

Bacteriocin and Quorum Sensing

-Struggle for existence of lactic acid bacteria-

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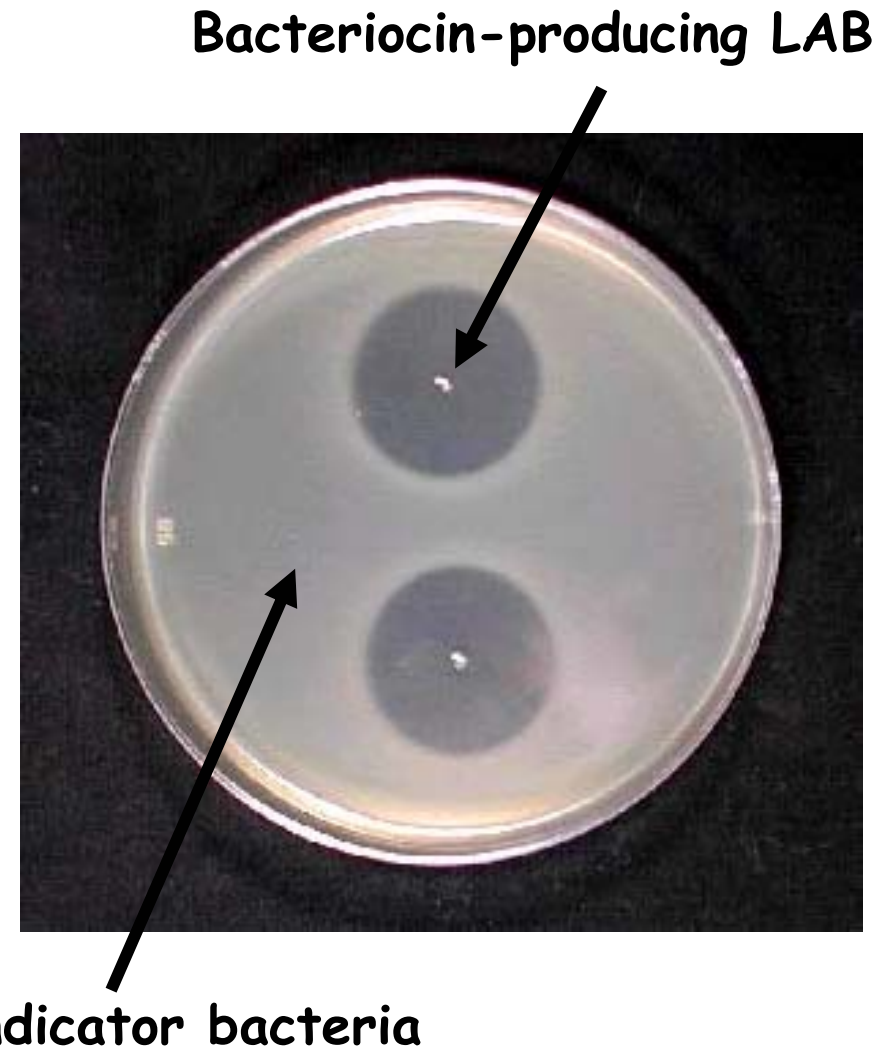


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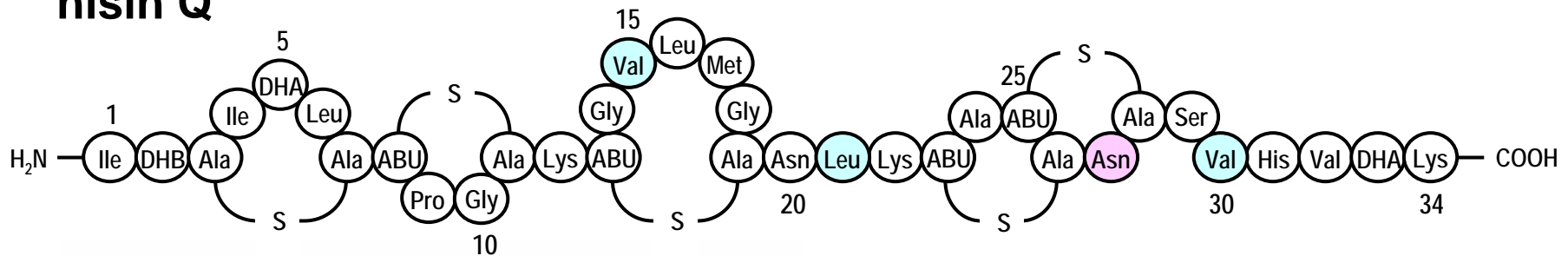
What is "Bacteriocin"

