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REVIEW ARTICLE

Bacteriocins from Gram-negative *Rhizobium* spp.

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ABSTRACT
Bacteriocins are ribosomally synthesized protein complexes or peptides produced by certain bacteria that have antimicrobial properties and antagonistic bacterial activity specific to competing bacteria. Bacteriocins range from simple protein to high molecular-weight complexes, where the active moiety of which is generally a protein in nature. The genetic determinants of most bacteriocins are located on native bacterial plasmids and notable few are located on chromosomes. Rhizobium spp. are Gram-negative soil bacteria that play important roles in nitrogen fixation of leguminous plants. Bacteriocins produced by different strains of Rhizobium are known to impart antagonistic effects against other closely related strains. In this review, we summarize previous work published on bacteriocins produced by Rhizobium spp; outline the prevalence of bacteriocinogenic Rhizobium spp. and discuss the classification, structure and genetic determinants and mode of action of bacteriocins. Furthermore, applications of bacteriocin producing Rhizobium spp. are discussed with a focus on their potential roles in inter-specific and intra-specific competition. Finally, future studies are highlighted that explore aspects of genetic manipulation for increased production and avenues to broaden the antimicrobial spectrum of bacteriocins.
Keywords: *Bacteriocin, Rhizobium, antagonism, Rhizobiocins, Bacteriocin like inhibitory substances (BLIS), intra-specific and inter-specific competition*

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INTRODUCTION

Legume *Rhizobium* symbiosis is a well-established plant microbe association. *Rhizobium*spp. have the ability to colonize root nodules of leguminous plants and are routinely applied as biofertilizers. These microbial bio-inoculants have the ability to compete with native microflora and successfully colonize root nodules. However, inoculated *Rhizobium* strains often fail to compete with indigenous rhizobia and fail to increase nodulation [22]. Bacteriocins are among the major factors affecting competition among rhizobia [40]. Bacteriocins are inhibitory agents that cause antagonism among closely related strains. Bacteriocins have a narrow spectrum of activity and are proteinaceous in nature, which differentiates them from antibiotics [59].

Almost 100 years ago, bacteriocins produced by *Escherichia coli* V were first identified as heat-labile and toxic to *E. coli* S. These bacteriocins were named colicin to identify the producing species [19]. In subsequent years, bacteriocins have been found in all types of bacteria and even found in some species of Archaea [43]. Bacteriocins can be defined as ribosomally synthesized proteinaceous toxins produced by Gram-positive and Gram-negative bacteria, which inhibit the growth of closely related bacterial species and strains [7]. Bacteriocins are classified in terms of size (small, medium and large), microbial target, mode of action, and release and immunity mechanisms [8].

Extensive work has focused on, for instance, lactic acid bacteria (LAB) bacteriocin genetics, biosynthesis, mode of action and, classification. Applications of LAB bacteriocins include use as biopreservatives in food. Nisin bacteriocin produced by *Lactococcus* spp. was approved for use in food by the FDA in 1988 [4]. Presently, this approach is widely used as a biopreservative in the cheese making industry [9]. Comparatively, little work has been conducted on Gram-negative bacteria, *Rhizobium* spp, as bacteriocin

producers. In this study, we attempt to compile all documented literature and potential applications of *Rhizobium* bacteriocins.

PREVALENCE OF *RHIZOBIUM* BACTERIOCINS

The first description of bacteriocin production by *Rhizobium* spp. was published by Roslycky [47]. Bacteriocin-producing strains have been identified in *R. leguminosarum* bv. *trifolii* [54, 55, 2, 29, 61], *R. japonicum* [21], *R. leguminosarum* bv. *viciae* [25, 68, 6], *Rhizobium lupine* (Lotz and Mayer 1972), *R. cicer* [55, 1], *R. meliloti* [60] and *R etli* [44]. Bacteriocins produced by *Rhizobium*spp. have been characterized as phage-like [54, 21] and having antibiotic properties [55, 61]. Many rhizobial species including *Rhizobium leguminosarum* and *Bradyrhizobium*spp.(bacteriocinogenic strains) produce bacteriocins, designated as rhizobiocins [58, 25, 17].

An antibacterial compound was reported to be a rhizobiocins by Roslycky [46, 47] who screened 136 strains of *Rhizobium* spp., belonging to various cross-inoculation groups for the production of bacteriocins. This researcher observed production of meliloticin by two strains of *R. meliloti,* trifolicin by three strains of *R. trifolii,* phaseolicin by 6 strains, japonicin by 13 strains, and lupinicin by three strains. Schwinghamer [54] screened 41 strains of *R. trifolii* and 270 isolates from clover nodules for intra-strain antagonism. This researcher observed 35% of the cultures produced antibiotics towards two indicator out of six strains used for intra-strain antagonism experiments, and 8% of the cultures were found to produce bacteriocin-like substances.

