In the Name of God

Antibacterial Activity of Antimicrobial Peptides (AMPs) isolated from human neutrophils of LCR5 Filters

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Antimicrobial Peptides

20th Century Challenge:
Microbial Resistance to the current Antimicrobial Agents

Amino glycosides,

Macrolides, Glycopeptides

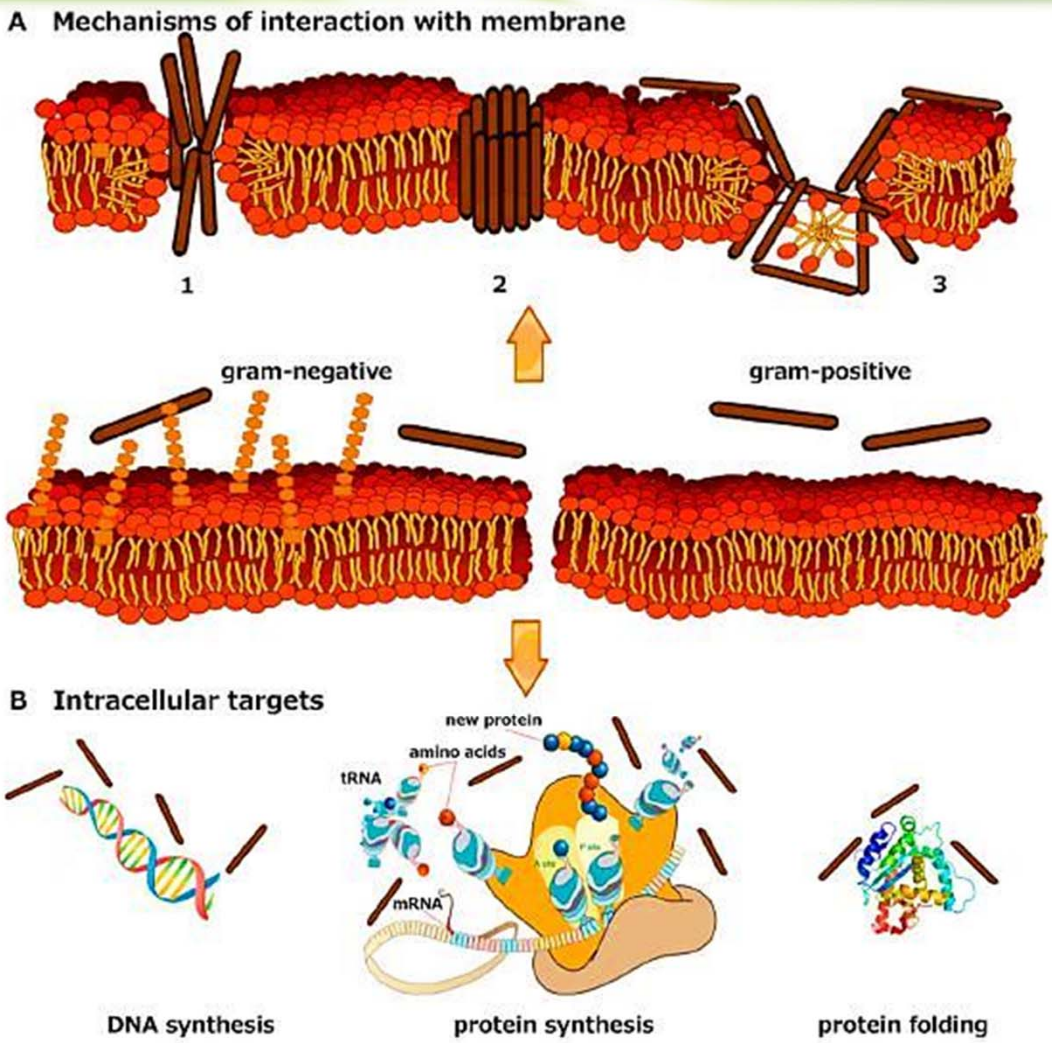
and Chemical Modifications

Investigation for Novel Antimicrobial classes with Novel Mechanisms

Antimicrobial Peptides

- In 1939, Dubos extracted an antimicrobial agent from Bacillus species.
- The agent could serve mouse from pneumonia.
- Years after, Hotchkiss and Dubos called it “Anti Microbial Peptide”.
- The peptide named as “Gramicidin”

Antimicrobial Peptides



N. Sasani, Antimicrobial activity assessment of Human PMN AMPs recovered from LCR5 filters

Antimicrobial Peptides

Name	Amino Acid Sequence [a]	Origin
α-defensin (HNP-2)	$C_1YC_2RIPAC_3IAGERRYGTC_2IYQGRLWAFC_3C_1$	Human
β-defensin (BD2)	$GIGDPVTC_1LKSGAIC_2HPVFC_3PRRYKQIGTC_2GLPGTKC_1C_3KKP$	Human
LL-37	LLGDFFRKSKEKIGKEFKIVQRIKDFLRNLVPRTES	Human
Protegrin	$RGGRLC_1YC_2RRRFC_2VC_1VGR$	Pig
Indolicidin	$ILPWKWPWWPWRR\text{-}NH_2$	Cattle
Magainin 2	GIGKFLHSAKKFGKAFVGEIMNS	African clawed frog
Cecropine A	$KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK\text{-}NH_2$	*Hyalophora cecropia*
Mellitin	GIGAVLKVLTTGLPALISWIKRKRQQ	Honey bee
Magainin II	GIGKFLHSAKKFGKAFVGEIMNS	African clawed frog
Polyphemusin	$RRWC_1FRVC_2YRGFC_2YRKC_1R$	Horseshoe crab
Gramicidin S	$cyclo\text{-}(Val\text{-}Orn\text{-}Leu\text{-}D\text{-}Phe\text{-}Pro)_2$	*Bacillus brevis*
Nisin A [b]	$I\text{-}DHB\text{-}A_1I\text{-}DHA\text{-}LA_1\text{-}ABA_2\text{-}PGA_2K\text{-}ABA_3\text{-}GALMGA_3NMK\text{-}ABA_4\text{-}A\text{-}ABA_5\text{-}A_4HA_5SIHV\text{-}DHA\text{-}K$	*Lactococcus lactis*