- Peptidic compounds ribosomally-synthesized in bacteria, which show antibacterial activity against normally closely-related species or different strains of same species.
- Generally NO effect on eukaryotic cells
- Degradable by proteolytic enzyme.
- LAB bacteriocins are notably applicable as safe food preservatives.

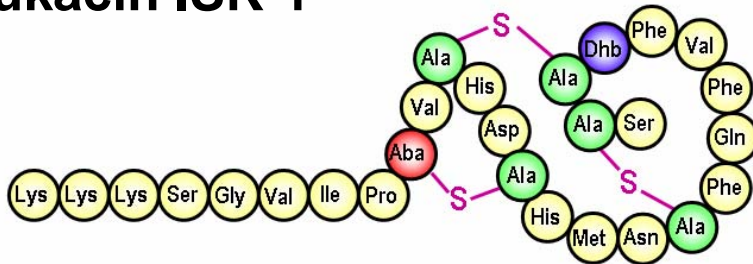


Class I (lantibiotic)

nisin Q



nukacin ISK-1



Ala-S-Ala: ランチオニン
 Abu-S-Ala: 3-メチルランチオニン
 Dha: デヒドロアラニン
 Dhb: デヒドロブチリン

Class IIb (two peptide bacteriocin)

Abp118 α : KRGPN CVGNFLGGLFAGAAAGVPLGPAGIVGGANLGMVGGALTCL

Abp118 β : KNGYGGSGNRWVHCGAGIVGGALIGAIGGPWSAVAGGISGGFTSCR

Class IIa

pediocin PA-1



Classification of bacteriocins produced by LAB

Class	Property	Bacteriocins
I	Lantibiotic; small peptides containing the unusual amino acid Lan, MeLan and dehydrated amino acids	Nisin A, Nisin Q, Nisin Z, Lacticin 481, Lactocin S
II	Small and heat-stable Non-Lan-containing a: antilisterial activity peptides with YGNGV consensus sequence b: two-peptide bacteriocins c: other bacteriocins	Pediocin PA-1, Sakacin P Enterocin A, Lactococcin Q, ABP-118, Durancin EF Lacticin Q
III	Large (>30kDa) heat-labile proteins	Helveticin J, Enterolysin A Millericin B
IV	Complex bacteriocins carrying lipid or carbohydrate moieties	Unknown
V	Circular peptides	Enterocin AS-48 Gassericin A

