A new psychrotolerant and histamine producing *Morganella* species

Results from the BIOCOM project in RTD pillar 3 'Seafood safety'

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Outline of the presentation

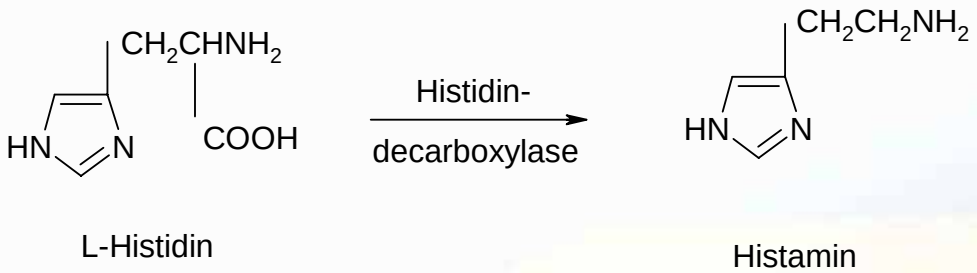
- Histamine Fish Poisoning (HFP)
- Outbreaks in Denmark 2004-2005
- *Morganella*
- IJSEM
- Identification of *Morganella*
- Perspectives

Histamine Fish Poisoning (HFP)

- Ingestion of high amounts of biogenic amines (histamine) can result in HFP
- HFP has clinical signs like an allergic reaction
- HFP has caused ~ 50 % of all finfish-borne cases of human disease in USA and UK

(Lehane and Olley 2000; Flick et al. 2001)

Histamine



- **Biogenic amine**
- **Potentiated by other biogenic amines?**
- **Produced by bacteria primarily in seafood**

Bacteria responsible for HFP are very rarely identified

Seafood	Bacteria	Place and time
Fresh tuna	*Morganella morganii*	Japan, 1955
Fresh tuna	*Hafnia* sp. ?	Prague, 1967
Fresh tuna	*Raoultella planticola*	California, 1977
Dried sardine	*Photobacterium phosphoreum*	Japan, 2002

Outbreaks/cases of HFP in Denmark (2004-2005)

Cases	Products	Histamine (mg/kg)	Histamine produced by
2	Tuna, cold-smoked	4500	*Photobacterium**
12	Tuna, cold-smoked	2000	*Morganella*-like*
2	Tuna sandwich	-	?
4	Escolar, fried	4100	?
9	Escolar	-	?
4	Tuna	3500	*Morganella morganii*
2	Tuna	-	?
10	Tuna, cold-smoked	1000	?
4	Sword fish	1500	?
7	Escolar	5000	?
2	Tuna	1700	?
5	Escolar, smoked	2300	?

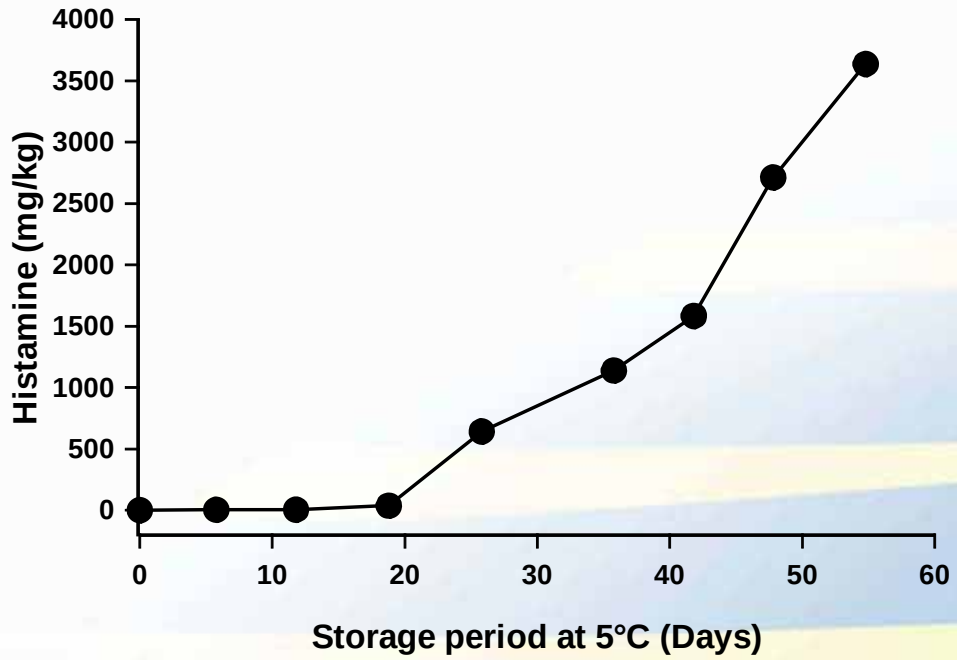
(* Emborg and Dalgaard 2006)