R. leguminosarum bv. *viceae* strain Z25, an inoculant used under field conditions, was reported to be one of the most effective isolates in antagonism experiments. Broad bacteriocinogenic activity on rhizobia was demonstrated by measuring nodule dry matter accumulation and total ARA (Acetylene-reducing activity) [45]. This strain exhibited such properties as non-dialysability, sensitivity to heat, and proteolytic enzymes, suggesting a proteinaceous nature. *Rhizobium* GR_4 in sterilized liquid broth and sterilized soil was observed to produce bacteriocins against the standard strain *Rhizobium* S24 of *Vigna* [1].

Bradyrhizobium and *Rhizobium* (bacteriocinogenic rhizobial strains) nodulating Green Gram and their antagonistic interaction with sensitive strains (*Rhizobium* strains VRF76) under co-growth conditions was reported by Goel *et al.*[17]. The effect of bacteriocin production on competitive ability for nodulation on Green Gram was studied by co-inoculation of a bacteriocin-producer strain (VRFlO and VRF76) with a sensitive strain under sterile conditions. Bacteriocin producer strain VRFlO had more nodules than sensitive strain VRF76, suggesting their role in nodulation competitiveness.

Rhizobium leguminosarum bv. *viciae* strain LC-31 produced medium-sized bacteriocins which inhibited some strains of *R leguminosarum* bv. *viciae* and *Agrobacterium* spp.. This bacteriocin-producer was among the ten strains of *Rhizobium*, *Bradyrhizobium* and *Agrobacterium*- used to study antagonism among mixtures of bacterial strains of *Rhizobiaceae* studied by Hafeez *et al.* [22]. A 50 KDa bacteriocin polypeptide was partially purified by chloroform and ammonium sulfate and assayed by SDS-PAGE.

Rhizobium isolated from rhizospheric soil of Horse Gram was reported to be bacteriocinogenic by Edulamundi *et al.* [11]. Thirty-two isolates produced bacteriocins against the remaining isolates. Two isolates, named HGR-4 and 9 exhibited the largest inhibition zone among all isolates. Bacteriocin production by *Rhizobium* from rhizosphere soil near non-leguminous plants (*Lupinus albus, Triticum aestivum and Zea mays*)also plays an important role in inter-specific competitions [3].

Two inhibitory strains of *Rhizobium* spp. STM 1081 and STM 1823 produced thermolabile bacteriocins. These bacteriocins were resistant to acidic pH, but weresensitive to basic pH, organic solvents, and proteolytic enzymes. The antimicrobial activity of these strains against soil bacteria *Clostridium* spp., *Vibrio* spp. and *Enterobacter* spp. was detected by Warda *et al.* [66].

Resistance to bacteriocins has also been reported. Lovisohn *et al.* [36] observed bacteriocinogenic properties and resistance of producer strains to their own bacteriocins. The resistance of the producer strains was not absolute, as certain strains were reported to be sensitive to a higher concentration of their own produced bacteriocins.

CLASSIFICATION OF *RHIZOBIUM* BACTERIOCINS

*Rhizobium*spp. produces bacteriocins, which have been grouped as small, medium and large based on their estimated sizes and diffusion characteristics [25, 53] (Table 1).

Fast-growing *Rhizobium* produce small-sized bacteriocins that were observed to be chloroform-soluble and heat-labile, and they are resistant to proteolytic enzymes and diffuse through cellophane. The presence of a bacteriocinogenic plasmid is detected in these *Rhizobium* spp. and is transmittable at frequencies of 10^{-1} to 10^{-2}. They bacteriocins have molecular masses of less than 2,000 daltons [25, 64, 53,

21]. They were shown to be acylated homoserine lactone (AHL) compounds related to quorum-sensing molecules [49, 20]. They are often called quorum-sensing pheromones and auto-inducers (induce genes encoding enzymes involved in their own synthesis). These bacteriocin diffuse rapidly in an agar plate containing sensitive bacteria and cause large inhibition zones.

A property of AHL molecules is growth inhibition of *Rhizobium* bacteria that harbor conjugative Sym plasmid pRL1JI. They play an important role as inducers of genes that are involved in cellularfunctions and in inter-cellular communication of Gram-negative bacteria [71]. Another small bacteriocin, which has been well-characterized in *R. leguminosarum* strain T24, is trifolitoxin [5, 52, 61, 62, 63]. This bacteriocin is a short peptide [5] similar to microcins and has been shown to be bacteriostatic to certain *Rhizobium* strains. It is used to enhance competitiveness of inoculant strains.

Table 1: Classification of bacteriocin on the basis of their size

S. No	Bacteriocin Category	Producer Species	Reference
1.	Small	*R. leguminosarum* *R. trifolii*	Schwinghamer and Brockwell [53], Gross and Vidaver [21], Hirsch [25], Van Brussel *et al.* [64], Gray *et al.* [20], Schripsema *et al.* [49], Goel *et al.* [17], Yajima *et al.* [71], Sridevi and Mallaiah [58]. Schwinghamer and Belkengren [52], Triplett and Barta [62], Triplett [60], Triplett [61].
2.	Medium	*R. leguminosarum* bv. *viciae* *R. japonicum*	Hirsch [25], Rodelas *et al.* [45], Oresnik *et al.* [40], Wisniewski [67], Hafeez *et al.* [21]. Roslycky [46]
3.	Large	*R. lupine*	Lotz and Mayer [35], Schwinghamer *et al.* [54]
4.	Bacteriocin like inhibitory substances (BLIS)	*R. trifolii* *R. japonicum*	Schwinghamer [50], Schwinghamer *et al.* [54], Schwinghamer [51], Schwinghamer and Brockwell [53], Joseph *et al.* [28], Hodgson *et al.* [26], Triplett and Barta [62], Warda *et al.* [66]. Gross and Vidaver [21]
5.	Rhizobiocins	*Rhizobium* spp.	Goel *et al.* [17], Sridevi and Mallaiah [58]