Bacteriocin-producing lactic acid bacteria in our laboratory

Species	Strain	Source	Bacteriocin	Class	Molecular Mass	Antibacterial Spectrum
<i>Lactococcus lactis</i>	IO-1	Kitchen sink	Nisin Z	I	3331	Broad
<i>Lactococcus lactis</i>	Chizuka, Yasaka	<i>Nukadoko</i> (Fermented rice bran)	Nisin Z	I	3331	Broad
<i>Lactococcus lactis</i>	QU 1	Seaweed	Nisin Z	I	3331	Broad
<i>Lactococcus lactis</i>	QU 3	Corn	Nisin Z	I	3331	Broad
<i>Lactococcus lactis</i>	QU 8	Carrot	Nisin Z	I	3331	Broad
<i>Lactococcus lactis</i>	g56-3	Chinese cabbage	Nisin Z	I	3331	Broad
<i>Lactococcus lactis</i>	KD12	<i>Komatsuna</i>	Nisin Z	I	3331	Broad
<i>Lactococcus lactis</i>	61-14	River water	Nisin Q	I	3327	Broad
<i>Lactococcus lactis</i>	QU 4	Corn	Lactococcin Q	IIb	4260/4018	Specific
<i>Lactococcus lactis</i>	QU 5	Corn	Lacticin Q	IIc	5926	Broad
<i>Enterococcus faecium</i>	WHE81	Soft cheese	Enterocin A, B	IIa,IIc	4829/5463	Moderate
<i>Enterococcus mundtii</i>	QU 2	Soybean	Mundticin KS	IIa	4287	Moderate
<i>Enterococcus durans</i>	QU 21	Flower	Durancin TW-21	IIc	5228	Moderate
<i>Enterococcus durans</i>	QU 49	Carrot	Durancin TW-49	IIc	5228	Moderate
<i>Lactobacillus curvatus</i>	k12-3	Flower	Curvacin A	IIa	4310	Moderate
<i>Lactobacillus salivarius</i>	AC21	Chicken intestine	Salivacin K21	IIb	4097/4283	Broad
<i>Enterococcus faecium</i>	NKR-5-3	Pla-ra (Fermented fish)	Brochocin A etc.	II	5243/etc.	Broad

Topics Today

- A variety of bacteriocins with different antimicrobial spectra and with different mode of actions, produced by *Lactococcus lactis* isolated from corn.
- Diversity of bacteriocins and their immunities in enterococci
- *Enterococcus faecium* NKR-5-3 isolated from Pla-ra: Multiple bacteriocins and their production control

Antibacterial spectra of various *L. lactis* strains isolated from corn

Indicator strain	Producer: Bacteriocin:	Activity (AU/mL)			
		QU1	OU4	OU5	OU6
		nisin Z	lactococcin Q	lacticin Q	Unknown
<i>Micrococcus luteus</i>		800	0	0	6400
<i>Bacillus subtilis</i>		3200	0	400	0
<i>Bacillus circulans</i>		12800	0	200	0
<i>Bacillus coagulans</i>		25600	0	819200	0
<i>Listeria innocua</i>		3200	0	800	0
<i>Lactobacillus sakei</i>		204800	0	6400	0
<i>Lactobacillus plantarum</i>		800	0	1600	6400
<i>Enterococcus faecalis</i>		400	0	800	3200
<i>Leuconostoc mesenteroides</i>		3200	0	400	200
<i>Pediococcus pentosaceus</i>		800	0	200	0
<i>Enterococcus faecium</i>		400	0	400	0
<i>Lactococcus lactis</i> ssp. <i>lactis</i>		800	6400	3200	0
<i>Lactococcus lactis</i> ssp. <i>lactis</i>		0	25600	3200	0
<i>Escherichia coli</i>		0	0	0	0

Lactococcin Q: novel class IIb bacteriocin

Lactococcin Q α (39 a.a.)

S I W G D I G Q G V G K A A Y W V G K A M G N M S D V N Q A S R I N R K K K H

Lactococcin Q β (35 a.a.)

