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Natural Cyclic Peptides from DBAASP Database

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pharmaceuticals MDPI

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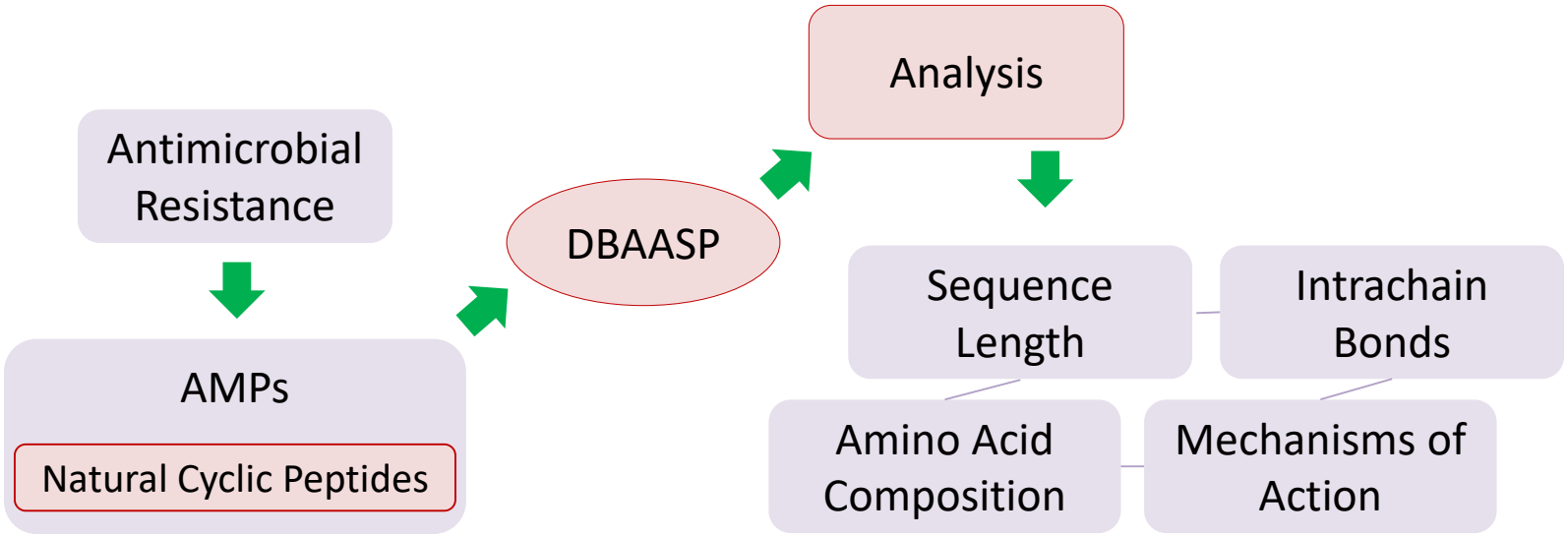
Natural Cyclic Peptides from DBAASP Database

Graphical Abstract



Abstract: Antimicrobial peptides (AMPs) have emerged as a novel class of anti-infectives to combat microbial resistance. However, their intrinsic characteristics, including low target affinity and limited *in vivo* stability, present obstacles to their therapeutic development. Macrocyclic peptides, a diverse peptide family, offer improved metabolic and proteolytic stability along with high target affinity. In this work, we performed an analysis of the DBAASP database to enhance our understanding of small cyclic peptides, nature's defense mechanisms for living organisms. To our knowledge, this is the first effort to characterize cyclic peptides originated from either non-ribosomal or post-ribosomal peptide synthesis and reveal shared and distinctive cyclization patterns. The Database of Antimicrobial Activity and Structure of Peptides (DBAASP) provides invaluable data on structural characteristics and experimental testing of AMPs, encompassing over 4,400 cyclic AMPs. Natural peptides within DBAASP are categorized based on their synthesis type: ribosomal and non-ribosomal. Currently, DBAASP contains 1346 ribosomal and 475 non-ribosomal cyclic AMPs. We conducted an analysis of this dataset, considering peptide length, amino acid composition, intrachain bonding for cyclization, and mechanisms of action. The methods of cyclization, types of cycles, and their peculiarities in the composition of small cyclic peptides have also been explored. Analysing this wealth of information not only deepens our understanding of cyclic AMPs but also inspires strategies for designing novel AMPs with enhanced therapeutic potential.

Keywords: AMPs; Cyclic Peptides; Macrocyclization; DBAASP

Introduction

Antimicrobial peptides (AMPs) are considered promising candidates to address the issue of microbial resistance. Unlike small molecular drugs, AMPs have the advantage of being able to act on large surfaces, such as protein-protein and lipid-lipid interactions, often referred to as 'undruggable' binding sites [1]. However, they face several limitations, including low target affinity and poor *in vivo* stability. Macrocyclization improves the pharmacological properties and bioactivity of peptides [2].

Macrocyclic peptides constitute a highly diverse family of peptides found in various organisms. Cyclic peptide natural products exhibit exceptional cell permeability and oral absorption [3]. Macrocyclization is employed to enhance metabolic stability.

