Chapter 1: Bacterial Aggregation Assay in the Presence of Cyclic

A lipopeptide is a molecule consisting of a lipid connected to a peptide. Bacteria express these molecules. They are able to self-assemble into different structures. Certain lipopeptides are used as antibiotics.

Methanol Sinopharm Chemical Reagent, catalog number: LP Agar powder Solarbio, catalog number: PN Autoclave sterilizer Zealway Instrument, model: H Biological safety cabinet Heal Force, model: Staphylococcus aureus and B. Aggregation assay of V. Prepare six sterilized 15 ml culture tubes. Add 3 ml bacterial suspension into each tube. Experimental design of V. Schematic representation of the steps in aggregation assay using well plates No. After incubation, discard the planktonic cells carefully with a pipettor and wash the aggregated cells in each well three times with sterile saline. Operate the other procedures as described above in Steps C4-C9 Figure 2. Quantitative cell aggregation assay at different concentrations of CLPs Scanning electron microscope observation Dilute overnight cultured cell of V. Centrifuge the cells at 1, x g for 4 min at room temperature, and resuspend the cells with a sterilized saline solution. Dry coverslips at room temperature. Wash with sterile saline solution see Recipes. Incubate the samples for 10 min in each grade ethanol solution. Observe the samples under a scanning electron microscope Figure 3. SEM images of V. Transmission electron microscope observation Perform the procedures as described above in Steps E1-E3. Drop samples on copper grids, and dried at room temperature. Observe the samples under a transmission electron microscope Figure 4. TEM images of V. Operate the other procedures as described above in Steps C2-C9 Figure 5. Action spectrum assays of CLPs. Notes To avoid other bacteria contamination, microbial operations must be carried out under aseptic conditions. All bacterial mediums and reagent solutions are sure to be prepared freshly and sterilized by autoclaving. It is necessary to homogenize the cell suspension with CLPs before incubation. Notice that the operation in discarding the planktonic cells and washing the aggregated cells with pipettor must be careful and gentle. Recipes Saline LB broth 1 L 10 g peptone.
Chapter 2 : Activity Booster: Lipopeptide

Cyclic lipopeptides, Cyclic lipopeptide antibiotic (product), Cyclic lipopeptide antibiotic (substance), Cyclic lipopeptide antibiotic, Cyclic lipopeptides (product), Cyclic lipopeptides (substance) Derived from the NIH UMLS (Unified Medical Language System).

The authors have declared that no competing interests exist. Conceived and designed the experiments: Received Feb 8; Accepted Mar This article has been cited by other articles in PMC. Abstract The rhizosphere isolate Pseudomonas putida BW11M1 produces a mixture of cyclic lipopeptide congeners, designated xantholysins. Properties of the major compound xantholysin A, shared with several other Pseudomonas lipopeptides, include antifungal activity and toxicity to Gram-positive bacteria, a supportive role in biofilm formation, and facilitation of surface colonization through swarming. The lipotetradecadepsipeptides are assembled by XtlA, XtlB and XtlC, three co-linearly operating non-ribosomal peptide synthetases NRPSs displaying similarity in modular architecture with the entolysin-producing enzymes of the entomopathogenic Pseudomonas entomaphila L. Relaxed fatty acid specificity during lipoinitiation by XtlA acylation with 3-hydroxydodecenoate instead of 3-hydroxydecanoate and for incorporation of the ultimate amino acid by XtlC valine instead of isoleucine account for the production of the minor structural variants xantholysin C and B, respectively. Remarkably, the genetic backbones of the xantholysin and entolysin NRPS systems also bear pronounced phylogenetic similarity to those of the P. This similarity includes the linked genes encoding the cognate LuxR-family regulator and tripartite export system components in addition to individual modules of the NRPS enzymes, and probably reflects a common evolutionary origin. Phylogenetic scrutiny of the modules used for selective amino acid activation by these synthetases indicates that bacteria such as pseudomonads recruit and reshuffle individual biosynthetic units and blocks thereof to engineer reorganized or novel NRPS assembly lines for diversified synthesis of lipopeptides. Introduction Certain genera of soil-dwelling and plant-associated bacteria, such as Streptomycetes, Myxobacteria, Bacilli, Pseudomonads, and Burkholderia, display a highly versatile secondary metabolism and, hence, have