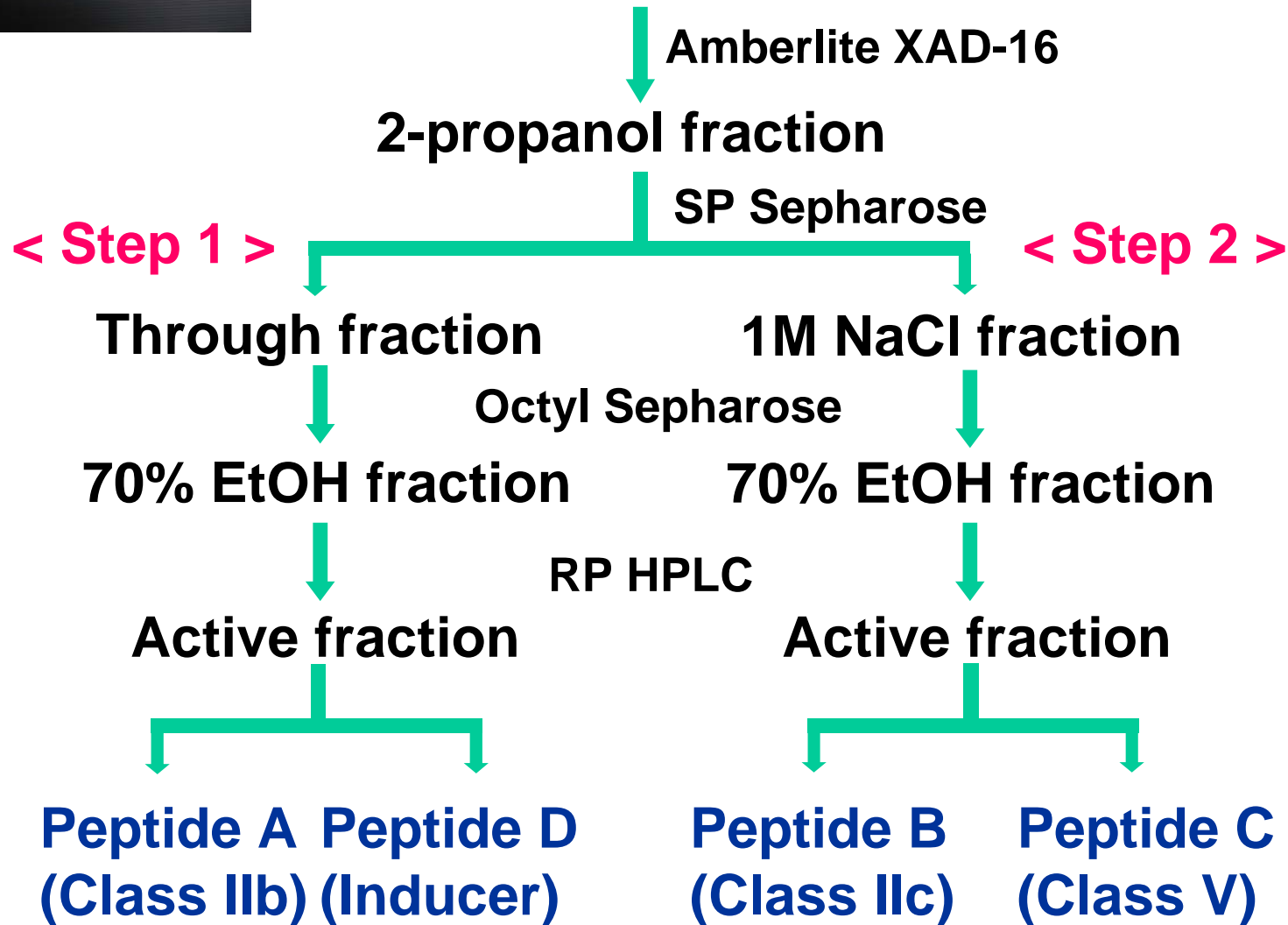
K K W G W L A W V E P A G E F L K G F G K G A I K E G N K D K W K N I

- Produced by *Lc. lactis* QU 4 isolated from corn
- Novel two-peptide (**class IIb**) bacteriocin
- Synergistic action of peptides α and β
- Active **only against** *Lactococcus lactis* strains



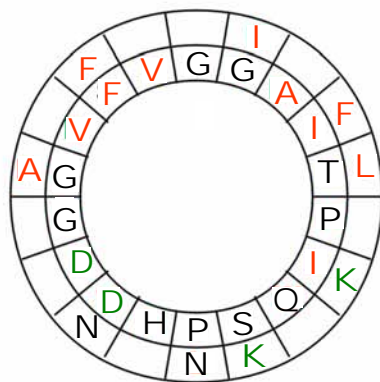
Purification of bacteriocins produced by *Enterococcus faecium* NKR-5-3