Comparatively, fewer strains produce medium-sized bacteriocins, suggested by cross-resistance patterns [25, 67]. Bacteriocins produced by *Rhizobium leguminosarum* bv *viciae* are generally bactericidal, heat labile and retained by cellophane. They are of intermediate size and have a sedimentation coefficient greater than 3.7S [25, 67]. These bacteriocins have shown different levels of sensitivity to proteases, trypsin, lysozymes, RNase I, and DNase I [51, 21, 26]. They have broad bacteriocinogenic activity. These bacteriocins can be produced in minimal or complete media, under a wide range of incubation temperatures using C and N as sole compounds. These bacteriocins produce a 2-10 mm wide inhibitory zone, which is more stable at alkaline pH [25, 45]. Roslycky [46] reported medium-sized bacteriocins to be true bacteriocins, i.e plasmid encoded. Oresnik *et al.*[40] detected bacteriocins similar to the RTX toxin, encoded on plasmid pRL1JI. Its protein nature is similar to the RTX protein (Calcium dependent cytolysins such as hemolysin and leukotoxin). Their bacteriocinogenic activity is enhanced by Ca^{2+} and the mode of action is via membrane depolarization [31].

Large-sized bacteriocins resemble defective bacteriophages of *Proteus mirabilis* and *Proteus vulgaris* [35, 40, 25, 53]. Electron microscopy of these bacteriocins revealed the presence of 123 nm long particles which resemble tails of T-even bacteriophages. The particles were not found attached to phage heads and were regarded as incomplete, thus they were named INCO particles [35, 54].

Rhizobium trifolii has been shown to produce bacteriocin-like inhibitory substances (BLISs) by Schwinghamer [50], Schwinghamer *et al.*[54], Schwinghamer [51], Schwinghamer and Brockwell [53], Joseph *et al.* [28] Hodgson *et al.* [26], Triplett and Barta [62], Warda *et al.* [66]. The bacteriocins of interest filtered through 0.01μm and did not produce plaques. Bacteriocins produced by these strains were in competition with soil bacteria (rhizobia and non-rhizobia) and were associated with soybeans [51]. *Rhizobium japonicum* has been shown to also produce BLIS by Gross and Vidaver [21]. These

particular BLIS passed through dialysis membranes and are resistant to heat and proteolytic enzymes. They produce inhibitory zones of 1 to 12 mm. They are sub-divided into the following categories: Group I, which has adoubling time of less than 14 min, have mucoid type growth, and a inhibitory zone of 2 to 9 mm, Group II, which has a doubling time and growth type similar to Group I, but has decreased culture viability compared with Groups I and III. Group III is easily distinguishable by their dry colony morphology, they exhibit long doubling time, but they lack bacteriocin production.

Bacteriocin produced by *Rhizobium* spp.. was termed as rhizobiocins by Goel *et al.* [17], and Sridevi and Mallaiah [5]. The bacteriocin production was detected after 48 hours of incubation and reached maximum levels after 96 hours of incubation. Bacteriocins produced by *Rhizobium* spp.. were observed to be sensitive to protease at concentration of 25 μgmL^{-1}, indicating its proteinaceous nature (Sridevi and Mallaiah 2008). Bacteriocins from *cicer-Rhizobium* were sensitive to protease and insensitive to DNAase and RNAase, indicating that they are not nucleoproteins (Nirmala *et al.* 2001). Bacteriocin activity was observed between 30 and 70°C [58].

STRUCTURE OF BACTERIOCINS AND THEIR MODE OF ACTION

The Gram-negative bacterium *Rhizobium leguminosarum* produces small bacteriocins, which form symbiotic nitrogen-fixing root nodules on leguminous plants. The structure was elucidated by Schripsema *et al.* [49] as N-(3R-hydroxy-7-cis-tetradecanoyl) L-homoserine lactone through inverse-detected carbon proton correlation spectrum methods. The spectrum displayed almost all expected signals due to two or three-bond couplings. The structure contains two asymmetric carbon atoms, one of which is in the homoserine lactone motif, which can be of D or L configuration (R or S, respectively). The other asymmetric carbon bears the ß-hydroxyl moiety in the fatty acid chain. Any of four possible stereoisomers or a mixture any of the four are possible. The configuration of both asymmetric carbon atoms was determined by the chiral solvating agents S-(1) and R-(2)-2,2,2-trifluoro-1-(9-anthryl)-ethanol.