Until now histamine produced by Enterobacteriacea in temperature abused seafood was generally considered the major reason for HFP

	Histamine production	
	< 10°C	> 10°C
Enterobacteriaceae (*Morganella*)	-/+	++++
Psychrotolerant bacteria incl. *Photobacterium*	++	++
Lactic acid bacteria	+/-	++

- Psychrotolerant bacteria growing at 0-5°C has been stated to be less important (Lehane and Olley 2000)

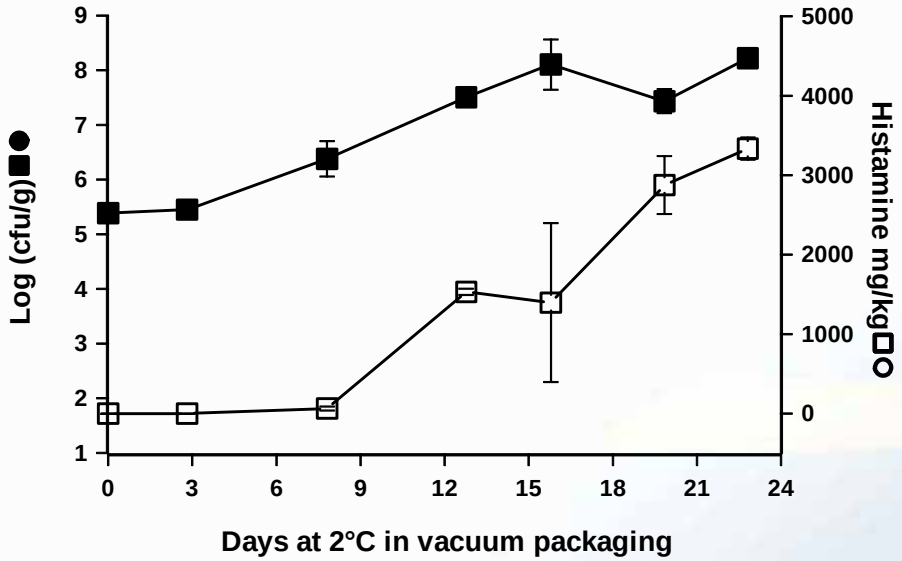
Histamine production by psychrotolerant bacteria

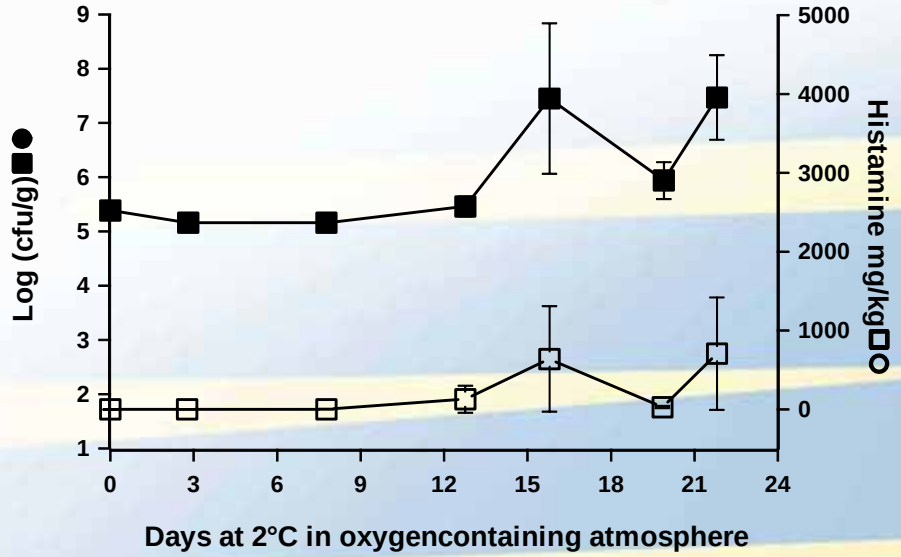


(Modified from Emborg and Dalgaard 2006)