M17 Culture supernatant

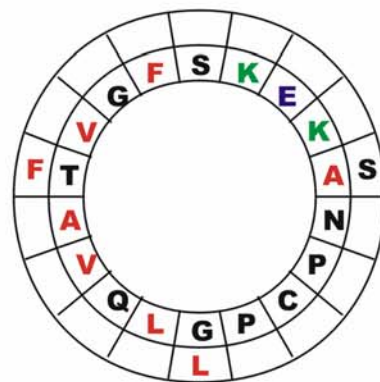


Known autoinducing peptides which induce bacteriocin production

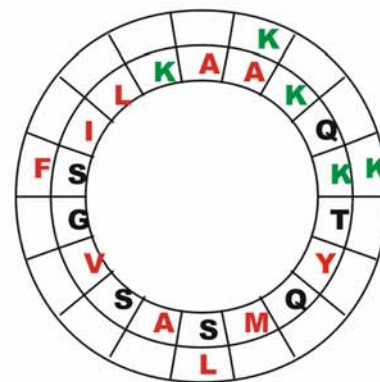
Inducer	Bacteriocin	Amino acid sequence	
		Leader peptide	Mature peptide inducer
EntF	Enterocin A	MEEKNRLNAKQCSDQELKKIKGG	AGT - KPOGKPASNLVECVFSLFKKCN
PisN	Piscicolin 126	MNDKKYLKLKECSEKLLKQIQGG	NKS - VIKGNPASNLAQCVFSFFKKC
CbaX	Carnobacteriocin A	MKI KYITRKQLIQIKGG	SIN - SQIGKATSSISKCVFSFFKKC
CbnS	CarnobacteriocinB2	MKI KTITKKQLIQIKGG	SKN - SQIGKSTSSISKCVFSFFKKC
Sap-Ph	Sakacin A	MKLN YIEKKQLTNKQLKLIIGG	- TN - RNYGKPNKDIGTCIWSGFRHC
AbpIP	ABP118	MKFEVLTEKKLQKIAGG	ATK - KGGFKR - - - WQCIFTFFGVCK
Pln A	Plantaricin EF/JK	MKI QIKGMKQLSNKEMQKIVGG	KSS - AYSLQMGATAIKQVKKLFFKKWGW
PLNC8IF	Plantaricin NC8	MKNINKYTELNDQKLQSLIGG	KTK - TISLMSGLOVPHAFTKLLKALGGHH
SppIP	Sakacin P	MMIFKKLSEKELQKINGG	- - - - -MAGNSSNF IHKIKQIFTHR
IP-TX	Sakacin TX	MTNRK TLPKEELKKIKGG	TPGGFDIISGGPHVAQDVLNAIKDFFK
PeptideD	NKR-5-3	MTNRKILPKEELKKIKGG	TPGGFDIISGGPHVAQDVLNAIKNFFK



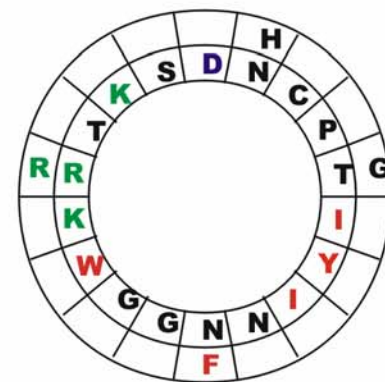
Peptide C
(*Ec. faecium*)



EntF
(*Ec. faecium*)