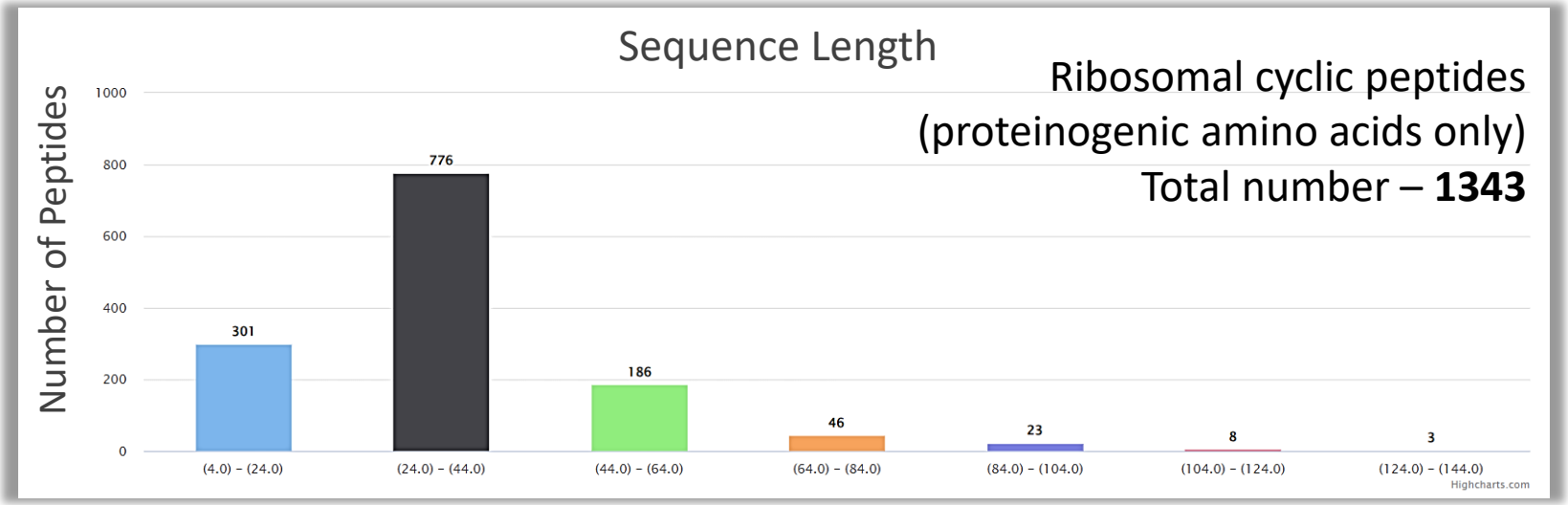
The Database of Antimicrobial Activity and Structure of Peptides (DBAASP) is a comprehensive repository for natural cyclic peptides, among which there are, currently, 470 non-ribosomal and 1354 ribosomal cyclic AMPs. The numbers are ever-growing. Several characteristics of these valuable drug candidates stored in DBAASP will be explored.

