AMRITA VISHWA VIDYAPEETHAM
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22BIO211: Intelligence of Biological Systems - 2

ANTIBIOTIC SEQUENCING

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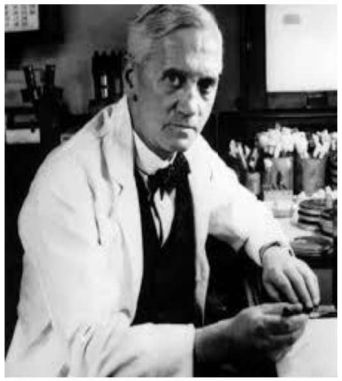
Discovery of Antibiotics

- ***Alexander Fleming - August 1928***
- Staphylococcus bacteria on a laboratory bench
  - *contaminated with Penicillium fungus*
  - *colony of Staphylococcus surrounding it had been destroyed!*
- Fleming named the bacteria-killing substance **penicillin**
- Fleming published his discovery in 1929 but he failed to isolate the antibiotic agent (i.e., the compound that actually killed bacteria) from the fungus.
- He abandoned his antibiotics research.



Discovery of Antibiotics

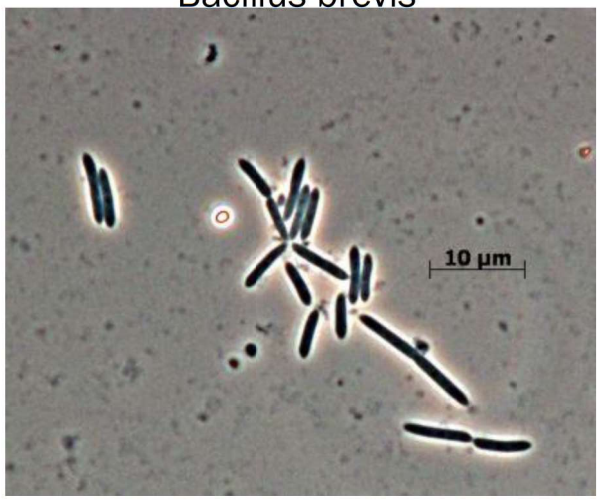
- World War II - search for antibiotics
- America
  - *American and British governments intensified their search for antibiotics. In March 1942, half of the total supply of penicillin owned by pharmaceutical giant Merck was used to treat a single infected patient.*
  - *Meanwhile a moldy cantaloupe in Illinois with a high concentration of penicillin was discovered.*
  - *2 million doses of penicillin in time for the Allied invasion of Normandy in 1944, thus saving thousands of wounded soldiers' lives.*

World War II Poster for Penicillin between 1942 and 1945

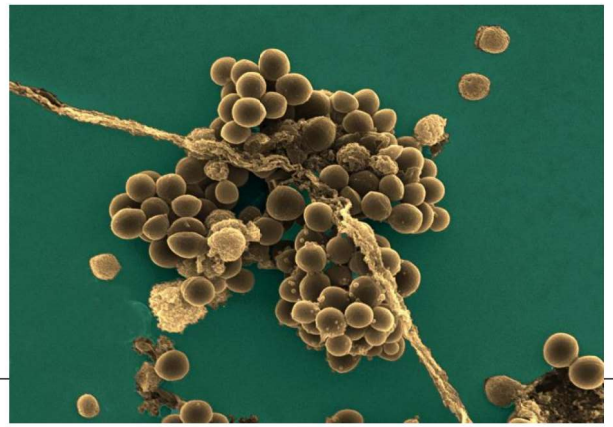
Discovery of Antibiotics

- World War II - search for antibiotics
- Russia
  - *in 1942, Russian biologists **Georgy Gause** and **Maria Brazhnikova** noticed that the **Bacillus brevis** bacterium killed the pathogenic bacterium **Staphylococcus aureus**.*
    - Bacillus brevis - It is rarely associated with infectious diseases. The antibiotics gramicidin and tyrocidine were first isolated from it.
    - Staphylococcus aureus – is a human pathogen responsible for food, respiratory and skin infections.
  - *contrast to Fleming's efforts with penicillin, they successfully isolated the antibiotic compound from Bacillus brevis and named it **Gramicidin Soviet.***
  - *Within a year, this antibiotic was distributed to Soviet military hospitals.*