R. leguminosarum 248 has been reported to produce medium-sized bacteriocins by Oresnik *et al.* [40]. Characterization of the pRL1JI plasmid indicated that there is only gene present, and expression of the gene reveals bacteriocin activity. Medium-sized bacteriocin is related to RTX-type proteins, which include calcium-dependent cytolysins, such as hemolysin and leukotoxin (Welch 1990). It contains a peptide sequence repeated up to 18 times within the protein. Additionally, a novel 19 to 25 amino acid motif was detected every 130 amino acids. Bacteriocin activity in the culture supernatants was associated with the presence of approximately a 100 kDa protein, which exhibited calcium dependence in *R. leguminosarum.* Nodulation competition experiments with *R. leguminosarum* wild types and strain 248 show the presence of bacteriocin activity in strain 248, which corresponds to its competitiveness. The RTX families of toxins are pore-forming cytolysins [70] that cause membrane depolarization [31]. The protein is produced in culture supernatants and there is no specific transport system. Bacteriocins are secreted by a general pathway and released by spontaneous cell lysis. The secretion and transportation mechanism of Rhizobiocin 248 are unclear.

Electron microscopy performed by Lotz and Mayer [35] on the large-sized bacteriocins of *Rhizobium lupini* revealed the presence of 123 nm long particles which resemble the tails of T-even bacteriophages. The particles were not found attached to phage heads. They were regarded as incomplete and were accordingly named INCO particles. INCO particles comprise a core, enveloped by a contractile sheath. One end of the sheath is attached to a base plate, to which six fibers (32 nm in length) are attached. These fibers connect the base plate of an adsorbing particle to the cell surface. As INCO particle cores are empty, specific adsorption of the particles to the bacterial surface is sufficient to irreversibly inactivate sensitive cells. Dimensions of INCO particles with an (a) extended and (b) contracted sheath are given in nanometers. INCO particle fibers are folded up against the extended sheath and its adsorption is triggered by a specific contact between the base plate of a particle and the cell surface, leading to structural changes of the plate. These structural changes of the base plate result in the downward orientation of the fibers, which attach to specific receptors of the cell wall. The base plate is then bound to the cell surface by the fibers. This contraction results in the penetration of the cell wall by the INCO core. The INCO particles possess bactericidal activity that inactivates the cells upon their contact with the bacterial surface.

GENETICS OF BACTERIOCIN PRODUCTION

Bacteriocins are agents encoded by genetic material located on plasmids, with the purpose of killing or inhibiting different or closely related species [7]. The genes encoding bacteriocin production and immunity are organized in epichromosomal operon clusters. However, certain genes are encoded on chromosomes [8, 38, 48].

Frederiq and Botz-Bareau [13, 14] initially suggested that the location of genetic elements that encode bacteriocins genes resides in an extra chromosomal genetic element. This conclusion was dependent on genetic analysis of various *E. coli* F^+ x F^- mating experiments, in which there were no linkages between any of the several chromosomal markers and the determinants of colicin production. Similar conclusions were made by transferring bacteriocinogenic determinants in *E. coli* by ^{14}C- labeled DNA [57]. The direct demonstration that the colicin determinant resided on a plasmid was also reported by Dewitt and Helenski [10].

Treatment with sodium dodecyl sulphate (SDS) eliminated bacteriocin production from *Rhizobium trifolii* strain B1 [72]. Conjugal transfer of bacteriocinogenic determinants showed that these determinants were self-transmissible at frequencies of 10^{-1} to 10^{-2} [25]. This finding indicated that bacteriocin production in *Rhizobium* is controlled by gene expression from plasmid DNA. These plasmids mobilized chromosomal genes at the frequencies of 10^{-7} to 10^{-8}, which further suggested that these plasmids were conjugative [25].

High frequency production of medium-sized bacteriocins from three different *Rhizobium leguminosarum* strains was demonstrated by Hirsch, [25]. Three bacteriocinogenic plasmids found in field isolates 248, 306 and 309 were named pRL1JI, pRL3JI and pRL4JI, respectively. Hirsch [25] and Brewin *et al.* [6] stated that these plasmids encode medium-sized bacteriocins and repress the production of small bacteriocins. These researchers have the ability of transfer between strains at high frequencies and mobilize chromosomal genes at low frequencies and cannot co-exist within the same cell without experiencing inter-plasmid recombination. Hirsch [25] observed a loss of ability to produce small bacteriocins due to repressive activity by trans-conjugated plasmids.

High frequencies of medium-sized bacteriocin production are attributed to conjugative bacteriocinogenic plasmids. No transfer regarding the ability to produce small bacteriocins was observed. Loss of production of small bacteriocins upon introduction of medium-sized bacteriocin plasmids was detected by Hirsch [25] which was attributed to the incompatibility between plasmids. Plasmid pRL1JI was found to carry symbiotic gene functions, capable of restoring mutations to a wild-type phenotype [6].