Results and discussion

1354 → Ribosomal — 4–133 AA

470 → Nonribosomal — 2–22 AA

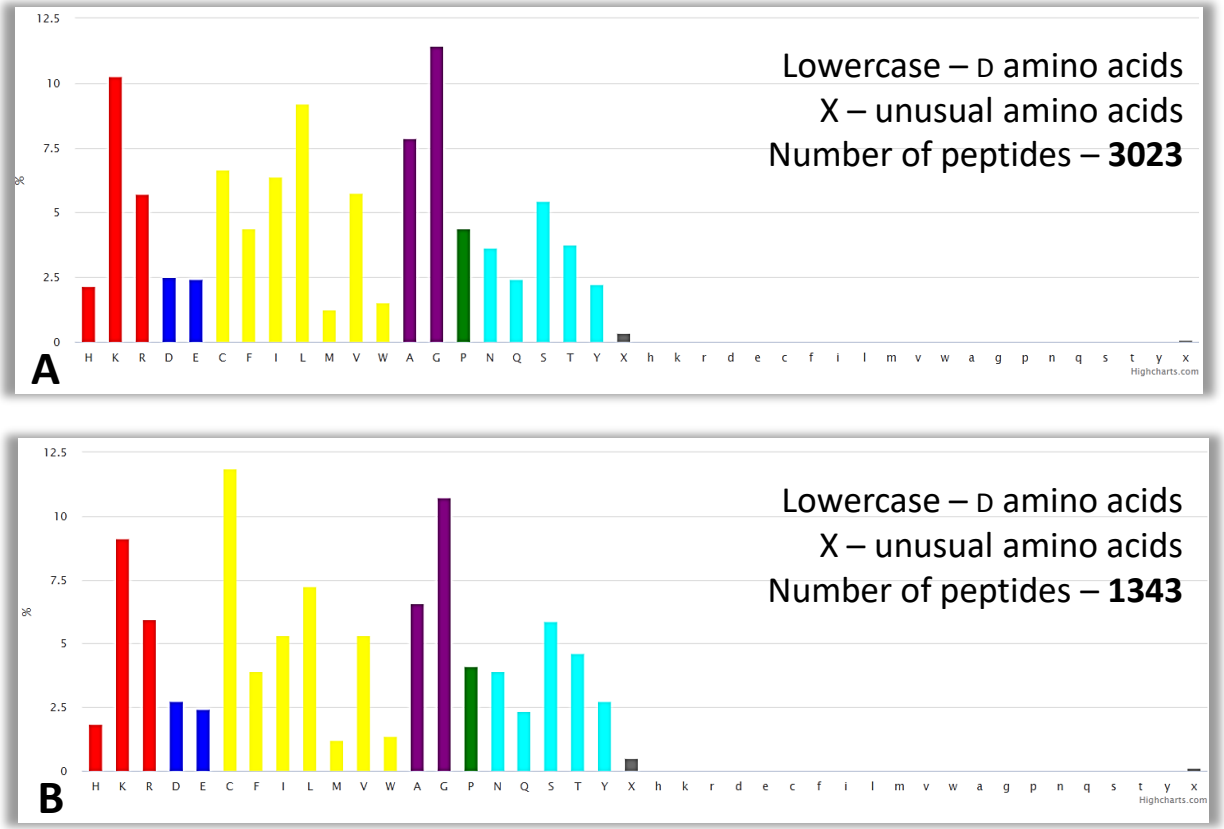
Length



For comparison, the ribosomal cyclic peptides in DBAASP tend to be longer than their non-ribosomal counterparts.

Results and discussion

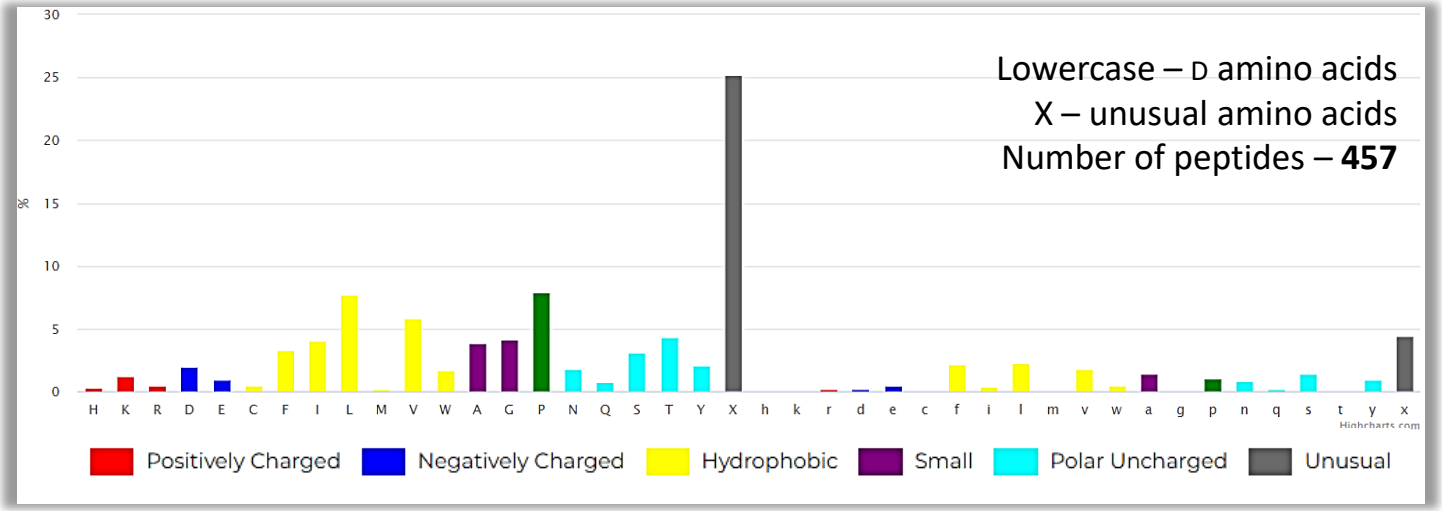
Amino Acid Composition of Ribosomal Linear **(A)** and Cyclic **(B)** Peptides



Cyclic peptides feature a more prominent presence of cysteine, mainly because many ribosomal peptides are cyclized through the formation of disulfide bonds.

Results and discussion

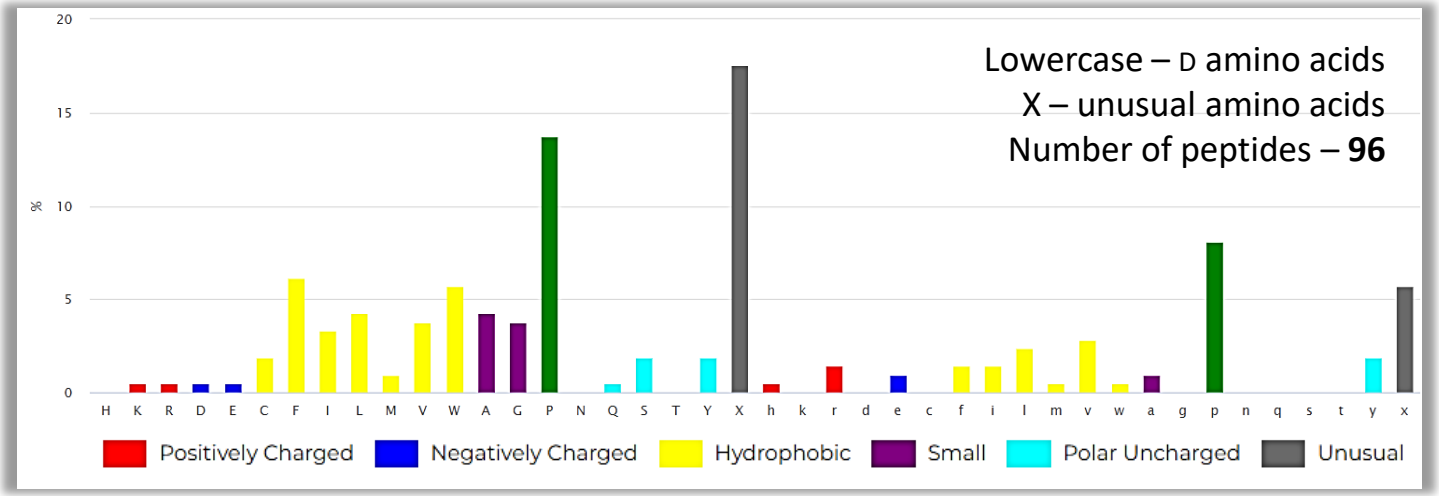
Amino Acid Composition of Non-ribosomal Cyclic Peptides



Unlike their ribosomal counterparts, non-ribosomal cyclic peptides are rich in unusual amino acids and D-enantiomers of natural amino acids.