proven valuable sources for structurally very diverse metabolites with useful biological activities, including antibiotics. Whereas Streptomycetes and other actinomycetes are major producers of clinical antibiotics, several antimicrobials from other soil bacteria, such as Bacillus and Pseudomonas, contribute to their capacity to suppress fungal plant diseases [1]. Such biocontrol effect on phytopathogenic fungi by Pseudomonas strains has been ascribed to in situ production of structurally diverse molecules: Together with Bacillus, Pseudomonas are prominent producers of lipopeptides with a range of different biological activities: Based on similarities in peptide length ranging from 8 to 25 residues and amino acid sequence, many of the lipopeptides produced by Pseudomonas strains can be assigned to specific groups, each named after a prototype compound: The largest group with six subtypes is represented by viscosin and its analogs massetolide, viscosinamide, pseudodesmin, pseudophomin, and WLIP white line-inducing principle. Quite often a particular Pseudomonas strains can produce variants of a particular lipopeptide by attaching a different fatty acid or by incorporation of a similar amino acid at a certain position due to relaxed substrate specificity of the biosynthetic enzymes. With the exception of syringafactins and its analogs, cichofactins [11], all of these molecules are cyclic lipopeptides, as they contain a macrocyclic lactone ring of variable size formed between the carboxyterminal residue of the peptide and an internal amino acid serine or threonine. The Pseudomonas biosynthetic genes for syringomycin, syringopeptin, syringafactin, arthrofactin, viscosin, orfamide, massetolide, putisolvin, entolysin, WLIP, and cichofactin have been described [5], [8] â€” [18]. The encoded nonribosomal peptide synthetases NRPSs are composed of multiple modules, each consisting of an adenylation A domain responsible for amino acid selection and activation, a thiolation T domain responsible for thioesterification of the activated substrate, and a condensation C domain that catalyzes peptide bond formation between two amino acids. The starter condensation domain in the initiating enzyme C1 catalyzes acylation of the first amino acid, thereby linking the lipid moiety to the oligopeptide [19], [20]. This indicates that the order and number of the NRPS modules are co-linear to the amino acid sequence of the peptide product [5], [21]. Another characteristic is the frequent
occurrence of a tandem thioesterase TE domain in the terminating NRPS domain required for release, usually with concomitant cyclization, of the mature peptide product [6]. Multiple D-configured amino acids have been identified in Pseudomonas lipopeptides with known stereochemistry, but the corresponding synthetases lack standalone epimerization E domains, as found in for instance Bacillus lipopeptide NRPSs [6] or Pseudomonas pyoverdine synthetases [22]. Several Pseudomonas lipopeptides are harmful to fungi and oomycetes but their antibacterial activities are mostly confined to Gram-positive bacteria, while in general Gram-negative bacteria seem to be better protected by their different cell envelope architecture with a surface-exposed outer membrane [24]. In a recent survey, three of its species were ranked among the top ten pathogenic bacteria of scientific and economic interest [28]. This report describes the characterization of Xanthomonas-antagonistic activity of Pseudomonas putida BW11M1, a strain isolated from banana rhizosphere in Sri Lanka, revealing the involvement of a new type of lipopeptide. Results and Discussion Xanthomonad-inhibitory Activity of P. Using an agar diffusion assay, spotted BW11M1 cells produced a growth inhibition halo with pathovars of different Xanthomonas species as indicator overlay Fig. The growth inhibitory pattern for some of these strains, such as X. As strain BW11M1 is capable of killing pseudomonads by production of the lectin-like toxin LlpA [34], it was verified whether this bacteriocin might also be responsible for the observed growth inhibition of xanthomonads, phylogenetic relatives of pseudomonads. However, the latter antagonism was not affected in a BW11M1 llpA mutant [35] and an Escherichia coli strain producing recombinant LlpA [35] displayed no activity against Xanthomonas species, demonstrating LlpA not to be involved data not shown. Furthermore, halo formation was not abolished by a non-specific protease spotted close to BW11M1 producer cells, pointing to a non-proteinaceous nature of the molecule causing halo formation in the lawn of Xanthomonas cells data not shown.