APPLICATIONS OF BACTERIOCIN

As stated earlier, bacteriocins are highly specific anti-bacterial proteins produced by strains of bacteria that are active against different strains of the same or related species [16]. Bacteriocin production plays an important role in inter-specific and intra-specific competition [11, 3, 66]. This has implications in the utilization of multiple strains or species to be used as bio-fertilizer. Bacteriocin-producing rhizobial strains have a competitive advantage as described in mixed culture [53]. Several innovative approaches were suggested by Schwinghamer and Belkengren [52], Hodgson *et al.* [26] and Triplett [61] to maximize nitrogen fixation by (1) using an inoculum consisting of a bacteriocin-resistant nitrogen fixing strains (2) using a bacteriocin producer which also fixes nitrogen, and (3) transfer of the genetic components for bacteriocin production to an efficient nitrogen fixer. Mixed cultures helped to improve legume inoculant potency, competitiveness and survival after inoculation [66]. *Rhizobium leguminosarum* bv. *viciae* strain LC-31 used as phosphate solubilizer bacteria (PSB), is added in bio-fertilizer, and inhibits the growth of *Rhizobium and Agrobacterium* spp.. [22]. *Rhizobium leguminosarum* produces a RTX-type toxin of approximately 100KDa which provides a competitive advantage in terms of nodule occupancy. Various studies have confirmed the role of Trifolitoxin, peptide bacteriocins produced by Gram-negative bacteria in competition with closely related species [44 24].

Bacteriocins are of interest as they affect bacterial population dynamics, survival and virulence [43, 15]. Certain bacteriocins have additional regulatory functions [23, 12, 34, 30]. Bacteriocins are exploited for their use to combat bacterial infections, e.g., in plant disease control caused by bacteria of the same or related groups [37]. Some bacteria associated with plants produce large bacteriocins that inhibit plant pathogenic bacteria [41]. Serracin, a phage tail-like bacteriocin was reported to inhibit *Erwinia amylovora*, the fire blight pathogen [27]. Lavermicocca *et al.* [33] reported olive-knot disease reduction by *Pseudomonas syringae* pv. *syringae* bacteriocins. *Xanthomonas campestris* pv.glycines produce glycinecin A, a bacteriocin that demonstrates bactericidal activity on phytopathogenic *Xanthomonas campestris* pv. vesicatoria [42].

FUTURE PROJECTIONS

Bacteriocins cause antagonism among closely related bacterial species and their production plays an important role in inter-specific and intra-specific competition. Bacteriocinogenic *Rhizobium* spp. alone or in different combinations has implications in the sustainability of agriculture, including their possible use as a cheap biofertilizer. These strains can be used to enhance competitiveness and survival after

inoculation. Bacteriocins produced by Gram-positive bacteria have yielded promising results as food bio-preservatives, exhibiting relatively narrow activity spectra. However, the scientific community is still in the initial stages of exploring bacteriocins produced by Gram-negative *Rhizobium*spp.. Manipulation of genetic determinants can pave the way for greater bacteriocin production. The synthetic routes have provided a hurdle in the widespread usage of bacteriocins, as it is costly to obtain them in purified form. Fermentative production of bacteriocins in natural media can provide economically feasible products. Information about structure can be used to determine the mode of action of bacteriocins.

Bacteriocinogenic *Rhizobium,* usedalone or in mixtures, can contribute to agriculture's need for more sustainable, effective and cheap bio-fertilizer production, not only as nitrogen fixers but also as bio-control agents. They can be used as a tool for plant disease control and potentially address current threats posed by multi-resistance bacterial pathogens. Bacteriocinogenic *Rhizobium* spp.may also be used in conjuction with transgenic crop plants that have nitrogen fixing and biocontrol capabilities.