Results and discussion

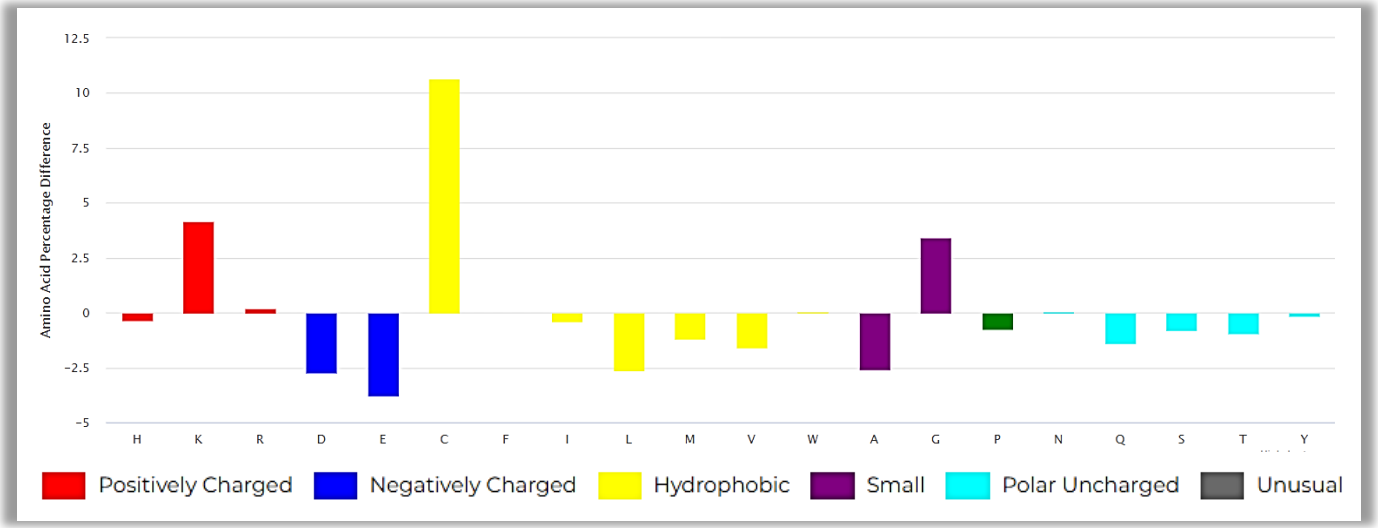
Amino Acid Composition of Non-ribosomal Cyclic Peptides of Length 2-3 AA



The amino acid composition of non-ribosomal cyclic dipeptides and tripeptides indicates a high abundance of proline, alongside unusual amino acids. This observation can be explained by the necessity of proline for cyclizing ultra-short peptides, as it has a propensity to induce sharp turns in the main chain of the polypeptide [4].

Results and discussion

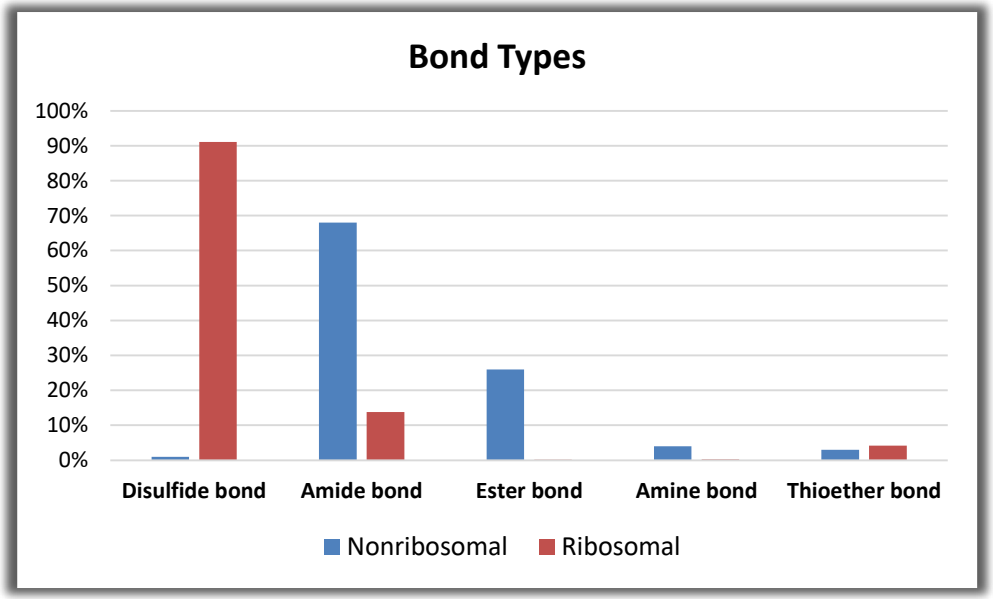
Amino Acid Composition of Ribosomal Cyclic Peptides from DBAASP Compared to UniProt Sequences



The comparison reveals that ribosomal cyclic peptides in DBAASP are more basic compared to the ‘average protein’ in UniProt, primarily due to the abundance of lysine. Additionally, they exhibit a relatively higher content of glycine and cysteine. The presence of glycine is likely associated with the need for flexibility to facilitate cyclic closure and to provide a ‘chameleonic’ structure, while the presence of cysteine is crucial for cyclization, contributing to the activity and stability of peptides.