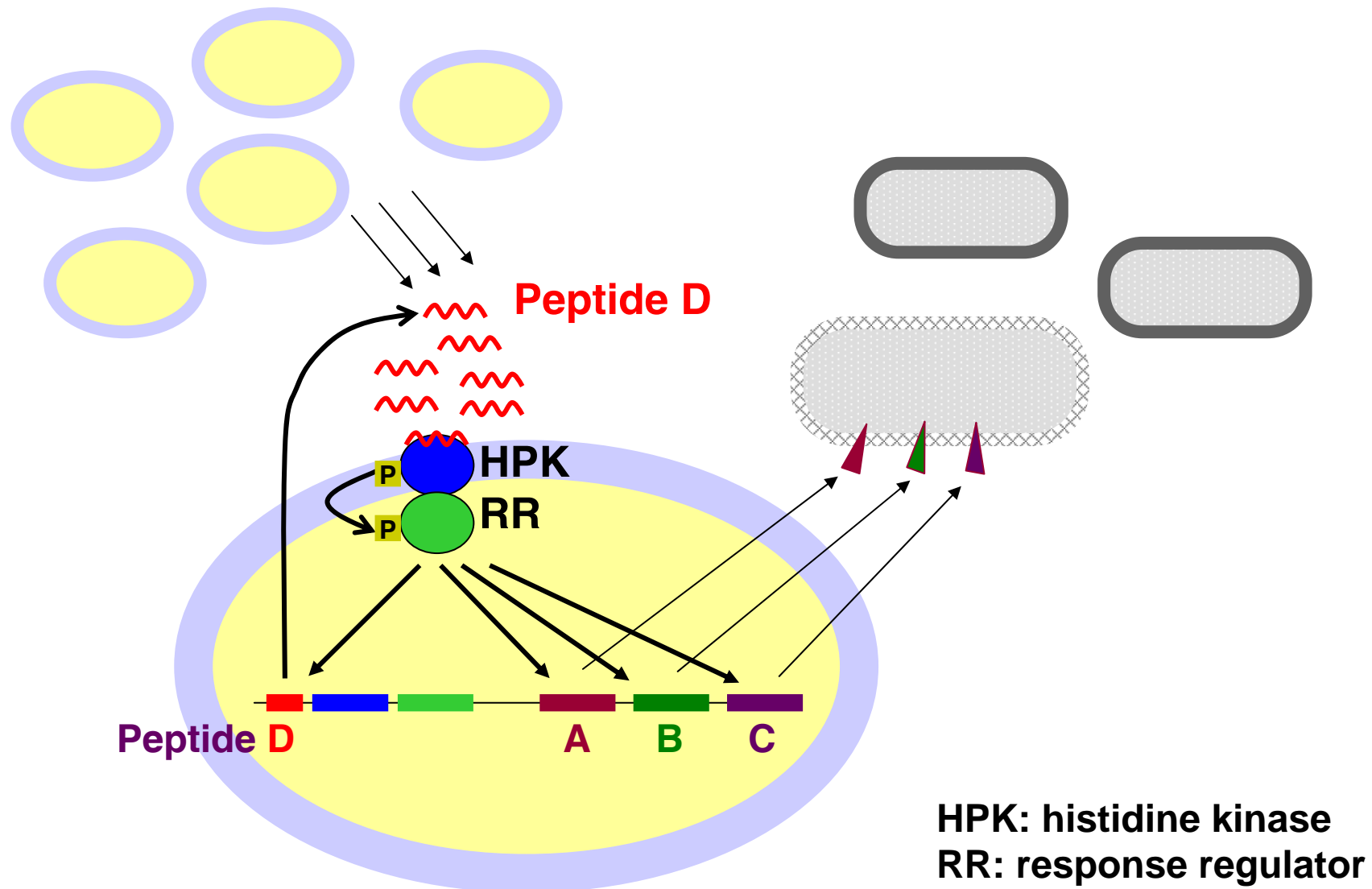


PlnA
(*Lb. plantarum*)



SapA
(*Lb. sake*)

Concerted control of bacteriocin production by peptide-mediated quorum sensing



Concluding Remarks

- Even same species produces different bacteriocins with different antimicrobial spectra and with different mode of actions.
(*L. lactis* QU3, QU4, QU5 and QU6 isolated from corn)
- One strain sometimes produces more than one bacteriocins.
(*E. durans* QU21, QU49, *E. faecium* NKR-5-3)
- A immunity gene sometimes showed cross-immunity against different bacteriocin. (*E. faecium* JCM5804)
- Multiple bacteriocins are sometimes regulated by one autoinducing peptide. (*E. faecium* NKR-5-3)
- Complex network organized by various bacteriocins with various antimicrobial spectrum, their immunities, and their quorum sensing control, would explain "struggle for existence" of LAB in complex ecosystem.