Chapter 3 : Lipopeptides (e.g. daptomycin) - GlobalRPH

Cyclic lipopeptides (CLPs) are a promising class of natural products with antibiotic properties. CLPs are amphiphilic molecules, composed of a fatty acid tail linked to a short oligopeptide which form a macrocyclic ring structure.

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Abstract A lot of crops are destroyed by the phytopathogens such as fungi, bacteria, and yeast leading to economic losses to the farmers. Members of the Bacillus genus are considered as the factories for the production of biologically active molecules that are potential inhibitors of growth of phytopathogens. Plant diseases constitute an emerging threat to global food security. Many of the currently available antimicrobial agents for agriculture are highly toxic and nonbiodegradable and thus cause extended environmental pollution. Moreover, an increasing number of phytopathogens have developed resistance to antimicrobial agents. The lipopeptides have been tried as potent versatile weapons to deal with a variety of phytopathogens. All the three families of Bacillus lipopeptides, namely, Surfactins, Iturins and Fengycins, have been explored for their antagonistic activities towards a wide range of phytopathogens including bacteria, fungi, and oomycetes. Iturin and Fengycin have antifungal activities, while Surfactin has broad range of potent antibacterial activities and this has also been used as larvicidal agent. Interestingly, lipopeptides being the molecules of biological origin are environmentally acceptable. Introduction Extensive use of chemicals to control plant diseases has disturbed the ecological balance of microbes inhabiting soil leading to development of resistant strains of pathogens, groundwater contamination, and obvious health risks to humans. One of the biggest ecological challenges being faced by the microbiologists and plant pathologists in the future is the development of environmental friendly alternatives to the currently used chemical pesticides for combating a variety of crop diseases [1]. The continuous increasing drug resistance seen in bacteria has prompted a pressing need to find out some alternative antimicrobial molecules like lipopeptides to be used for clinical applications as well as in food preservation and dairy products [2]. Demand of lipopeptides is also surging due to their utility in human welfare, too. Lipopeptides were approved in the USA as antibiotics in the year Daptomycin, the first cyclic lipopeptide antibiotic was approved in USA by Food and Drug Administration FDA for the treatment of serious blood and skin infections caused by certain Gram-positive microorganisms [3]. Members of the Bacillus genus are considered as efficient microbial factories for the large scale production of such type of bioactive molecules [4,5]. In the context of biological control of plant diseases, the three families of Bacillus lipopeptides, that is, Surfactin, Iturin, and Fengycin, were studied for their potent antagonistic activities against various phytopathogens [1]. Therefore, these compounds are widely considered as potential alternatives to the growing problem of resistance to the conventional antibiotics, fungal infections, and life-threatening diseases. Generally, bactericidal activity of the lipopeptide increases with the addition of a lipid moiety of appropriate length typically C10â€“C12 and lipopeptides containing higher carbon atoms, such as 14 or 16, in lipid tail exhibit enhanced antifungal activity in addition to antibacterial activity [2]. Actinobacteria are species of the genus Streptomyces that has been reported to produce diverse antimicrobial lipopeptides with their applications in pharmaceutical industries [6]. Another lipopeptide Polymyxin interacts with an indispensable bacterial outer membrane component lipopolysaccharide LPS. Polymyxin binds to LPS in Gram negative bacteria by electrostatic interaction by involving its N-terminal fatty acid tail that leads to its bactericidal action because of inhibition of synthesis of outer membrane [7]. Synthetic lipopeptides are widely used as vaccine adjuvants to enhance immune response, but bacterial derived recombinant lipopeptide, such as Lipo-Nter, is a novel adjuvant that can be used to induce superior antitumor effects as compared to a synthetic lipopeptide [8]. The principal representative of the lipopeptide family is Surfactin, which is produced by a bacterium Bacillus subtilis. Surfactin shows remarkable membrane-active and surface-interface properties resulting in a number of excellent biological activities, which are of great relevance in health care and biotechnology-based processes. These properties make Surfactin a potent candidate drug for the resolution of a number of global issues in medicine [9,10], industry [11,12], and environmental protection [13].