- Maximum concentrations found within 3 packages
- Inoculated cold smoked tuna
- Sensory shelf life = 30 days

Histamine production of psychrotolerant bacteria (2)





(Modified from Emborg et *al.* 2005)

- Fresh tuna stored at 2°C
- Oxygen seems to delay the growth of psychrotolerant *Morganella*

Morganella

- **Enterobacteriaceae**
- **Until now only one species – *Morganella morganii***
- **Growth at 4 - 45°C**
- **Histamine production above 7 - 10°C**

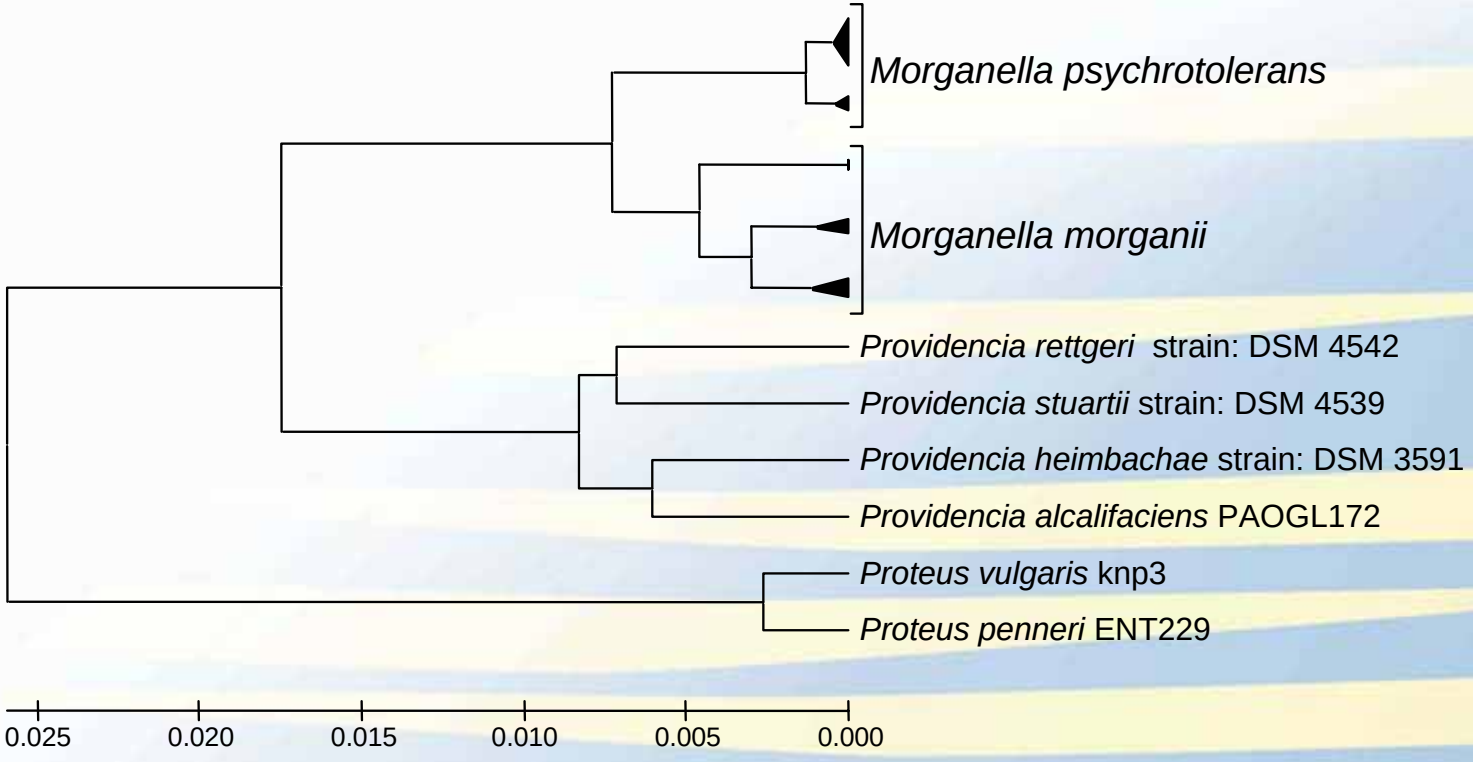
Biochemical identification of *Morganella*