Results and discussion

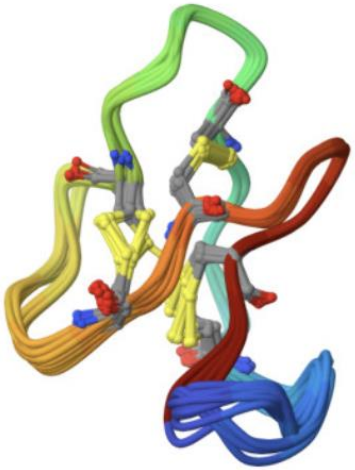
In ribosomal cyclic peptides, cysteine residues are primarily utilized for cyclization, resulting in the formation of disulfide bonds and the creation of a cystine ring. This feature is notably less common in non-ribosomal cyclic peptides.



Conversely, amide bonds serve as the primary means of cyclization in non-ribosomal cyclic peptides. Approximately half of them feature head-to-tail closed lactam rings, while the other half adopt diketopiperazine rings and sidechain-to-mainchain closed lactam rings.

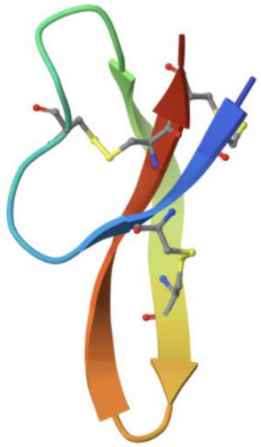
Results and discussion

The 3D structures of over 700 ribosomal cyclic AMPs, with a length >26 AA and ≥3 disulfide bonds, are complex.



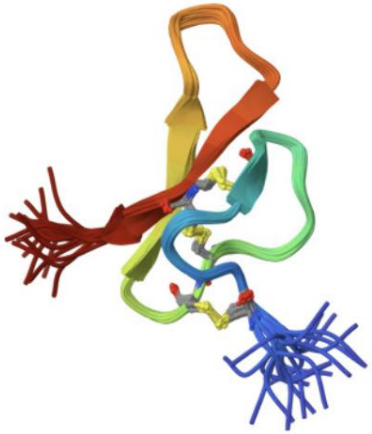
Kalata B1

PDB ID – 1JJZ



Alpha-defensin-4

PDB ID – 1ZMM



Tachystatin b

PDB ID – 2DCV

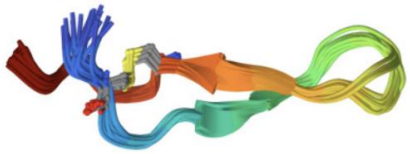
Cyclic peptides with a length <25 AA are preferred for designing new drug candidates.

Results and discussion

More than 450 ribosomal cyclic peptides are small, with a length <25 AA.
The majority of them, approximately 400, are cyclized by disulfide bonds.
Small disulfide-bonded cyclic ribosomal peptides can be classified as peptides with:

1 disulfide bond

Lactoferricin B
PDB ID – 1LFC

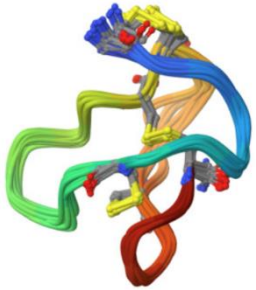


2 disulfide bonds

Protegrin PG-5
PDB ID – 2NC7

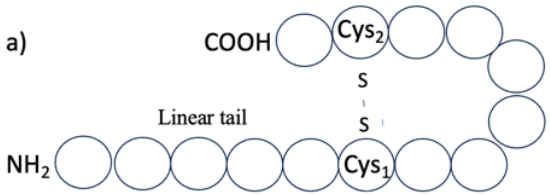
3 disulfide bonds

Omega-conotoxin MVIIA
PDB ID – 1TTK



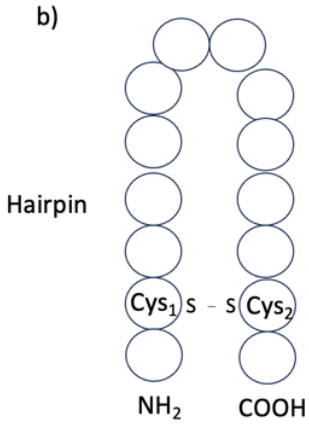
Results and discussion

Schematic representation of the structures of small AMPs containing a single disulfide bond: a) lasso-like, and b) hairpin-like.



