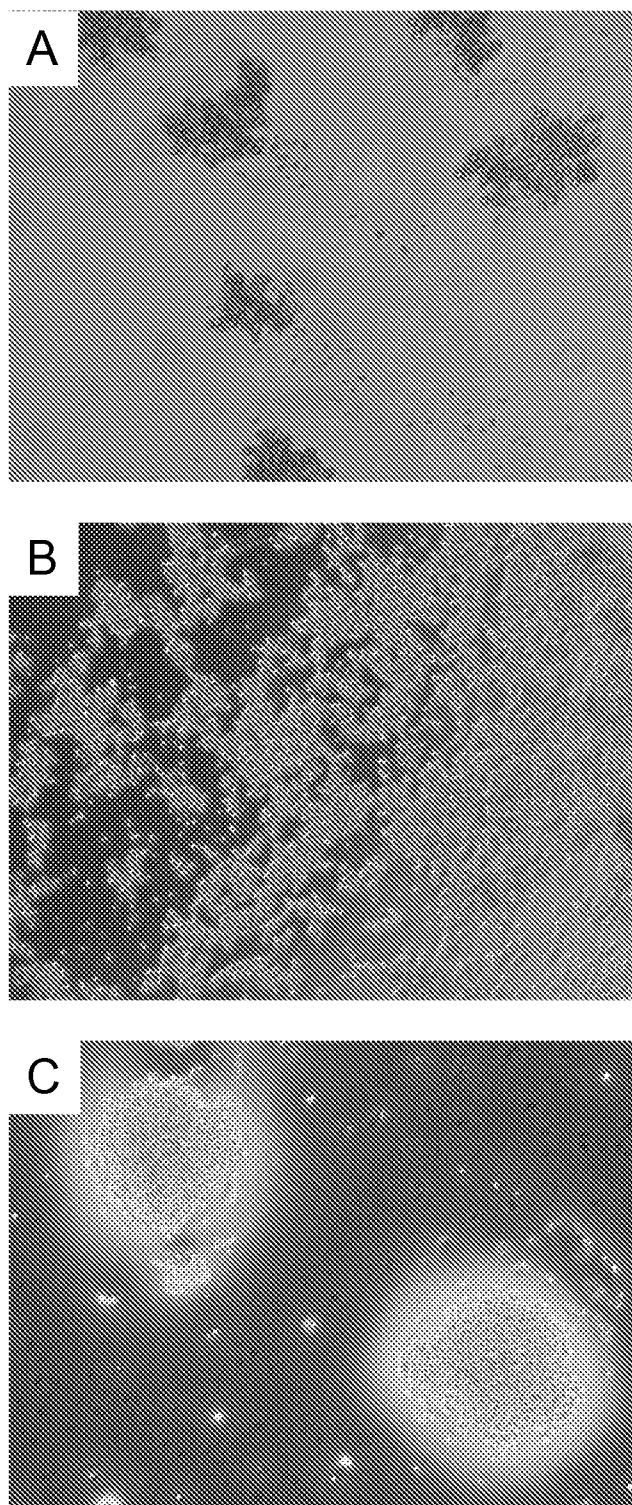


Figure 24

31 /44

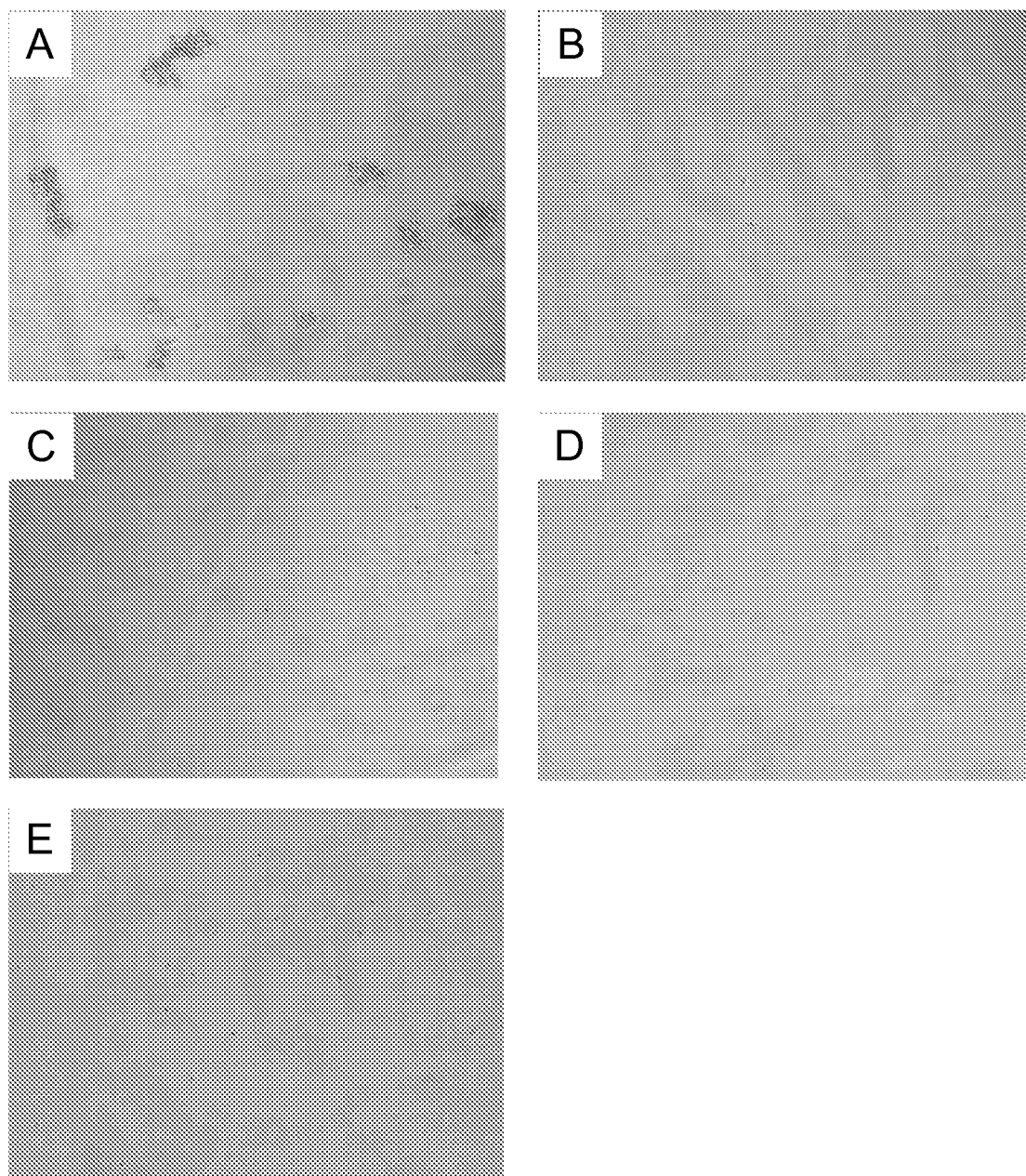


Figure 25

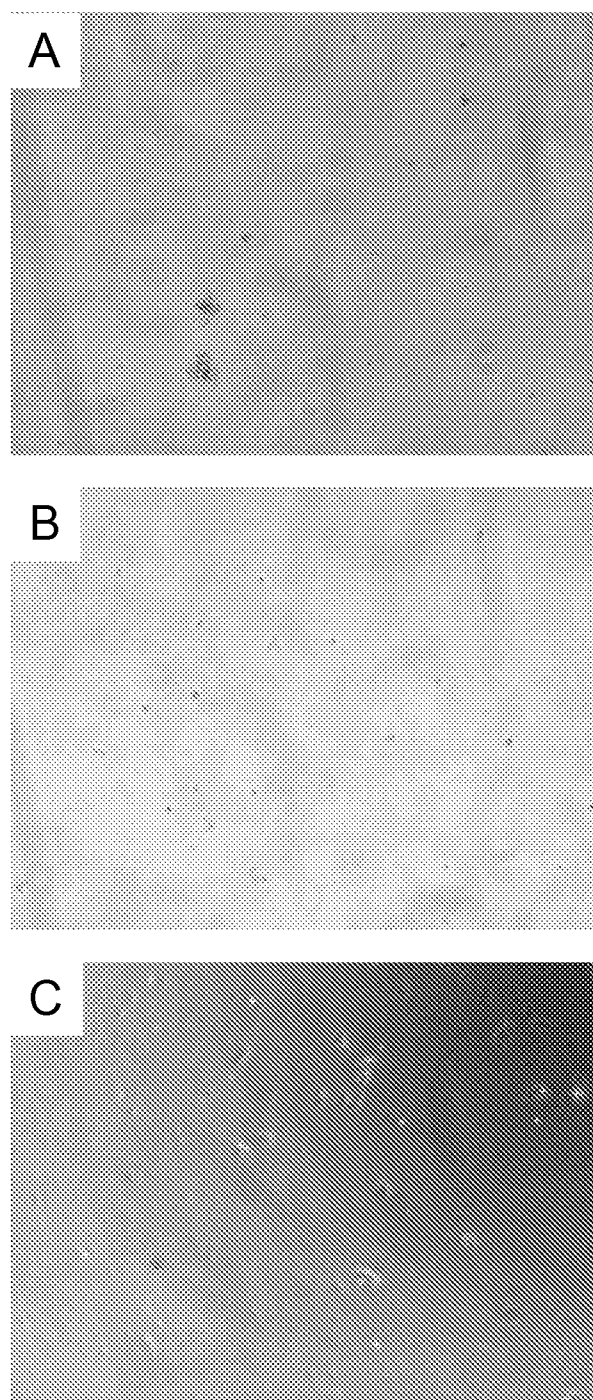


Figure 26

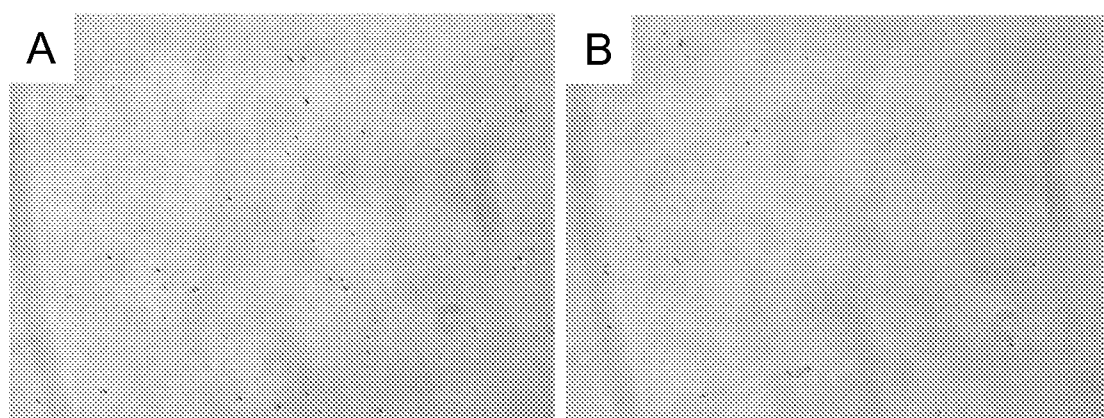


Figure 27

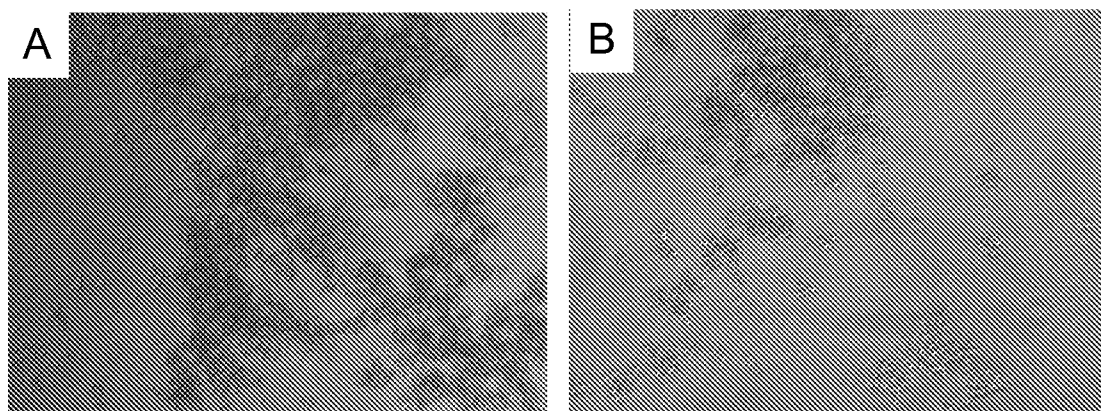


Figure 28