- Gram-negative motile rods
- Glucose and a few other sugars are fermented
- Phenylalanine-deaminase positive
- Citrate negative
- Variable in lysin and ornithin decarboxylase, trehalose fermentation

Biochemical differentiation of *Morganella* species

Characteristic	*Morganella psychrotolerans*	*Morganella morganii*
Growth at:		
2°C	+	-
37°C	-	+
Growth in:		
8.5% NaCl	-	+
Fermentation of:		
Trehalose (48h)	(-)	Subspecies variable
Galactose (48h)	-	+

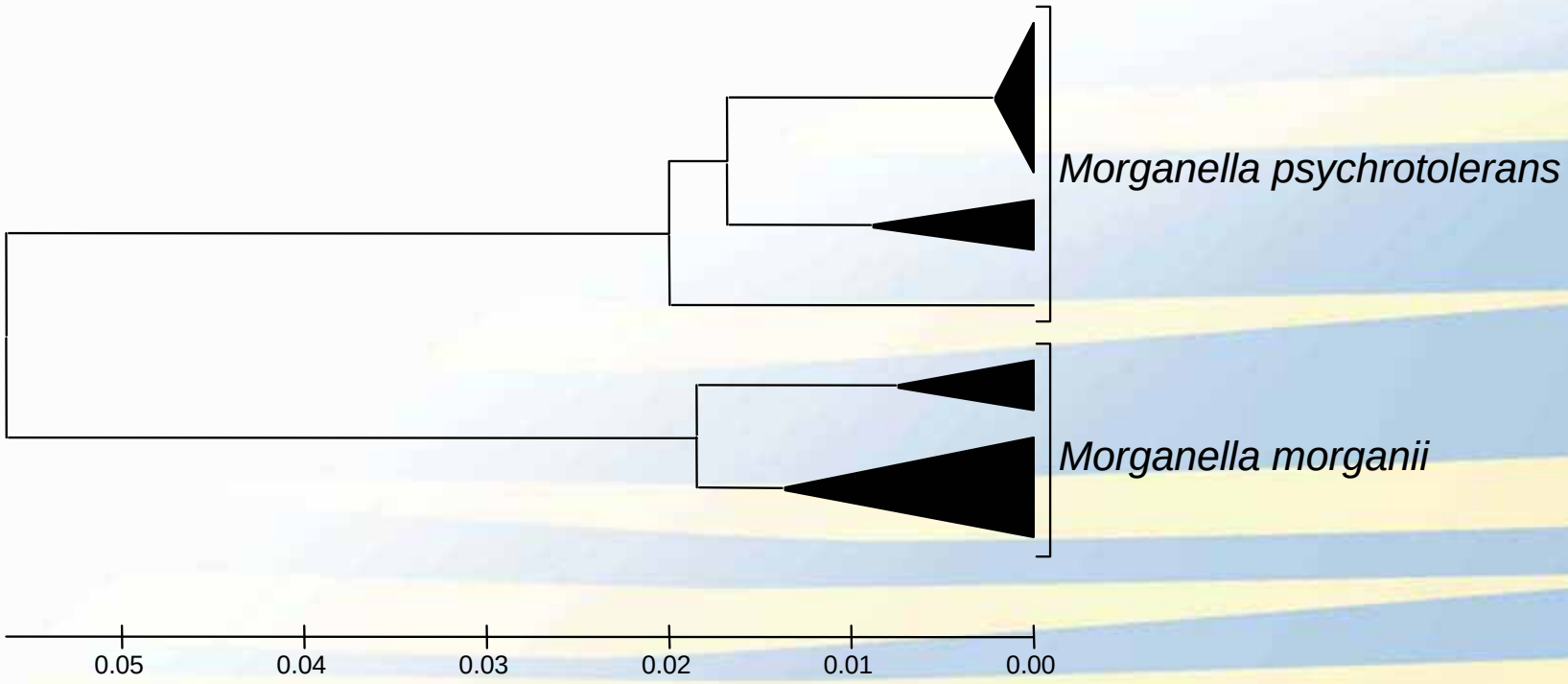
Identification of *Morganella* by 16S rDNA sequencing