Types of Lipopeptides

Broadly there are three types of lipopeptides namely Surfactin, Iturin and Fengycin that are produced by various bacterial species. Iturin A consists of two major parts: This structure clearly indicates an amphiphilic character of this compound, thus pointing towards the cellular membranes as the most probable site of their action [14]. Such molecules are of great interest because of their biological and physicochemical properties, which can be exploited in food, oil, and pharmaceutical industries. All strains of Bacillus subtilis produce this family of lipopeptides. Cyclic structure of lipopeptide Iturin, containing seven amino acid residues attached to a carbon chain indicates its amphiphilic nature. The same sequence of amino acids is found in a strain of Bacillus sp. The type of Surfactin may also vary according to the order of amino acids and the size of lipid portion [17]. Hydrophobic amino acids of Surfactin molecule are located at positions 2, 3, 4, 6 and 7 while the Glu and Asp residues are located at positions 1 and 5, respectively. Usually, Surfactin isoforms coexist in the cell as a mixture of several peptidic variants with a different aliphatic chain length [18]. Heptapeptide cyclic structure of Surfactin, containing both hydrophobic and hydrophilic amino acids. The structure containing amino acids: Fengycin Fengycin is a bioactive lipopeptide produced by several strains of Bacillus subtilis. It has antifungal activity against filamentous fungi [20]. It represents the third family of lipopeptides after the Surfactin and Iturin and is also called Plipastatin Figure 3. The structure of Fengycin contains a peptide chain of 10 amino acids linked to a fatty acid chain [21]. The length of the fatty acid chain can vary from C6 to C12 carbon atoms for Fengycins, thus giving different homologous compounds and isomers.

Fengycins are cyclic decapeptide formed by lactonization [22]. On the basis of variation at single amino acid at the 6th position in peptide ring, Fengycins have been classified in two classes, namely, Fengycin A and Fengycin B. Primary cyclic structure of Fengycin A. Lipopeptides-Based Biosurfactants Lipopeptides as biosurfactants have been used in biomedical and pharmaceutical applications as antimicrobial, antimycoplasma, antiadhesive, and antitumor agents [41]. These biosurfactants from Bacillus spp. These lipopeptides surfactants are environmental ecofriendly alternatives to synthetic surfactants. There has been an increasing interest to study the effect of lipopeptide biosurfactants on human and animal cell lines. Some of the roles of these biosurfactants include their use as antiadhesive agents to pathogens, thus making them useful therapeutic, probiotic, and pharmaceutical agents [43]. Lipopeptides as Biocontrol Agents Lipopeptides act as biocontrol agents because of their property of inhibition of growth of a variety of microorganisms including phytopathogens Table 1. Lipopeptides role in food safety for inhibiting the growth of phytopathogens. Applications of Lipopeptides Out of the three lipopeptides Iturin, Surfactin, and Fengycin, Surfactin has been preferentially considered for various commercial applications Figure 4. Several recent reviews summarize the high interest in the use of biosurfactant for applications [44] in foods [12], environmental management [13, 45], biomedical fields [32], and cosmetics [46]. During its long history, Surfactin was first studied for its potential pharmaceutical applications antibacterial, antitumor, and cholesterol lowering activities. The discovery at the end of s of its antimycoplasma and antiviral properties leads to the proposal of its use to ensure the safety of biotechnological or pharmaceutical products. The presence of lipopeptides in fermented food products [47] was also considered for their applications in the food sector. Moreover, their ability to induce systemic resistance in plants and their use in the spreading of the bacterial cells leading to rhizosphere colonization could open new fields of applications for their use as promising phytopharmaceutical products. Broader applications of Surfactin in food and pharmaceutical industries. Applications are shown in different areas such as phytosanitation, pharmaceuticals, food, and cosmetics. This sensitivity can be prevented by using peptides having cyclic ring-structure such as lipopeptides [48]. Lipopeptides in the food industry are well characterized in the terms of their antiadhesive, antimicrobial, antiviral, and antitumor activities, which ensure their position and important roles in the industries such as pharmaceutical and cosmetics [48]. In the food industry, lipopeptides can be used as emulsifiers in the processing of raw materials. In the baking industry, Surfactins are used to maintain the texture, stability, and volume and also to help in the emulsification of fat in order to control the aggregation of fat globules [48]. Recently, some lipopeptides isolated from bacterial group, Enterobacteriaceae, have been introduced into the food industry with their high emulsifying properties at enhanced viscosity at an acidic pH [48]. Often various food preservatives are used by food manufacturers during processing to avoid rapid food spoilage. Among biopreservatives, several