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REFERENCES

1. Ahlawat, O.P., Sharma, H.R. &Dadarwal, K.R. (1992) Bacteriocin producing mutants of cowpea miscellany *Rhizobium*- role in strain competition. Ind. J. Microbiol.,32(3): 263-268.
2. Ahlawat, O.P. & Dadarwal, K.R. (1996) Bacteriocin production by *Rhizobium* species *cicer*and its role in nodule competence. Ind. J. Microbiol.,36:17-23.
3. Ambika, R., Kavitha, P., Panneerselvam, A. & Sengottaian, N. (2014) Production of bacteriocin by *Rhizobium* isolated from rhizosphere soil of maize in Lalgudi Taluk, Trichy district, Tamil Nadu, India. Inter. J. Curr. Res.6: 5346-5348.
4. Balciunas, E.M., Martinez, F.A.C., Todorov, S.D., Franco, B.D.G.M., Converti, A. & Oliveira, R.P.S. (2013) Novel biotechnological applications of bacteriocins: A review. Food Control 32: 134-142.
5. Breil, B.T., Ludden, P.W. & Triplett, E.W. (1993) DNA sequence and mutational analysis of genes involved in the production and resistance of the antibiotic peptide trifolitoxin. J. Bacteriol.,175: 3693-3702.
6. Brewin, N., Beringer, E.J., Buchanan-Wollaston, A.V., Johnston, A.W.B. & Hirsch, P.R. (1980) Transfer of symbiotic genes with bacteriocinogenic plasmids in *Rhizobium leguminosarum*. J. Gen. Microbiol.,116: 261-270.
7. Butt, A.M., Khan, I.B., Haq, F. & Tong, Y. (2011) De novo structural modeling and computational sequence analysis of a bacteriocin protein isolated from *Rhizobium leguminosarum bv. viciae* strain LC-31. Afr. J. Biotech.,10: 7381-7388.
8. Chassy, B., Hlywka, J.J., Kleter, G.A., Kok, E.J., Kuiper, H.A., McGloughlin, M., Munro, I.C., Phipps, R.H., Reid, J.E., Stein, J. & Zabik, J. (2005) Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology. Food Nut. Bul.,26: 436-442.
9. Chen, H. & Hoover, D.G. (2003) Bacteriocins and their food science and food safety. Comp. Rev. Food Sci. Food Safety.,2: 82-100.
10. Dewitt, W. & Helenski D. R. (1965) Characterization of colicinogenic factor E1 from a non-induced and a Mitomycin C induced *Proteus* strain. J. Mol. Bio.,13: 692-703.
11. Edulamudi, P., Masilamani, A.J.A., Divi, V.R.S.G. & Konada, V.M. (2011) Production bacteriocin by rhizobia obtained from root nodules of *Macrotyloma uniflorum* (Lam,) Verdc. (Horse Gram). Ban. J. Microbiol.,28: 76-79.
12. Eijsink, V.G.H., Axelsson, L., Diep, D.B., Havarstein, L.S., Holo, H. & Nes, I.F. (2002) Production of class II bacteriocins by lactic acid bacteria; an example of biological warfare and communication. Antonie Van Leeuwen Inter. J. Gen. Mol. Microbiol.,81:639-654.
13. Frederiq, P. & Betz-Bareau, M. (1953a) Transfért génétiqué de la propriété de produire un antibiotique. Comptes. Rendus des Seances de la Societe de Biologie et de ses Filiales147: 1653.
14. Frederiq, P. & Betz-Bareau, M. (1953b) Transfert génétique de la propriété colicino-gène en rapport avec la polarité F des parents. Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 147: 2043.
15. Gardner A, West, S.A. & Buckling, A. (2004) Bacteriocins, spite and virulence. Proceed. Royal Soc. Bio. Sci.,271: 1529-1535.
16. Gaur, Y.K., Narayan, S., Chauhan & Ali, A. (2004) Bacteriocinogeny: concept, nomenclature, prevalence and application. Ind. J Microbiol.,44: 1-30.
17. Goel, A.K., Sindhu, S.S. & Dadarwal K.R. (1999) Bacteriocin-producing native rhizobia of Green Gram (*Vigna radiata*) having competitive advantage in nodule occupancy. Microbiol Res.,154: 43-48.
18. Gordon, D.M. & O'Brien, C.L. (2006) Bacteriocin diversity and the frequency of multiple bacteriocin production in *Escherichia coli*.Microbiol., 152: 3239-3244.
19. Gratia, A. (1925) Sur un remarquable example d'antagonisme entre deux souches de colibacille. Comptes Rendus Bio.,93: 1040-1042.