Multilocus Sequencing

- 24 isolates of *Morganella*
- 7 genefragments from protein encoding housekeeping genes were sequenced by the Sanger method
- Aligned and analysed by Kimura´s 2 parameter distances, the methods of parsimony maximum and neighbour-joining.
- Reliability assessed by the bootstrap method

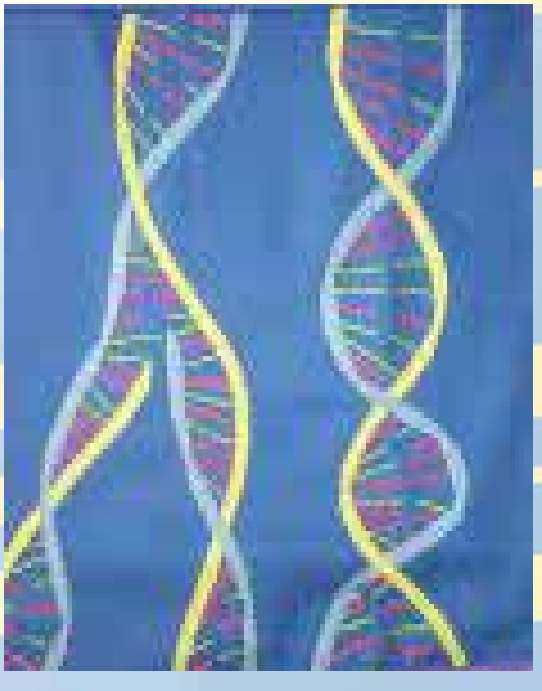
Multilocus sequencing of *Morganella*



DNA-DNA hybridisation

- **Treshold value for species seperation = 70%**

- ***M. psychrotolerans* have 18 - 41% DNA similarity to *M. morganella***



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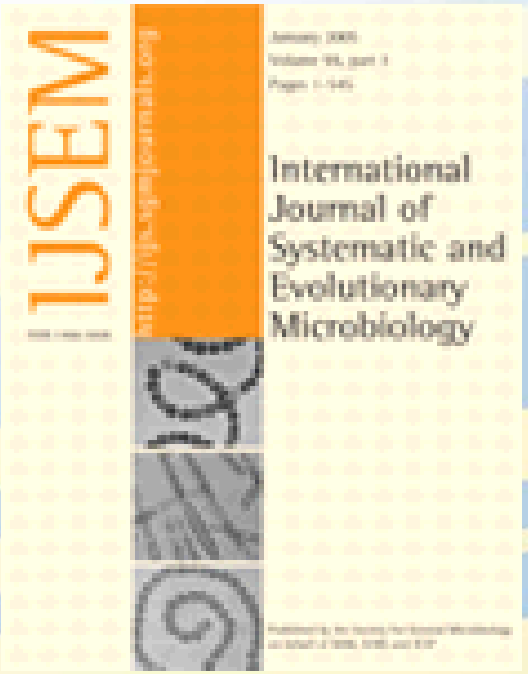
- Taxonomy
- Nomenclature
- Identification
- Characterization
- Culture preservation



Conclusions and perspectives

- New psychrotolerant and histamine producing species of *Morganella* with significant impact on food safety

- Reduce/minimize the frequency of HFP
  - Temperature
  - Salting
  - Packaging

- Prediction of
  - Histamine formation and concentration
  - Exposure assessment

- Detection
  - Polymerase chain reaction

Thanks to….

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And to you for your attention

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