35 /44

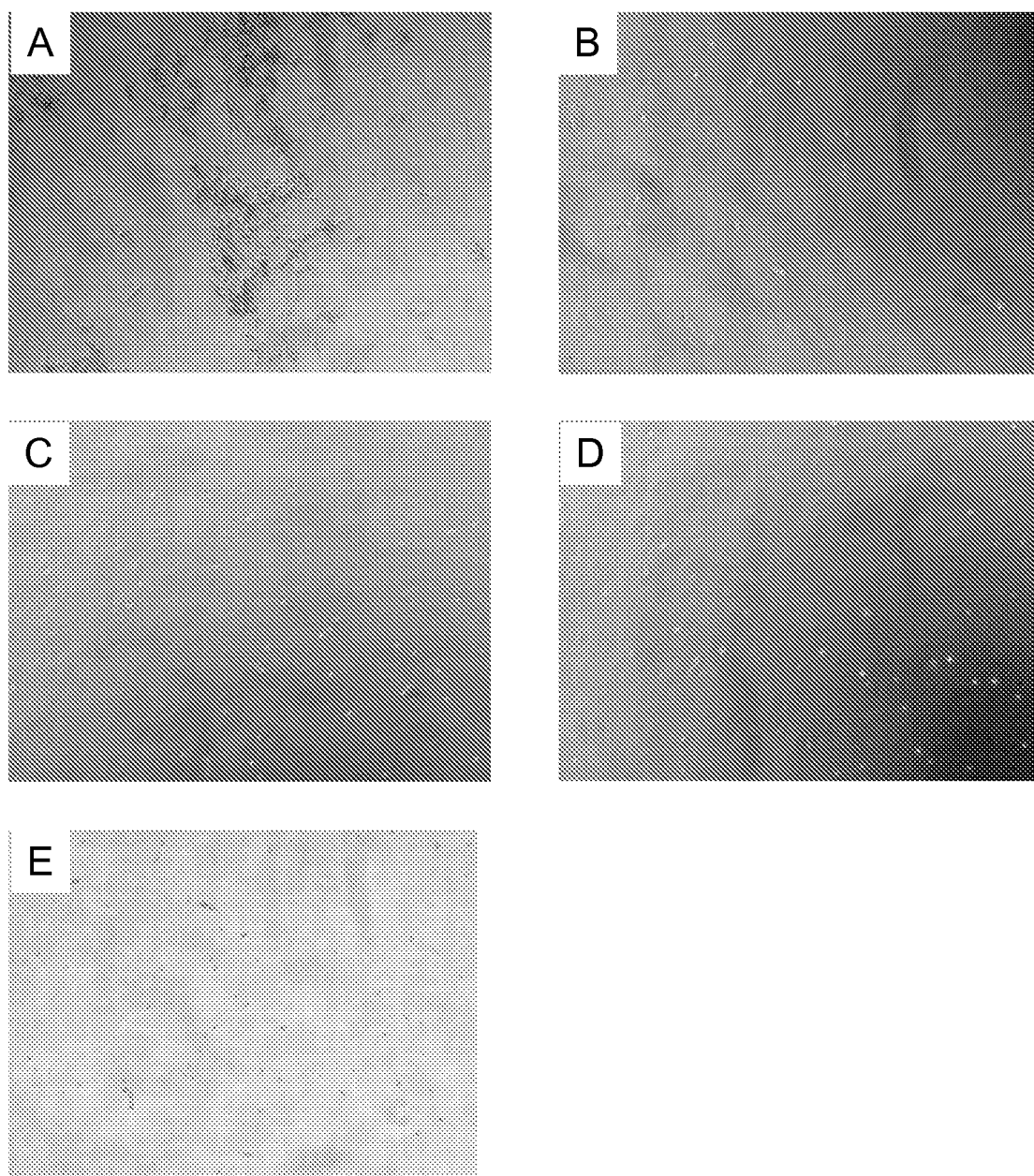


Figure 29

36 /44

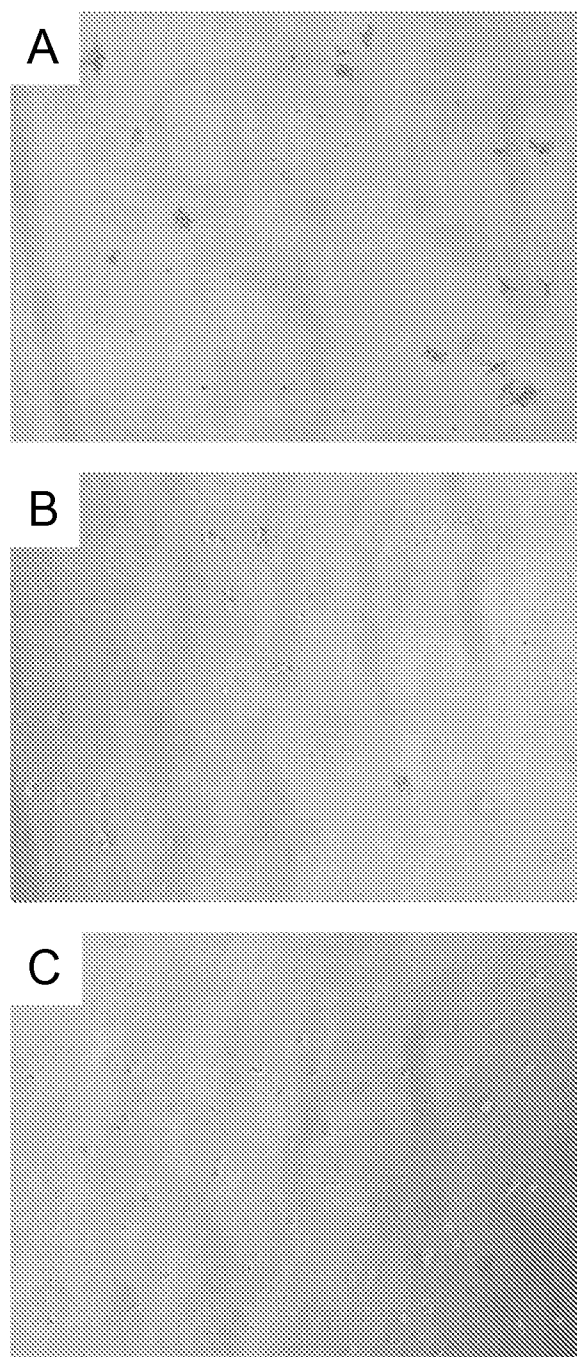


Figure 30

37 /44

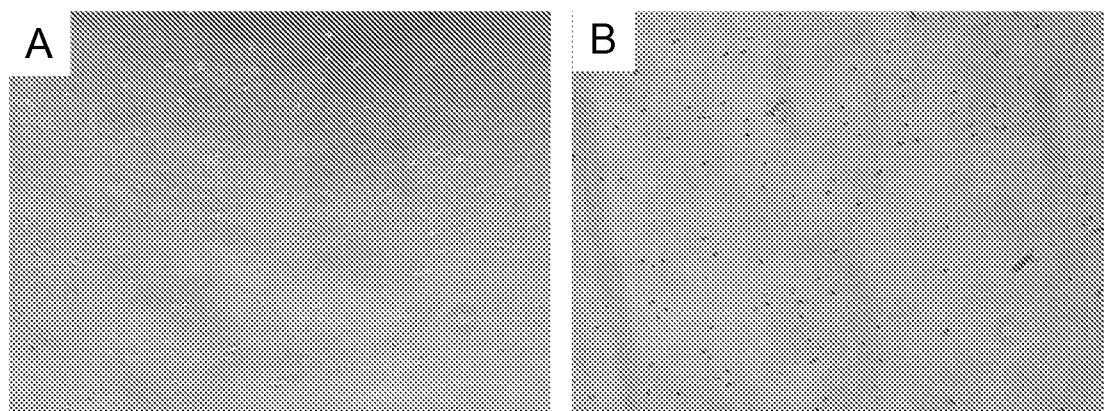


Figure 31

38 /44

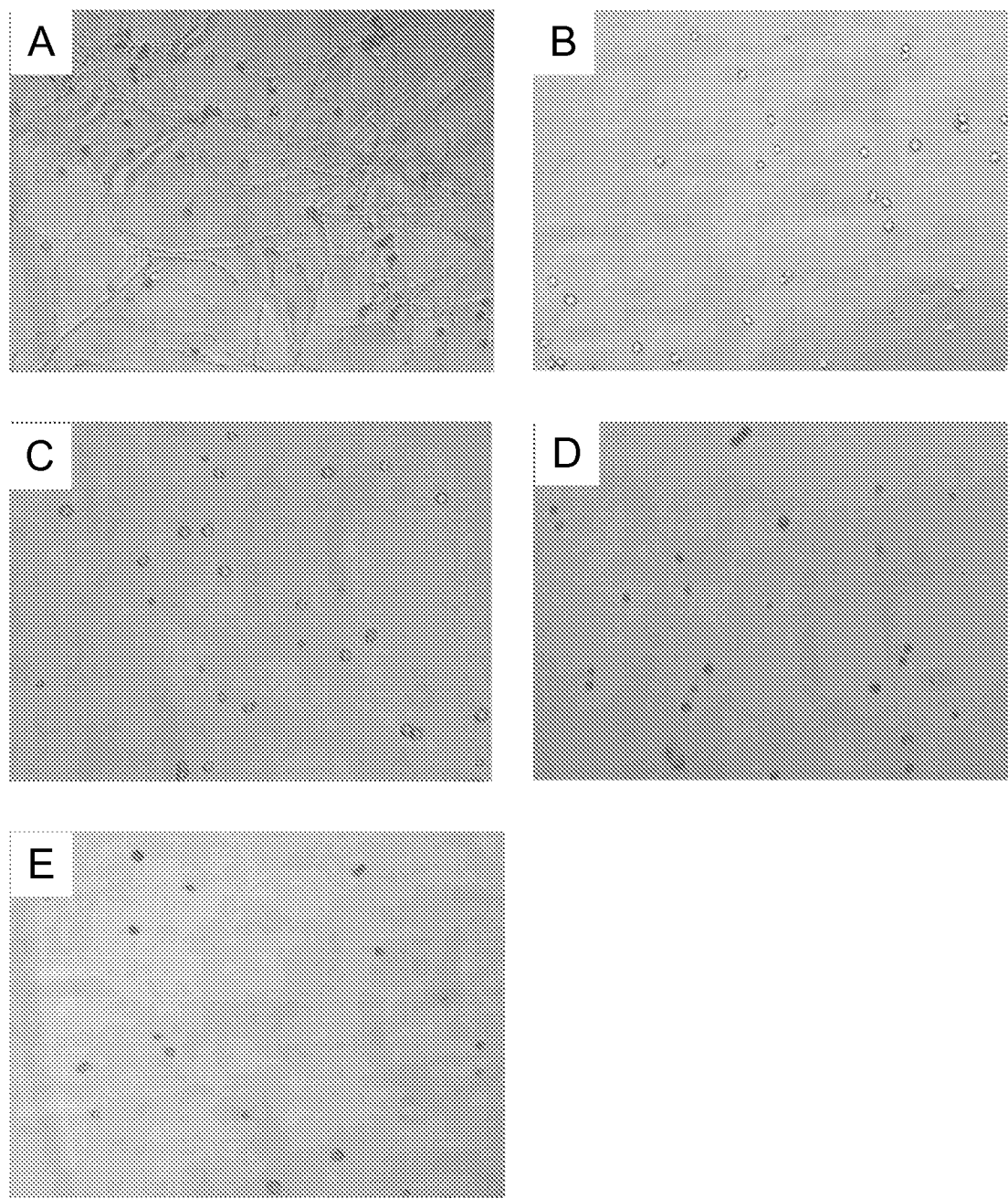


Figure 32