antimicrobial compounds have been accepted till date. These compounds effectively control food poisoning microbes [49]. In terms of market increase, the most significant growth rates in food additives were observed for emulsifiers and hydrocolloids [50]. It is quite likely that lipopeptides, in the near future, will represent significant percentage of food additives in the market. Biomedical and Therapeutic Applications of Surfactins and Iturins Among several categories of biosurfactants, lipopeptides are particularly interesting because of their high surface activities and antibiotic potential against an array of phytopathogens. Surfactins can act as antiviral agents, antibiotics, antitumor agents, immunomodulators or specific toxins inhibitors Table 2. Surfactin was found to be more efficient than Iturin A in modifying the B. Conjugates of lipopeptide and T-cell epitopes also constituted effective adjuvants for the in vitro immunization of either human mononuclear cells or mouse B cells and resulted in an increased yield of antibody-secreting hybridoma. Applications of lipopeptides in medical field. An Antimycoplasma Agent Mycoplasma is the smallest free-living organism and parasite of eukaryotic cells and is one of the major contaminants that affect mammalian tissue culture cells. Mycoplasmas are serious causative agents of diseases of both humans and animals, such as acute respiratory inflammation including pneumonia, urogenital tract infections and AIDS [52, 53]. Treatment with antibiotics is the most effective procedure for eliminating or suppressing mycoplasma infection in the cell cultures. Surfactin is used commercially for curing of cell cultures and cleansing of biotechnological products of mycoplasma contamination [54]. Surfactins have versatile bioactive properties with significant antimycoplasma activity [57]. Their disintegration is obviously due to the physicochemical interaction of the membrane-active Surfactin with the outer part of the lipid membrane bilayer, which causes permeability changes and at higher concentrations leads finally to disintegration of the mycoplasma membrane system by its detergent-like effect. Mosquito Larvicidal Agents Mosquitoes are blood feeding insects and serve as vectors for spreading human diseases such as malaria, yellow fever, dengue fever, encephalitis, West Nile fever, and lymphatic filariasis. The culture supernatant of a Surfactin-producing Bacillus subtilis strain was found to effectively kill the larval and pupal stages of mosquito species such as Culex quinquefasciatus, Anopheles stephensi, and Aedes aegypti [16]. As some biocontrol agents or insecticides are effective against mosquito pupae, this could be a good tool for application in malaria control programmes [58]. Further, growing public awareness about the environmental and human risk associated with chemical pesticides, emergence of pesticide resistant insect populations and rising prices of chemical pesticides has invariably stimulated the search for new eco-friendly vector control biological tools [59]. In this respect, several biological control agents have been tested in India and in many other parts of the world to evaluate their potential to control the mosquito vectors [60]. Toxins from certain strains of bacteria, such as Bacillus thuringenesis var. However, the biolarvicide formulation from Bs strain is reported to be less effective against Anopheles culicifacies and hardly effective against Aedes aegypti [59]. A potential key strategy for delaying resistance to mosquitocidal proteins is to use a mixture of toxins that act at different targets within the insects [62]. Antiparasitic Activity of Surfactin Microsporidia are defined as highly specialized fungi [63]. Nosema ceranae is one of the etiologic agents of nosemosis, a worldwide disease [64]. Surfactin is considered as a molecule capable of reducing parasitosis development, acting either by direct exposure to spores or by its incorporation in the luminal of bee midgut [65]. Surfactin was also found to be a potent inhibitor of intraerythrocytic growth of P. Surfactin can also be used as alternative treatment for nosemosis. When exposed to Surfactin, the spores of Nosema ceranae, the causative agent of parasitic infection in Apis mellifera, revealed a significant reduction in infectivity [65]. Moreover, when Surfactin is administered and is introduced into the digestive tract of a bee, it also leads to a reduction in parasitoids development [65]. The length of the carbon chain in cyclic Surfactin lipopeptide influences its capacity for viral inactivation [67].
Cyclic lipopeptide antibiotics can be classified structurally into several groups. The enormous structural diversity in this group of antibiotics leads to wide variation in biological activity. The present review describes the structural characteristics, chemistry, biological activity and pharmacology of natural cyclic lipopeptides.