20. Gray, K.M., Pearson, J.P., Downie, J.A., Boboye, B.E.A. & Greenberg, E.P. (1996) Cell-to-cell signalling in the symbiotic nitrogen fixing bacterium *Rhizobium leguminosarum*: autoinduction of stationary phase and rhizosphere-expressed genes. J. Bacteriol.,187: 372-376.
21. Gross, D.C. & Vidaver, A. K. (1978) Bacteriocin-like substance produced by *Rhizobium japonicum* and other slowgrowing rhizobia. Appl. Environ. Microbiol., 36: 936-943.
22. Hafeez, F.Y., Naeem, F.I., Naeem, R., Zaidi, A.H. & Maalik, K.A. (2005) Symbiotic effectiveness and bacteriocin production by *Rhizobium leguminosarum* bv. *viciae* isolated from agriculture soil in Faisalabad. Env. Exper. Bot.,54: 142-147.
23. Hauge, H.H., Mantzilas, D., Moll, G.N., Konings, W.N., Driessen, A.J.M., Eijsink, V.G.H. & Nissen-Meyer, J. (1998) Plantaricin A is an amphiphilic alpha-helical bacteriocin-like pheromone which exerts antimicrobial and pheromone activities through different mechanisms. Biochem.,37: 16026-16032.
24. Herlache, T.C. & Triplett, E.W. (2002) Expression of a crown gall biological control phenotype in an avirulent strain of *Agrobacterium vitis* by addition of the trifolitoxin production and resistance genes. Bio Med Central Biotech.,2: 2.
25. Hirsch, P.R. (1979) Plasmid determined bacteriocin production by *Rhizobium leguminosarum*J. Gen. Microbiol.,113: 219-228.
26. Hodgson, A.L.M., Roberts, W.P. & Waid, J.S. (1985) Regulated nodulation of *Trifolium subterraneum* inoculated with bacteriocin producing strains of *Rhizobium trifolii*. Soil Bio. Biochem.,20: 19-24.
27. Jabrane, A., Sabri, A., Compere, P., Jacques, P., Vandenberghe, I., Van Beeumen, J. & Thonart, P. (2002) Characterization of serracin P, a phage-tail-like bacteriocin, and its activity against *Erwinia amylovora*, the fire blight pathogen. App. Env. Microbiol.,68: 5704-5710.
28. Joseph, M.V., Desai, D.J. & Desai, A.J. (1983) Production of antimicrobial and bacteriocin-like substance by *Rhizobium trifolii*. App Environ. Microbiol.,45: 532-535.
29. Joseph, M.V., Desai, D.J. & Desai, A.J. (1985) Possible involvement of phage like structure in antagonism of cowpea rhizobia by *Rhizobium trifolii*. App. Env. Microbiol.,49: 459-468.
30. [30]. Kodani, S., Lodato, M.A., Durrant, M.C., Picart, F. & Willey, J.M. (2005) SapT, a lanthionine-containing peptide involved in aerial hyphae formation in the *Streptomycetes*. Mol. Microbiol.,58: 1368-1380.
31. Konisky, J. (1982) Colicins and other bacteriocins with established modes of action.Ann. Rev. Microbiol., 36: 125-144.
32. Lally, E.T., Hill, R.B., Kieba, I.R. & Korostoff, J. (1999) The interaction between RTX toxins and target cells. Trend. Microbiol.,7: 356-361.
33. Lavermicocca, P., Lonigro, S.L., Valerio, F, Evidente, A. & Visconti, A. (2002) Reduction of olive knot diseaseby bacteriocin from *Pseudomonas syringae* pv. *ciccaronei*. App. Env. Microbiol.,68: 1403-1407.
34. Linares, J.F., Gustafsson, I., Baquero, F. & Martinez, J.L. (2006) Antibiotics as intermicrobial signaling agents instead of weapons. Proceed. Nation. Acad. Sci. USA103: 19484-19489.
35. Lotz, W. & Mayer, F. (1972) Isolation and characterization of a bacteriophage tail like bacteriocin from a strain of *Rhizobium*.J Virology.,9: 160-173.
36. Lovisohn, R., Konisky, J. & Nomura, M. (1968) Interaction of colicins with bacterial cells IV. Immunity breakdown studied with colicins Ia and Ib. J. Bacteriol.,96: 811-821.
37. Montesinos, E. (2007) Antimicrobial peptides and plant disease control. FEMS Microbiol. Letters.,270: 1-11.
38. Nes, I.F., Diep, D.B., Havarstein, L.S., Brurberg, M.B., Eijsink, V. & Holo, H. (1996) Biosynthesis of bacteriocins in lactic acid bacteria. Ant Van Leeuwen.,70: 113-128
39. Nirmala, J., Gaur Y.D. & Lawrence P.K. (2001) Isolation and characterization of a bacteriocin by *cicer Rhizobium*. World J. Microbiol. Biotechnol.,17:795-799.
40. Oresnik, I.J., Twelker, S. & Hynes, M.F. (1999) Cloning and characterization of *Rhizobium leguminosarum* gene encoding a bacteriocin with similarities to RTX toxin. App. Env. Microbiol.,65: 2833-2840.
41. Parret, A.H., Temmerman, K. & De Mot, R. (2005) Novel lectin-like bacteriocins of biocontrol strain *Pseudomonas fluorescens* Pf-5. Appl. Env. Microbiol.,71: 5197-5207.
42. Pham, H.T., Riu, K.Z., Jang, K.M., Cho, S.K. & Cho, M. (2004) Bactericidal activity of glycinecin A, a bacteriocin derived from *Xanthomonas campestris* pv. *glycines*, on phytopathogenic *Xanthomonas campestris* pv. vesicatoria cells. App. Env. Microbiol.,70: 4486-4490.
43. Riley, M.A. & Wertz J.E. (2002) Bacteriocin diversity: ecological and evolutionary perspectives. Biochimie.,84: 357-364.
44. Robleto, E. A., Kmiecek, K., Oplinger, E.S., Nienhuis, J. & Triplett, E.W. (1998) Trifolitoxin production increases nodulation competitiveness of *Rhizobium etli* CE3 under agricultural conditions. Appl. Env. Microbiol.,64: 2630-2633.
45. Rodelas, B., Gonzalez-Lopez, J., Salmeron, V., Martinez-Toledo, M.V. & Pozo, C. (1998) Symbiotic effectiveness and bacteriocin production by *Rhizobium leguminosarum* bv.*viceae* isolated from agricultural soils in Spain.Appl. Soil Eco.,8: 51-60.
46. Roslycky, E.B. (1965) Lysogeny in the rhizobia bacteria. *Canadian Society of Microbiology*15th Ann. Meeting, 47.
47. Roslycky, E.B. (1967) Bacteriocin production in the rhizobia bacteria. Canadian. J. Microbiol.,13: 431-432.
48. [49]. Sahl, H.G. & Bierbaum, G. (1998) Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from Gram-positive bacteria. Ann. Rev. Microbiol.,52: 41-79.