C-Lasso

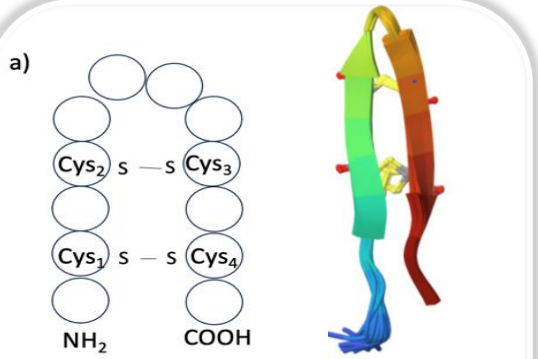
Brevinin-1BYa
PDB ID – 6G4U



Lactoferricin B
PDB ID= 1LFC

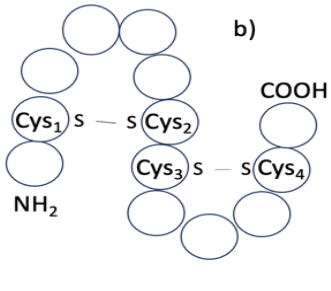
Results and discussion

Schematic representation of the structures of cysteine-containing small AMPs, with a network of ≥2 intrachain bonds: a) ladder-like (hairpin-like), b) string of cycles, and c) knot-like.



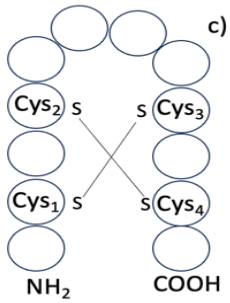
Ladder-like

Protegrin PG-5
PDB ID – 2NC7



String of Cycles

LP2006
PDB ID – 5JPL



Knot

Sviceucin
PDB ID – 2LSI

Results and discussion

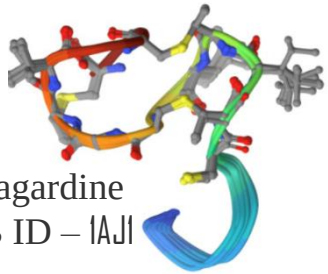
Structural types of small ribosomal peptides cyclized by disulfide bonds:

- The majority of small disulfide-bonded DBAASP peptides are ‘lasso-like’ (approximately 230).
- Only a few disulfide-bonded peptides result in structures characterized as a ‘string of cycles’.
- Approximately 120 peptides adopt a ‘hairpin-like’ structure, including those referred to as ‘ladder-like’.
- Around 50 small AMPs exhibit a ‘knot-like’ structure.

Results and discussion

Similar topological features in peptide chains can also be achieved through other types of chemical bonds.

- Thioether bonds play a pivotal role in forming a 'string of cycles' structure in lantibiotics, with data on approximately 40 lantibiotics stored in the DBAASP.



Actagardine
PDB ID – 1AJ1

Lassomycin
PDB ID – 2MAI

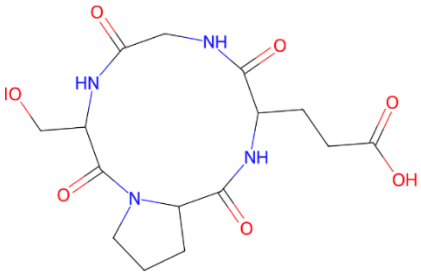
- Amide bonds, specifically those between N-terminal amino groups and the side-chain carboxyl groups of Asp and Glu residues, are responsible for cyclizing peptides into the category known as ‘lasso' peptides. The DBAASP database contains information on around 30 ‘lasso' peptides.

- Amide bonds also participate in head-to-tail cyclization, contributing to the stabilization of certain hairpin-like (ladder-like) topologies.

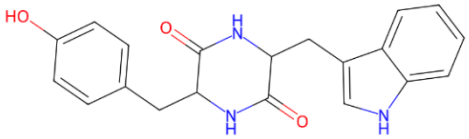
Theta-defensin RTD-1
PDB ID – 2LYF

Results and discussion

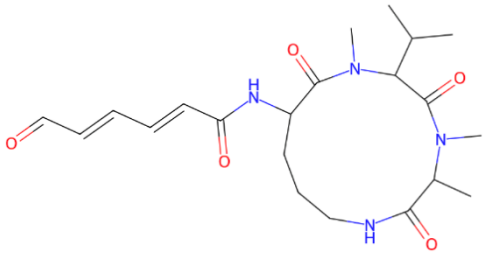
Non-ribosomal peptides, cyclized by amide bonds, can be categorized into three groups: head-to-tail linked AMPs, head-to-tail linked dipeptides (diketopiperazines), and sidechain-to-mainchain linked AMPs.



Head-to-tail linked AMP
from marine bacteria
DBAASP ID – 10055



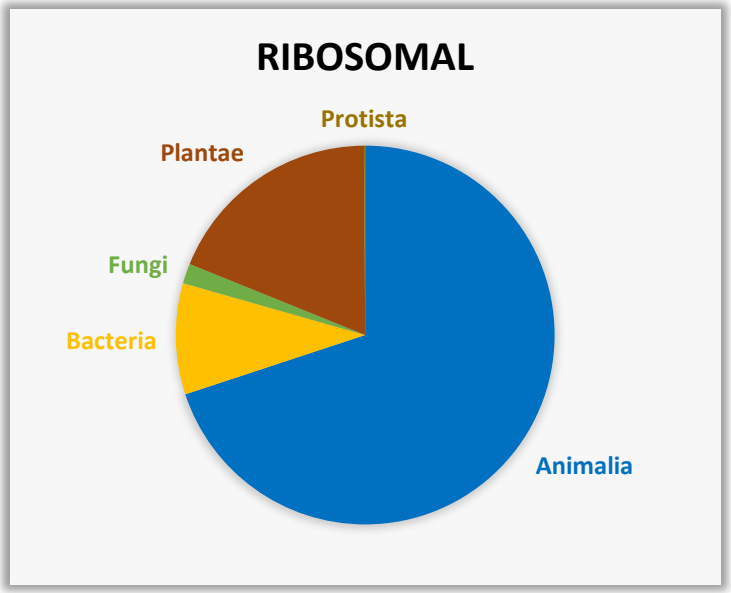
Head-to-tail linked dipeptide
Diketopiperazine (WY)
DBAASP ID – 7132