The mechanisms by which the bacteria manage to infest this alternative host, to overcome its immune system, and to ultimately kill the insect are still largely unknown. However, the investigation of the few virulence factors discovered so far, points to a highly multifactorial nature of insecticidal activity. Antimicrobial compounds produced by fluorescent pseudomonads are effective weapons against a vast diversity of organisms such as fungi, oomycetes, nematodes, and protozoa. Here, we investigated whether these compounds also contribute to insecticidal activity. CMR12a, defective for individual or multiple antimicrobial compounds, for injectable and oral activity against lepidopteran insect larvae. Moreover, we studied expression of biosynthesis genes for these antimicrobial compounds for the first time in insects. Our survey revealed that hydrogen cyanide and different types of cyclic lipopeptides contribute to insecticidal activity. Mutants of CMR12a and PCL impaired in the production of the cyclic lipopeptides sessilin and clp, respectively, showed reduced virulence in injection and feeding experiments. Although virulence of mutants lacking one or several of the other antimicrobial compounds, i. In summary, our study identified new factors contributing to insecticidal activity and extends the diverse functions of antimicrobial compounds produced by fluorescent pseudomonads from the plant environment to the insect host. Introduction Root-colonizing fluorescent pseudomonads are well known for their plant-beneficial traits, which include inhibition of root-pathogens, induction of resistance in the plant and solubilization of mineral nutrients. Nevertheless, during the last decade evidence arose that we should widen our view on their life-style since plant roots apparently are not the only environment colonized by these bacteria. Especially strains of sub-clade I Loper et al. How exactly plant-associated pseudomonads colonize insects and which factors are decisive for fatal infections is still largely unknown. Fit mutants of P. However, all Fit mutants retained significant virulence, and in oral infections of Drosophila melanogaster by P. This indicates the involvement of additional virulence factors and points to a certain specificity of virulence factors to individual insect species Keel, Accordingly, a Fit-GacA double mutant was strongly reduced in virulence Ruffner et al. Recently, chitinase C was identified as one of the Gac-regulated factors contributing to toxicity of P. While some factors, such as the Fit toxin or chitinase C, are present throughout all strains of the P. Rhizoxin seems to be such a factor as it strongly contributes to insecticidal activity of P. Generally, antimicrobial compounds produced by strains of the P. These compounds exhibit toxic effects toward a broad spectrum of organisms Gross and Loper, and their production is Gac-regulated Hassan et al. Besides their well-demonstrated contribution to biocontrol activity of pseudomonads against fungal pathogens, also activity against bacteria, protists, nematodes, arthropods, plants, and mammalian cells is reported Keel et al. The aim of this study was to investigate whether antimicrobial compounds are also important for these pseudomonads when infecting an insect host. The highly insecticidal P. CMR12a and Pseudomonas sp. CMR5c Flury et al. Since the different species produce distinct sets of antimicrobial compounds we selected one representative strain per species: All three strains produce Hcn and P. Recently, orfamide production was also demonstrated for P. Here, we screened newly generated as well as existing mutants deficient for one or several of the different antimicrobial metabolites for their toxic activity toward insects either when injected directly into the hemocoel or when taken up orally. Furthermore, virulence of mutants deficient for additional compounds or enzymes contributing to biocontrol, i. Finally, expression of biosynthetic genes for a selection of the investigated metabolites was studied in CHA0 during the process of insect infection. Our findings highlight major contributions to insecticidal activity for all investigated Clps as well as for Hcn, but not for any of the other metabolites or enzymes. These cultures were used to inoculate 10 mL LB medium Bertani. Cells were washed twice or three times in sterile 0. Strains used in this study. Construction of Deletion Mutants of P. Briefly, using the primer pairs listed in Supplementary Table S1, the â€”bp upstream and downstream regions flanking the genomic region to be deleted were