Bacillus brevis

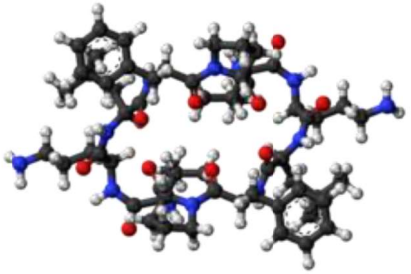


Staphylococcus aureus



Discovery of Antibiotics

- English biochemist Richard Synge studied Gramicidin Soviet and a wide array of other antibiotics produced by Bacillus brevis.
- A few years after World War II ended, he demonstrated that they represent **short amino acid sequences (i.e., mini-proteins) called peptides.**
  - *Gause received the Stalin Prize in 1946, and Synge won the Nobel Prize in 1952.*



Challenges in Developing new Antibiotics

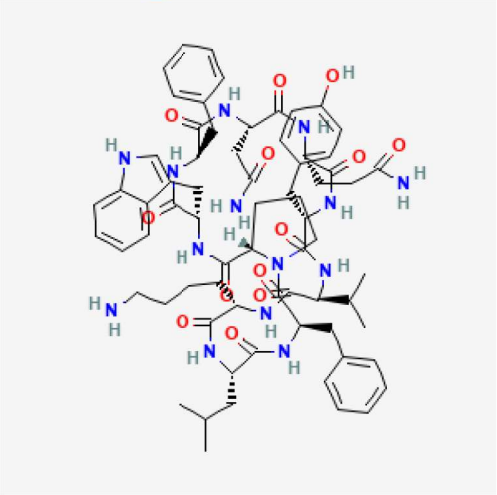
- Evolutionary arms race between pharmaceutical companies(***develop new antibiotic drugs,)*** and pathogenic bacteria (***acquire resistance against these drugs)***
- Alarming rise in antibiotic-resistant bacterial infections that cannot be treated even by the most powerful antibiotics.
  - *Staphylococcus aureus bacterium that Gause had studied in 1942 mutated into a resistant strain known as* ***Methicillin-resistant Staphylococcus aureus (MRSA).***
  - *MRSA is now the leading cause of death from infections in hospitals; its death rate has even passed that of AIDS in the United States.*

Challenges in Developing new Antibiotics

- **De**veloping new antibiotics represents a central challenge to modern medicine.
- A difficult problems in antibiotics research
  - *sequencing newly discovered antibiotics*
  - *determining the order of amino acids making up the antibiotic peptide.*

How do bacteria make Antibiotics

- ***Tyrocidine B1***, one of many antibiotics produced by ***Bacillus brevis.***
- Tyrocidine B1 is defined by the 10 amino acid-long sequence shown below