❖Leukoreduction Filters

- ❖ To deplete leukocytes from donated blood bags
- ❖ The product is to be applied in patients with certain criteria
- ❖ Potential source for cell research and drug discovery

Leukodepletion Filters

❖LCR5 filter

- ❖ Dominant LRF in Iranian Blood Transfusion System
- ❖ Made by Macopharma, France
- ❖ ≥ 99% leukocyte depletion capability
- ❖ 27 layers:
  - ❖ 5 layers with 30 micrometers in polyethylentrephetalate
    - ❖ *Clot extraction*
  - ❖ 22 layers with 9 micrometers in polypropylene
    - ❖ *Extraction mechanisms of size, affinity and trapping*

Objective

- **High yielded PMN recovery from LCR5 filters**
- **AMP extraction from recovered PMNs**
- **Antimicrobial Activity assessment of the extracted AMPs**

PMN recovery

- **Optimized Leukocyte Recovery system from LCR5**
  - Granulocytes were recovered
  - Viability was assessed

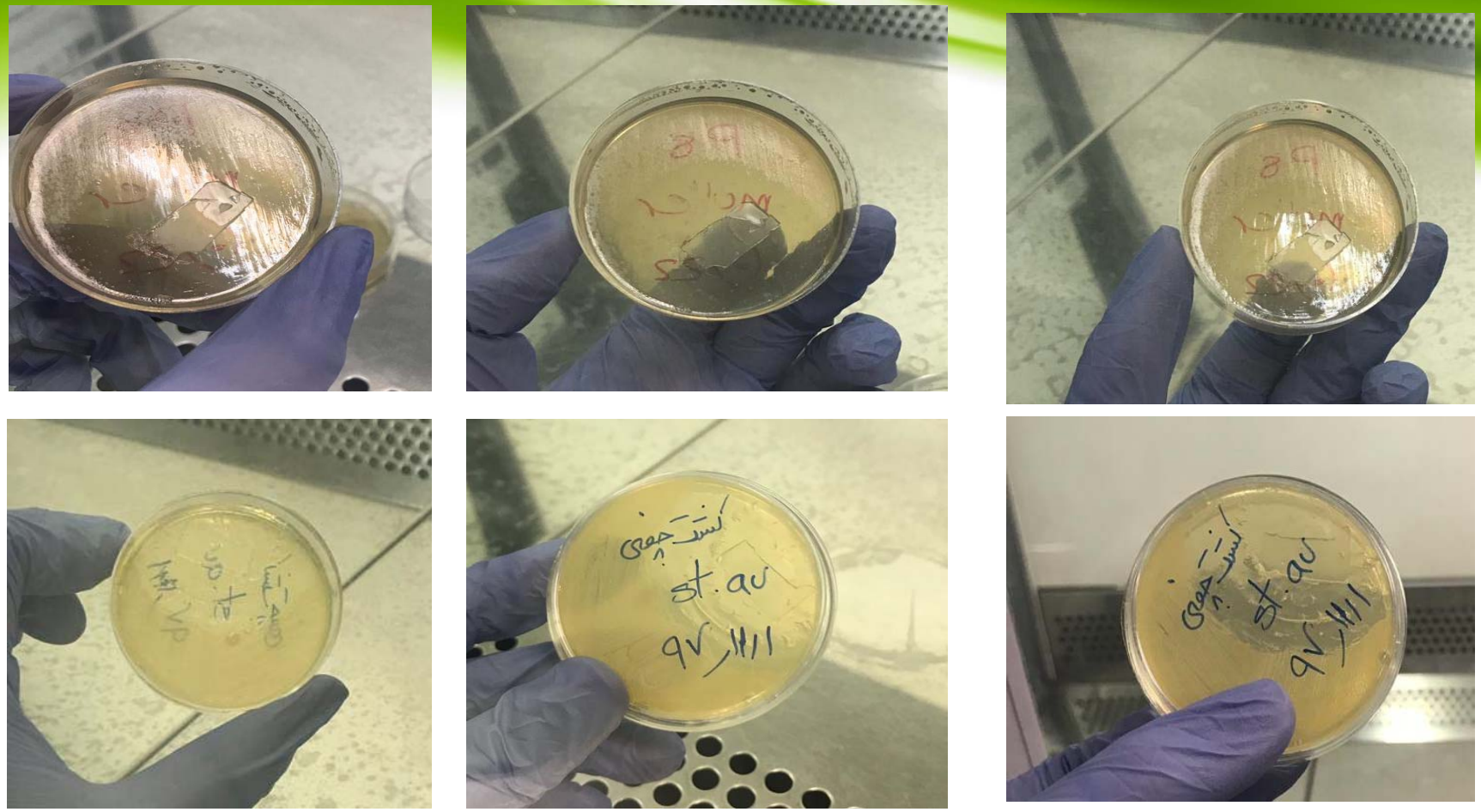
AMP Extraction

- Granules were separated by time and power controlled sonication
- Granules were broken by time and power controlled sonication
- Having been centrifuged, the supernatant was applied to SDS-PAGE

Antimicrobial Activity Assessment

- Extracted peptide in the specific region on the gel was cut
- The piece was directly put on to Muller Hinton Agar medium
- Resistant strains of Gram positive and Gram negative bacteria were checked for antimicrobial activity.

Results



To be Continued…