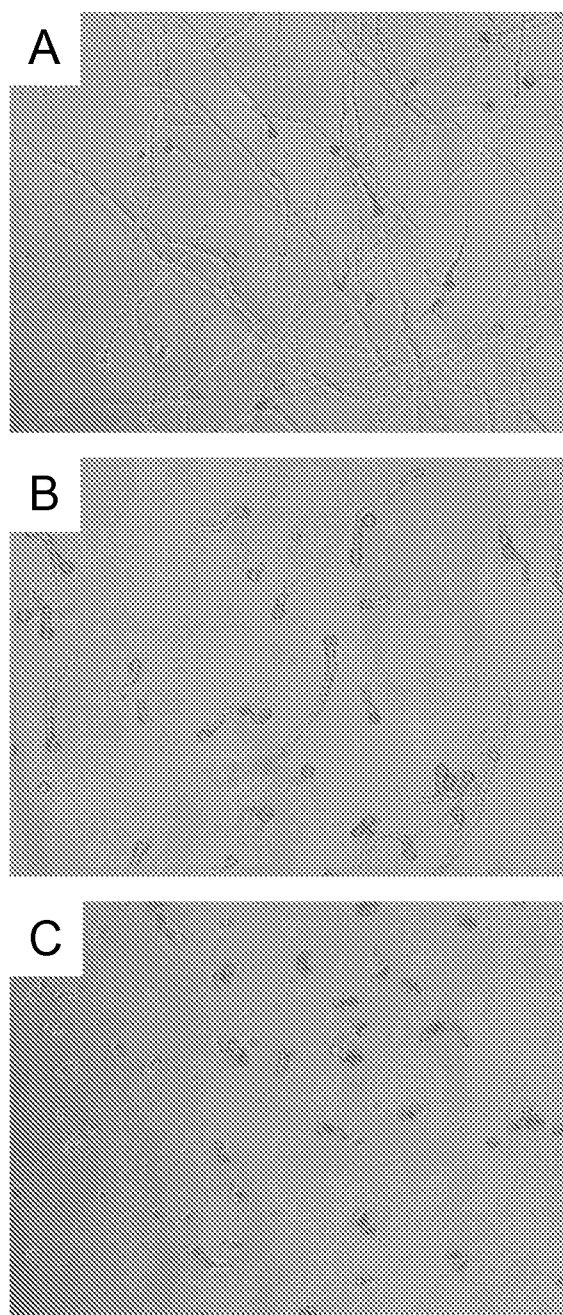


Figure 33

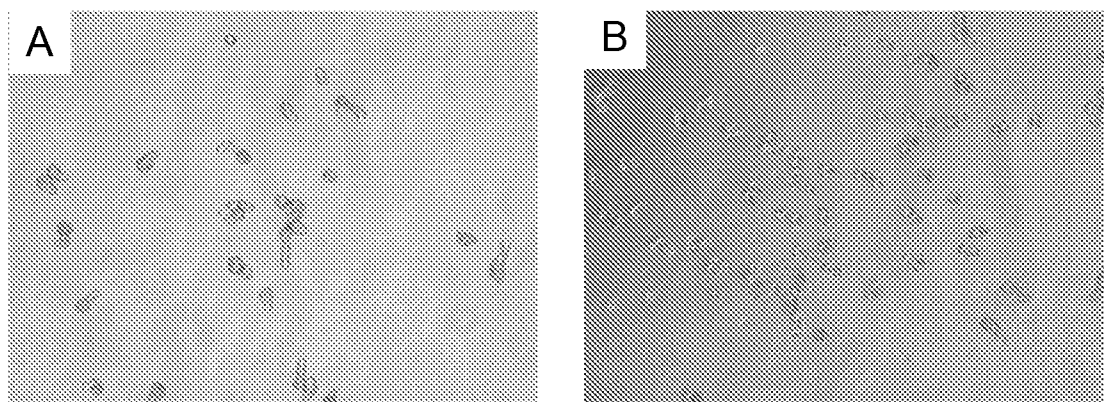


Figure 34

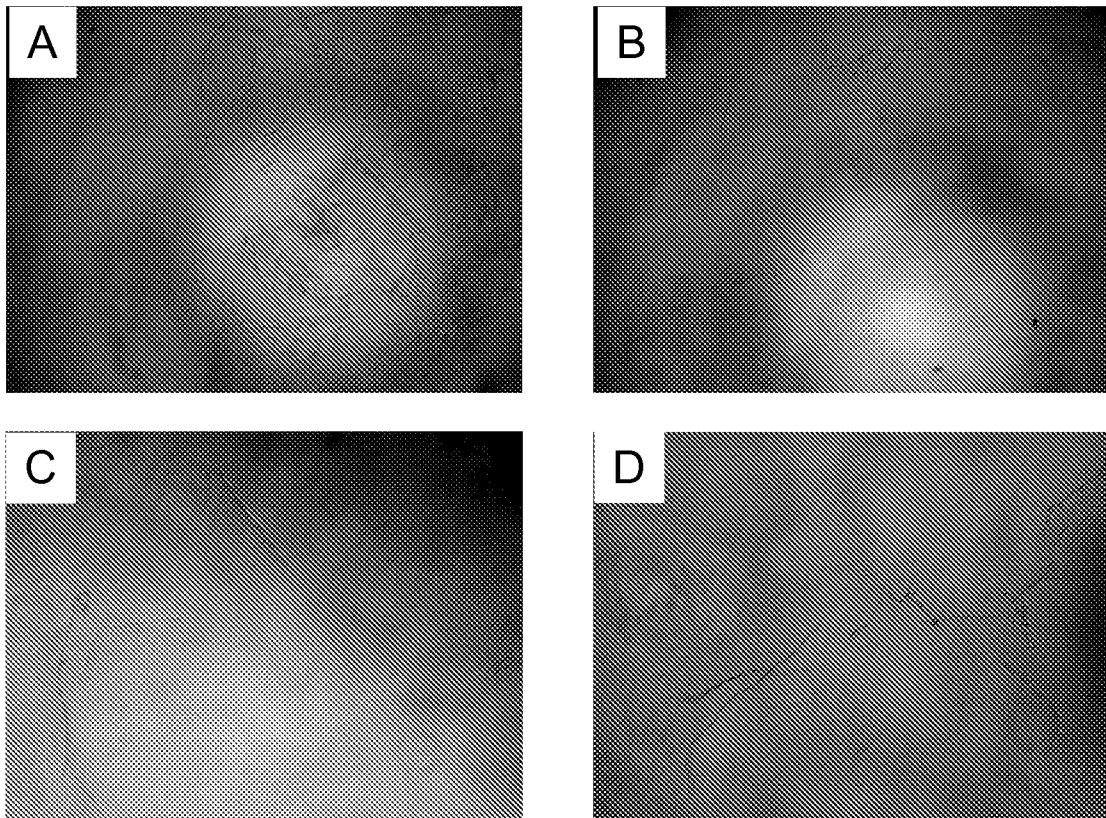


Figure 35

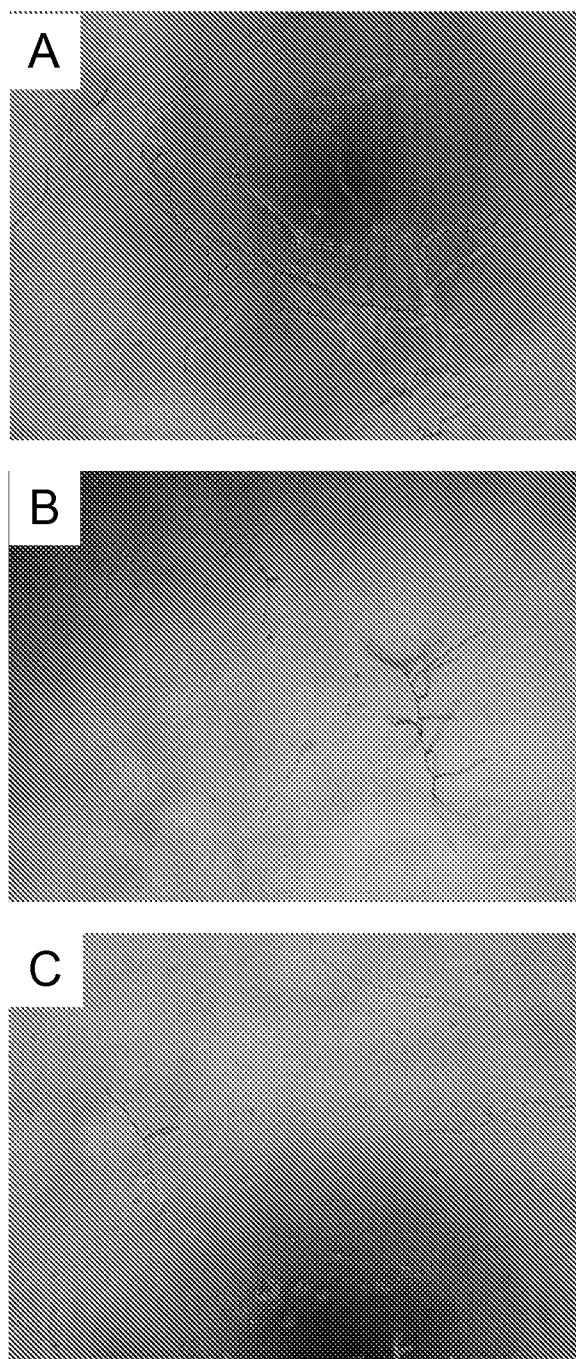


Figure 36

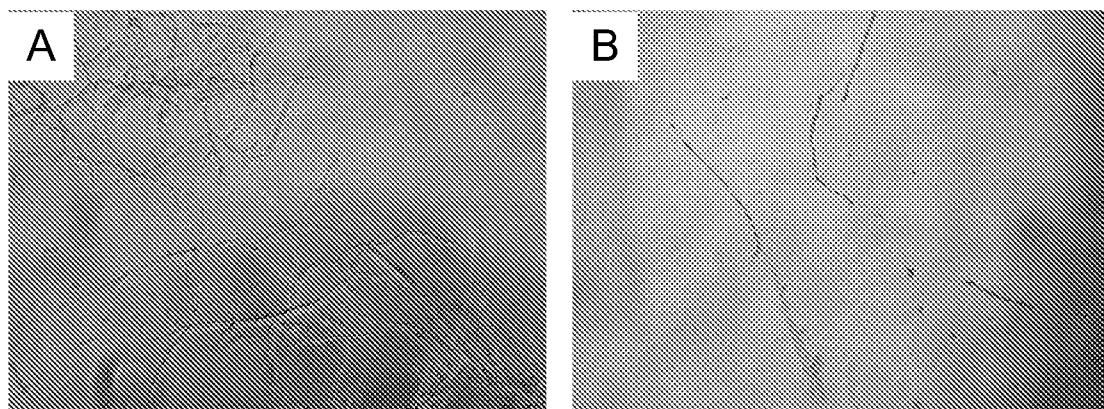


Figure 37

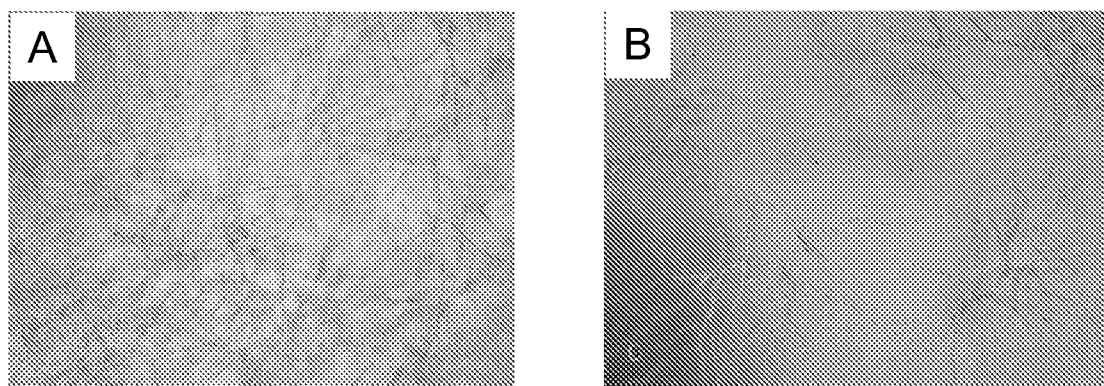


Figure 38

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/058417

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K38/16 A01N37/46 A01N65/20 A61P31/04 A61P31/10 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, MEDLINE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MERGAERT P ET AL: "A novel family in Medicago truncatula consisting of more than 300 nodule-specific genes coding for small, secreted polypeptides with conserved cysteine motifs" PLANT PHYSIOLOGY 20030501 AMERICAN SOCIETY OF PLANT BIOLOGISTS US,, vol. 132, no. 1, 1 May 2003 (2003-05-01), pages 161-173, XP002550829 cited in the application * abstract "Biochemical Role of NCR peptides" on pages 170-171 ----- -/--	1-22
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents :		
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/058417

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ALUNNI BENOIT ET AL: "Genomic organization and evolutionary insights on GRP and NCR genes, two large nodule-specific gene families in <i>Medicago truncatula</i>" MOLECULAR PLANT-MICROBE INTERACTIONS, APS PRESS, ST. PAUL, MN, US LNKD-DOI:10.1094/MPMI-20-9-1138, vol. 20, no. 9, 1 January 2007 (2007-01-01), pages 1138-1148, XP009124365 ISSN: 0894-0282 cited in the application * abstract "What are the functions for so many genes?" on pages 1146-1147 -----	1-22