Val-Lys-Leu-Phe-Pro-Trp-Phe-Asn-Gln-Tyr

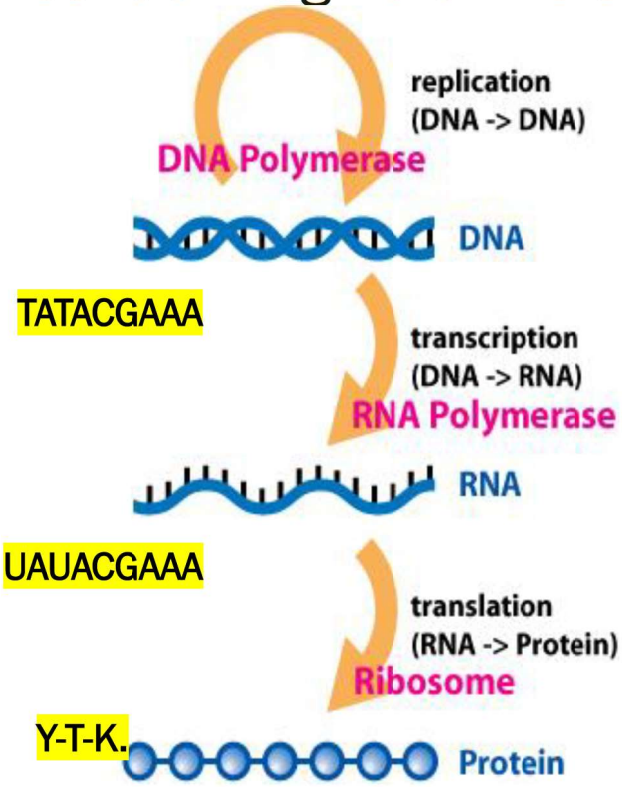
V K L F P W F N Q Y

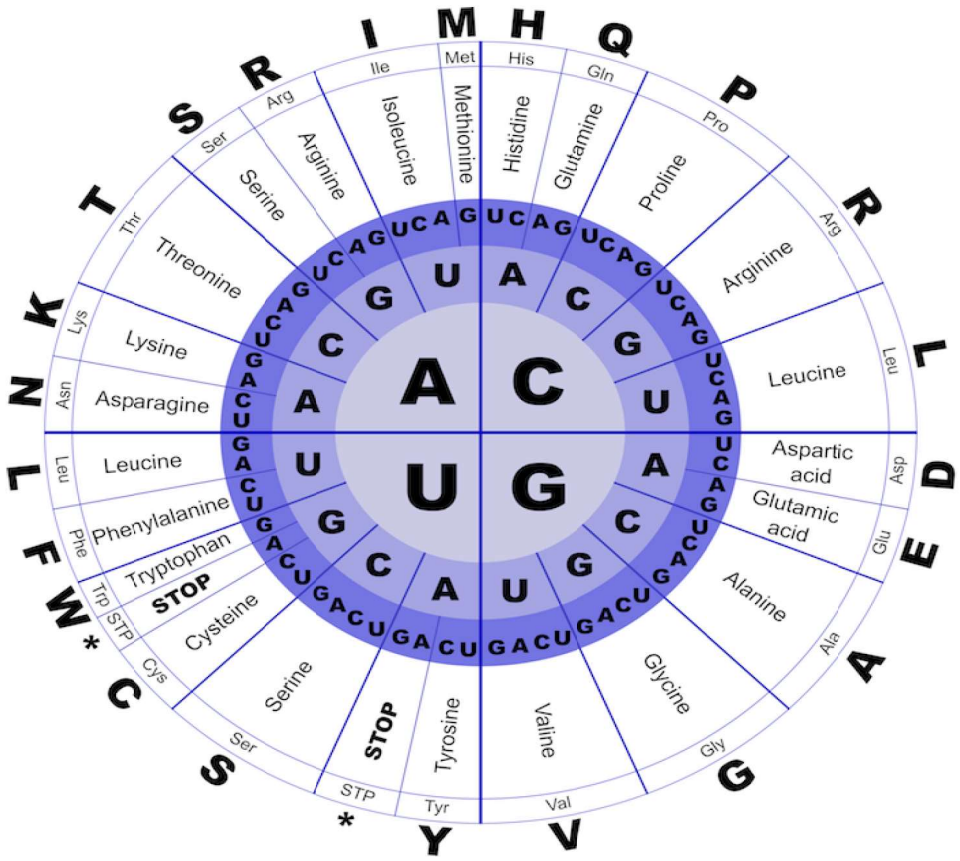
Valine – Lysine – Leucine- Phenylalanine- Proline- Tryptophan-Phenylalanine- Asparagine- Glutamine- Tyrosine

How Bacillus brevis could have made this antibiotic?

How do bacteria make Antibiotics

- Central Dogma of Molecular Biology





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How do bacteria make Antibiotics

- The array GeneticCode contains 64 elements, each of which is an amino acid or a stop codon.

0	AAA	K	16	CAA	Q	32	GAA	E	48	UAA	*
1	AAC	N	17	CAC	H	33	GAC	D	49	UAC	Y
2	AAG	K	18	CAG	Q	34	GAG	E	50	UAG	*
3	AAU	N	19	CAU	H	35	GAU	D	51	UAU	Y
4	ACA	T	20	CCA	P	36	GCA	A	52	UCA	S
5	ACC	T	21	CCC	P	37	GCC	A	53	UCC	S
6	ACG	T	22	CCG	P	38	GCG	A	54	UCG	S
7	ACU	T	23	CCU	P	39	GCU	A	55	UCU	S
8	AGA	R	24	CGA	R	40	GGA	G	56	UGA	*
9	AGC	S	25	CGC	R	41	GGC	G	57	UGC	C
10	AGG	R	26	CGG	R	42	GGG	G	58	UGG	W
11	AGU	S	27	CGU	R	43	GGU	G	59	UGU	C
12	AUA	I	28	CUA	L	44	GUA	V	60	UUA	L
13	AUC	I	29	CUC	L	45	GUC	V	61	UUC	F
14	AUG	M	30	CUG	L	46	GUG	V	62	UUG	L
15	AUU	I	31	CUU	L	47	GUU	V	63	UUU	F

How do bacteria make Antibiotics

- **Protein Translation Problem**

Translate an RNA string into an amino acid string.

Input: An RNA string Pattern and the array GeneticCode.

Output: The translation of Pattern into an amino acid string Peptide.

How many DNA strings of length 30 transcribe and translate into Tyrocidine B1?

Where is Tyrocidine encoded in the Bacillus brevis genome?