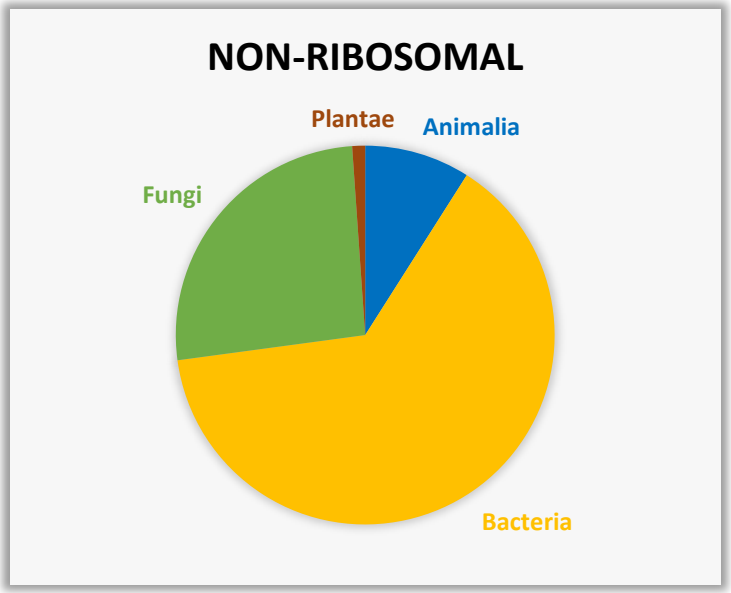


Sidechain-to-mainchain linked AMP
Sclerotiotide F
DBAASP ID – 18453

Results and discussion

Source Organisms (Kingdoms) of Ribosomal and Non-ribosomal Cyclic Peptides in DBAASP

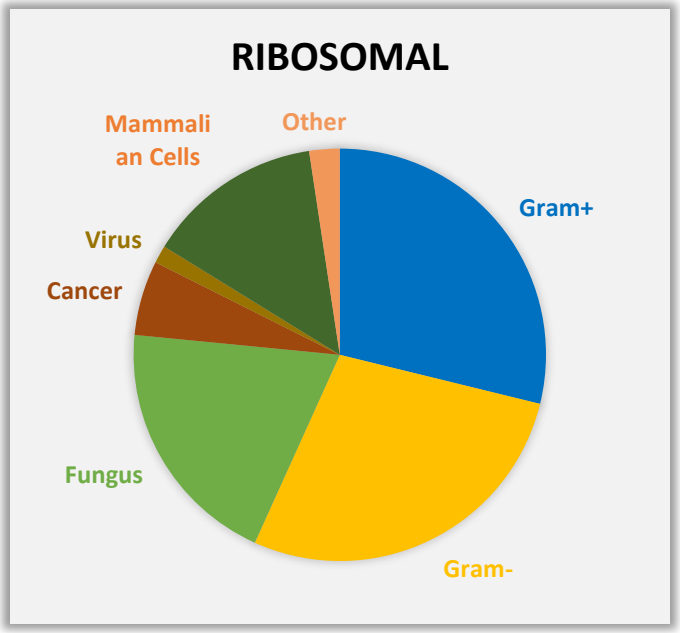


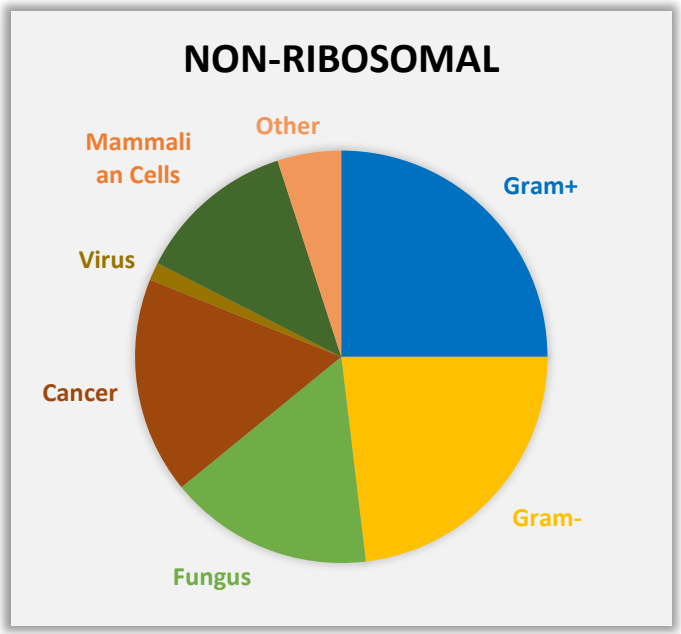


The majority of ribosomal cyclic peptides are synthesized in multicellular organisms, including animals and plants, while non-ribosomal peptide synthesis primarily occurs in microorganisms, such as bacteria and fungi.