amplified by PCR. After digestion with the indicated restriction enzymes Supplementary Table S1, the fragments were cloned into pEMG via triple ligation yielding the final suicide plasmid which was verified by sequencing. Plasmid pRLA Wolk et al. A library of Tn5luxAB mutants of PCL was screened for loss of drop collapsing activity, which resulted in the selection of biosurfactant mutants. To recover the regions flanking the transposon insertion site, chromosomal DNA of the mutant was isolated and digested with EcoRI. After re-circulation and transformation into E. The primers used, i. The flattening and spreading of the droplet drop collapsing activity was assessed on soft agar LB plates 0. Galleria Injection Assay Injection assays with G. When they started to become melanized they were scored as live or dead every hour. Each experiment consisted of three replicates per treatment with 10 larvae per replicate. Every mutant strain was tested at least in three independent experiments and two representative experiments are shown in Table 2. To exclude a human bias, the experiments were performed double-blind, i. Lethal time 50 LT50 for Galleria mellonella larvae injected with wild-type and mutant Pseudomonas strains. Plutella Feeding Assays Feeding assays were performed as described by Flury et al. One-week-old larvae deriving from four different egg batches were used for experiments during which each larva was kept separately in cell bioassay trays Frontier Agricultural Sciences, Delaware, USA to prevent injuries due to cannibalism. For insect diet preparation ml of sterile ddH2O containing 7. The hot mixture was poured into Petri dishes to a height of approximately 1. From the solidified insect diet food pellets were cut with a sterile cork borer 4 mm diameter. Larvae were scored as live or dead regularly over time. Every mutant strain was tested at least in three independent experiments and two representative experiments are shown in Table 3. To exclude a human bias the experiments were performed double-blind as described above. Lethal time 50 LT50 for Plutella xylostella larvae upon oral uptake of wild-type and mutant Pseudomonas strains. Gene Expression Analysis To study bacterial gene expression in the insect host, G. For experiments with G. At 42 h infected larvae had died while control larvae were still alive. Three independent experiments were performed and each time a pool of 10 larvae was analyzed per time point. Three independent experiments were performed and each time a pool of 32 larvae was analyzed per time point. Larvae were washed in ddH2O, homogenized and frozen in liquid nitrogen. The presence of the amplicon was verified by electrophoresis in 1. Statistics Data analysis was performed in RStudio version 0. To test for significant differences between survival curves of wild-type and mutant strains, the Log-Rank test of the Survival package of R was applied. When injected into the hemocoel of G. A similar trend was observed in feeding assays with P. Hence, the lack of Hcn seems to have a pronounced impact on injectable insecticidal activity, but rather a minor effect in oral infections. Hydrogen cyanide-deficient mutants exhibit reduced injectable insecticidal activity. A,B Injectable activity against Galleria mellonella. C,D Oral activity against Plutella xylostella. This figure shows the survival curves of one representative experiment per strain and insect system. The LT50 values corresponding to these experiments and to a repetition of them are depicted in Tables 2, 3. Here, a mutant of CHA0 deficient for all three peptide synthases required for the production of orfamide A Ma et al. This mutant neither reduced surface tension as indicated by a droplet collapse test nor showed swarming motility on soft agar plates Supplementary Figures S1A,B. However, swarming ability was regained when plates were supplemented with orfamide A Supplementary Figure S1A. A detailed characterization of Clp and the mutant PCL will be presented elsewhere. Cyclic lipopeptides contribute to insecticidal activity of three plant-beneficial pseudomonads. A,B,C Injectable activity against Galleria mellonella. D,E,F Oral activity against Plutella xylostella. In feeding experiments with P. Similar to the results for injectable insecticidal activity, Ses seems to make a major contribution to oral insecticidal activity of strain CMR12a. Thus, after 28 h already. In feeding experiments with larvae of P. However, in our experiments with Phz-deficient mutants of both strains, we did not find any reduction of virulence in infection assays with G. We generated new in frame deletion mutants of CHA0 lacking biosynthesis genes needed for the production of these metabolites and screened them for insecticidal activity. All three mutants retained full toxicity in injection and feeding experiments Tables 2, 3, Supplementary Figure S2. Because the production of different antifungal metabolites in P. However, a mutant that in addition to Phl, Prn, and Plt also lacks Ofa and Hcn production exhibited strongly reduced virulence compared to the wild type Table 3. It further displays reduced
virulence compared to single mutants defective for either Ofa or Hcn indicating an additive effect of these two mutations in the five-fold mutant. In contrast, the toxicity of the five-fold mutant did not differ from that of the wild type in injection experiments Table 2. The fact, that not even a reduction in virulence due to the lack of Hcn production was observed, cannot be explained at present, but might be attributable to possible adverse effects on the production of other, yet to be discovered factors.