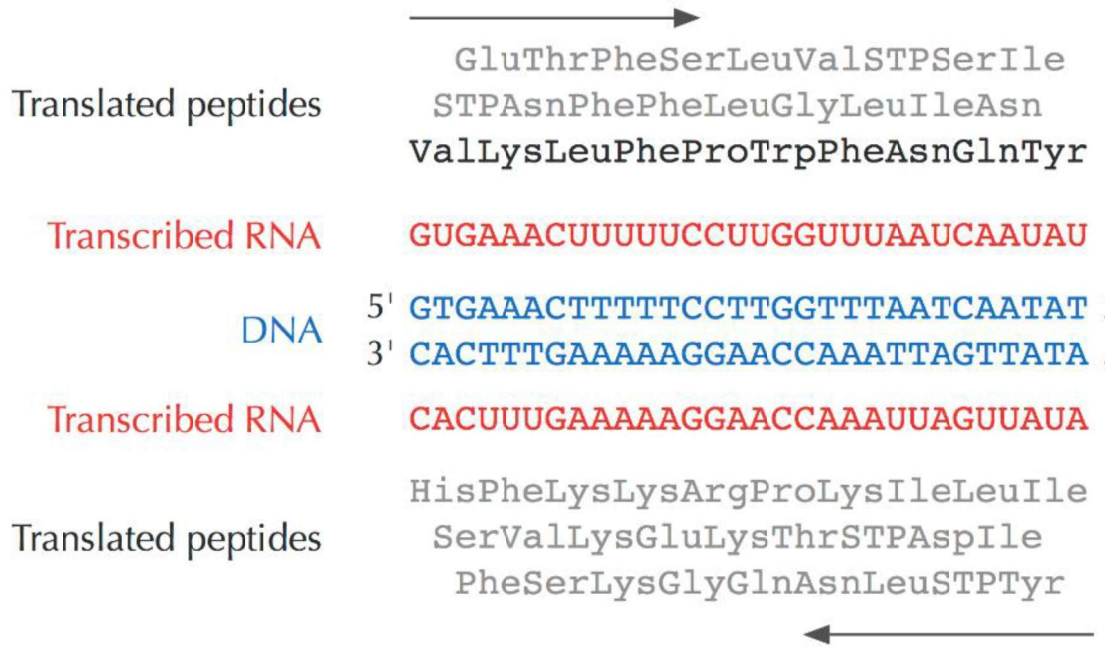
- Thousands of different DNA 30-mers could code for Tyrocidine B1

```
Val-Lys-Leu-Phe-Pro-Trp-Phe-Asn-Gln-Tyr
 V   K   L   F   P   W   F   N   Q   Y
```

- **Reading frames**
- There are three different ways to divide a DNA string into codons for translation,
  - *one starting at each of the first three starting positions of the string*.
- Since DNA is double-stranded, a genome has six reading frames (three on each strand)

```
1. ATG CAA TGG GGA AAT GTT ACC AGG TCC GAA CTT ATT GAG GTA AGA CAG ATT TAA
2. A TGC AAT GGG GAA ATG TTA CCA GGT CCG AAC TTA TTG AGG TAA GAC AGA TTT AA
3. AT GCA ATG GGG AAA TGT TAC CAG GTC CGA ACT TAT TGA GGT AAG ACA GAT TTA A
```

Where is Tyrocidine encoded in the Bacillus brevis genome?



Six reading frames give six different ways for the same fragment of DNA to be transcribed and translated. The highlighted amino acid string spells out the sequence of Tyrocidine B1

Where is Tyrocidine encoded in the Bacillus brevis genome?

- We say that a DNA string ***Pattern*** encodes an amino acid string ***Peptide*** if the RNA string transcribed from either *Pattern* or its reverse complement translates into Peptide.
- For example, the DNA string **GAAACT** is transcribed into **GAAACU** and translated into **ET**.
- The reverse complement of this DNA string, **AGTTTC**, is transcribed into **AGUUUC** and translated into **SF**.
- Thus, GAAACT encodes both ET and SF.

Peptide Encoding Problem

- **Peptide Encoding Problem**:

Find substrings of a genome encoding a given amino acid sequence.

Input: A DNA string Text, an amino acid string Peptide, and the array GeneticCode.

Output: All substrings of Text encoding Peptide (if any such substrings exist).

Solving the Peptide Encoding Problem for Tyrocidine B1

- How many starting positions in Bacillus brevis encode this peptide?
- Result
- No 30-mer in the Bacillus brevis genome encoding Tyrocidine B1

How could a bacterium produce a peptide that is not encoded by the bacterium's genome?

Summary

- Discovery of Antibiotics
- How do bacteria make Antibiotics
- Peptide Encoding Problem
- Solving the Peptide Encoding Problem for Tyrocidine B1