49. Schripsema, J., De Rudder, K.E.E., Van Vliet, T.B., Lankhorst, P.P., De Vroom, E., Kijne, J.W. & Van Brussel, A.A.N. (1996) Bacteriocin small of *Rhizobium leguminosarum* belongs to the class of N-acyl-L-homoserine lactone molecules, known as autoinducers and as quorum sensing co-transcription factors. J Bacteriol.,178: 366-371.
50. Schwinghamer, E. A. (1971) Antagonism between strains of *R. trifolii* in culture. Soil Bio Biochem.,3, 355-363.
51. Schwinghamer, E.A. (1975) Properties of some bacteriocins produced by *Rhizobium trifolii*.J. Gen. Microbiol.,91: 403-413.
52. Schwinghamer, E. A. & Belkengren, R.P. (1968) Inhibition of rhizobia by a strain of *R. trifolii*. Some properties of the antibiotic and of the strain.Arch. Microbiol.,64: 130-145.
53. Schwinghamer, E.A. & Brockwell, J. (1978) Competitive advantage of bacteriocin and phage-producing strains of *Rhizobium trifolii* in mixed culture. Soil Bio. Biochem.,10: 383-387.
54. Schwinghamer, E. A., Pankhurst, C. E. & Whitfeld, P. R. (1973) A phage-like bacteriocin of *R. trifolii*. Can. J. Microbiol.,19: 359-368.
55. Schwinghamer, E. A. & Reinhardt, D.J. (1963) Lysogeny in *Rhizobium leguminosarum* and *R. trifolii*. Aus. J. Bio. Sci.,16: 597-605.
56. Scupham, A.J. & Triplett, E.W. (2006) Determination of the amino acid residues required for the activity of the anti-rhizobial peptide antibiotic trifolitoxin. J. App. Microbiol.,100: 500-507.
57. Silver, S. & Ozeki, H. (1962) Transfer of deoxyribonucleic acid accompanying the transmission of colicinogenic properties by cell mating. Nature195: 873-874.
58. Sridevi, M. & Mallaiah, K.V. (2008) Production of bacteriocins by root nodule bacteria. International J. Agri. Res.,3:161-165.
59. Tagg, J.R., Dajani, A. & Wannanaker, L.W. (1976) Bacteriocins of Gram positive bacteria. Bacteriol. Rev.,40: 722-756.
60. Thakar, A.J., Dube, H.C. & Patel, R.J. (1990) Bacteriocin-dependent inhibition of *Rhizobium meliloti* strains in mixed cultures. Ind. J. Exp. Bio.,28: 55-57.
61. Triplett, E.W. (1988) Isolation of genes involved in nodulation competitiveness from *Rhizobium leguminosarum* bv. *trifolii* T24. Pro. Nat. Acad. Sci.,85: 3810-3814.
62. Triplett, E.W. (1990) Construction of a symbiotically effective strain of *Rhizobium leguminosarum* bv. *trifolii* with increased nodulation competitiveness. Appl. Environ. Microbiol.56: 98-103.
63. Triplett, E. W. & Barta, T. M. (1987) Trifolitoxin production and nodulation are necessary for the expression of superior nodulation competitiveness by *Rhizobium leguminosarum* bv. *trifolii* strain T24 on clover. Plant Physiol.,85: 335-342.
64. Van-Brussel, A.A.N., Zaat, S.A.J., Wijffelman, C.A., Pees, E. & Lugtenberg, B.J.J. (1985) Small bacteriocins of fast growing rhizobia is chloroform soluble and is not required for effective nodulation. J. Bacteriol., 162: 1079-108.
65. Van Sluys, M.A., Monteiro-Vitorello, C.B., Camargo, L.E., Menck, C.F., Da Silva, A.C., Ferro, J.A., Oliveira, M.C., Setubal, J.C., Kitajima, J.P. &Simpson, A.J., (2002) Comparative genomic analysis of plant-associated bacteria. Ann. Rev. Phyto.,40:169-189.
66. Warda, A., Zoubida, B., Faiza, B.Z., Yamina, A. & Bekki, A. (2014) Selection and characterization of inhibitor agents (bacteriocin like) produced by rhizobial strains associated to Medicago in western Algeria. Int. J. Agri. Crop Sci.,7:393-401.
67. Wijffelman, C.A., Pees, E., Van-Brussel, A.A.N. & Hooykaas, P.J.J. (1983) Repression of small bacteriocin excretion in *Rhizobium leguminosarum* and *Rhizobium trifolii* by transmissible plasmids. Mol. Microbiol.,192: 171-176.
68. Wilson, R.A., Handley, B.A. & Beringer, J.E. (1998) Bacteriocin production and resistance in a field population of *Rhizobium leguminosarum* biovar *viciae*. Soil Bio. Biochem., 30: 413-417.
69. Wisniewski-Dyé, F. & Downie, J.A. (2002) Quorum-sensing in *Rhizobium*. Antonie van Leeuwenhoek81: 397-407.
70. Welch, R. A. (1990) Pore-forming cytolysins of Gram negative bacteria.Mol. Microbiol.,5: 521-528.
71. Yajima, A., Van Brussel, A.A.N., Schripsema, J., Nukada, T. & Yabuta, G. (2008) Synthesis and stereochemistry activity relationship of small bacteriocin, an autoinducer of the symbiotic nitrogen-fixing bacterium *Rhizobium leguminosarum.* Organic letters10:2047-2050.
72. Zolazane-Kowalska, I. (1979) Bacteriocinogeny of *Rhizobium trifolii.* Acta Microbiologia Polonica28(1): 39-45.