Results and discussion

Target Groups of Ribosomal and Non-ribosomal Cyclic Peptides in DBAASP

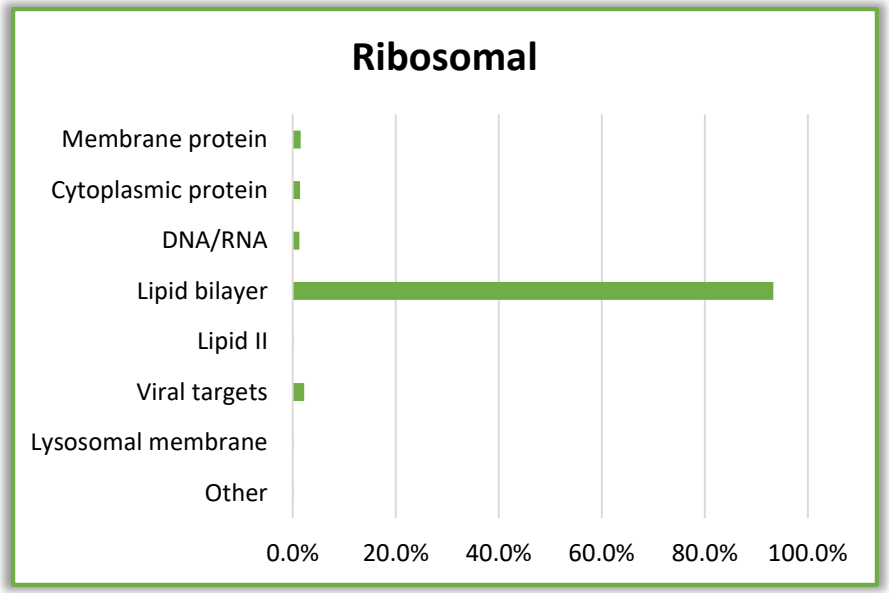


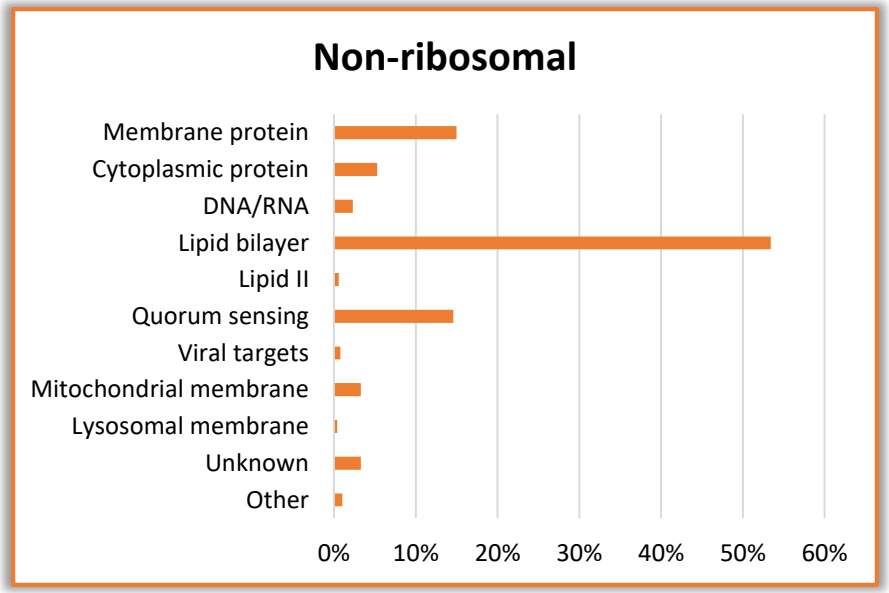


Like other AMPs in general, both ribosomally and non-ribosomally synthesized cyclic peptides display a broad spectrum of activity, primarily targeting bacteria and fungi, as well as cancer cells.

Results and discussion

Target Objects of Ribosomal and Non-ribosomal Cyclic Peptides in DBAASP





The majority of both ribosomal and non-ribosomal cyclic peptides tend to exhibit membrane-active properties. However, this propensity is notably more pronounced in ribosomal cyclic AMPs.
Conversely, another significant class of targets for non-ribosomal cyclic peptides, primarily those of shorter lengths (2-5 AA), are membrane or cytoplasmic proteins. These peptides often adhere to the so-called Rule-of-5 and can act on 'druggable' targets such as enzymes and receptors. Furthermore, non-ribosomal cyclic dipeptides containing a diketopiperazine ring frequently demonstrate an ability to inhibit regulators of bacterial cell population density, a phenomenon known as ‘quorum sensing’.

Conclusions

- Non-ribosomal cyclic peptides are generally shorter in length compared to their ribosomal counterparts;
- Amino acid composition reveals that ribosomal cyclic AMPs are rich in lysine, cysteine, and glycine, whereas non-ribosomal cyclic AMPs feature proline and unusual amino acids;
- Regarding cyclization, ribosomal cyclic AMPs favor disulfide bonds, while non-ribosomal cyclic AMPs prefer amide bonds;
- In both synthesis methods, similar types of bonds are formed, but by different protein systems that are not homologous. This suggests a potential case of convergent evolution;
- Both types of natural cyclic peptides exhibit a broad spectrum of activity;
- Ribosomally synthesized cyclic AMPs primarily target membranes, while non-ribosomal ones have a broader range of target objects beyond lipid bilayers, including membrane and cytoplasmic proteins.

Acknowledgments

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