Chapter 5 : Lipopeptide - Wikipedia

Cyclic Lipopeptide Antibiotics Aminoglycoside antibiotics are used to treat bacterial infections like skin and skin structure infections, and infections in the bloodstream and heart. They disrupt cell membrane function, killing the bacteria cells causing the infection.

It is designed for energetic those who are searching out a mighty resource of their quest for speedy weight loss. Potential Side Effects of Lipopeptide The potential side effects of lipopeptide are constipation, nausea, diarrhea, vomiting, dyspepsia, abdominal pain, headache, insomnia, dizziness, anxiety, rash, pruritus, abnormal liver function test results, CK elevations, renal failure, anemia, dyspnea, fever. Lipopeptide is a molecule with a lipid and peptide attached to each other. TLR 1 and other toll-like receptors create the bond. TLRs are essential for the initial invasion of pathogens in the body. TLRs recognize and the invading microorganisms and assist in defending against them. The cosmetic lipopeptide is also referred to as biopeptide EL. It is expressed by the presence of bacteria. Lipopeptide is an interesting molecule because it has the ability to rearrange themselves into different configurations. In scientific studies, researchers found that lipopeptide is uniquely situated to increase cell production naturally. It is not a water-soluble peptide, which means it has high bioavailability properties that make it biocompatible on the surface. Lipopeptides interact directly with cell membranes to boost activity, repair, and reproduce. Each variation has a unique use. The three types of lipopeptides are: The different structure can be used for different reasons. The most common reasons for lipopeptide use and research are: Study the increase in natural collagen and hyaluronic acid bioavailability Cell membrane interaction for renewal and regrowth To control plant disease Obviously the lipopeptides used are not the same for each individual use. When studying the antimicrobial and biocompatibility of lipopeptide, the common dosage is 10 mg. Common lipopeptide studies for biocompatible use also use mg of lipopeptide Biopeptide EL. The manufacturing stage and the structural range of synthesized LPs have been depending on the medium composition. Surfactins have been produced by means of both lines as a mixture of 4 homologues CC16 with the domination of variation C The highest iturin attention In all cultures best iturin A was identified. The acquired records indicate that the waste composition has a power on both the types and amounts of biosurfactants produced through studied B. Lipopeptides constitute a structurally various organization of metabolites produced with the aid of various bacterial and fungal genera. In the past many years, research on lipopeptides has been fueled with the aid of their antimicrobial, antitumour, immunosuppressant and surfactant sports. However, the herbal functions of lipopeptides within the existence of the manufacturing microorganisms have received significantly much less interest. The good sized structural range of lipopeptides indicates that these metabolites have different natural roles, a number of which may be particular to the biology of the manufacturing organism. This overview gives a detailed assessment of the flexible capabilities of lipopeptides within the biology of Pseudomonas and Bacillus species, and highlights their role in aggressive interactions with coexisting organisms, which includes micro organism, fungi, oomycetes, protozoa, nematodes and flora. Their functions in cell motility, leading to colonization of novel habitats, and in the formation and improvement of exceptionally based biofilms are discussed in element. Finally, this evaluate offers an update on lipopeptide detection and discovery in addition to on novel regulatory mechanisms and genes involved in lipopeptide biosynthesis in these bacterial genera. Final Review of Lipopeptide The lipopeptides are among the class of strong versatile guns to address a variety of phytopathogens. These lipopeptides seem to be promising biopesticides in agriculture practices for replacing dangerous chemical pesticide and therefore they can be taken into consideration as amazing opportunity gear to conquer growing chemical resistance of phytopathogens. Lipopeptides are riskless, biodegradable, fairly solid, ecofriendly, and nonpolluting biomolecules. These residences of the lipopeptides cause them to more efficient biologics for use in phytosanitation, pharmaceuticals, foods, bioremediation, and so forth. However, producing and making use of those lipopeptides at a much wider scale at present appear to be superb challenges that want suitable scale-up technology to be evolved at industrial scale. Also, the said benefits makes the ingredient a very good supplementation for everyone.
Chapter 6 : Cyclic Lipopeptide

Daptomycin (1) Definition (MSH) A cyclic lipopeptide antibiotic that inhibits GRAM-POSITIVE BACTERIA. Definition (NCI) A semi-synthetic cyclic lipopeptide antibiotic isolated from the bacterium Streptomyces roseosporus with broad-spectrum antibiotic activity against Gram-positive bacteria.