



Survival kinetics of *Mycobacterium bovis* in raw milk cheese

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Background

- If a herd whose milk is destined for raw milk cheese production has a TB reactor what happens to the stock of cheese from the last test negative result?
- *M. bovis* – hazard group 3.
- Previous experience with *M. avium* subsp. *paratuberculosis* (hazard group 2).

Tasks

- Produce protocols for pilot-scale production that meet H&S standards.
- Devise media that will allow enumeration from a cheese matrix without decontamination.
- Demonstrate cheese produced has physicochemical properties of commercial cheese.

Experimental design

- High inoculum levels (10^6 cfu/ml).
- 3 strains x 2 cheeses x 3 replicates = 18.
- Preincubation of raw milk (22°C/16h).
- TVC, coliforms, LAB, antibiotics.
- Chemical analysis of irradiated cheese.
- Process control – titratable acidity.
- Validation by artisan cheesemaker.

Behaviour of *M. bovis* during raw milk cheesemaking

Cheesemaking under containment conditions.

Improved Cheese vat



Behaviour of *M. bovis* during raw milk cheesemaking

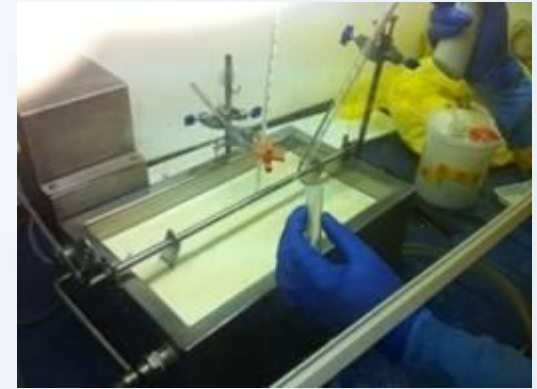
Cheesemaking under containment conditions.



1 Prepare vat



2 Add Milk



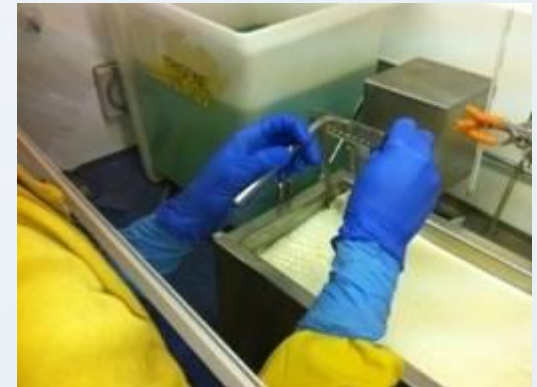
3 Add Inoculum



4 Add Starter



5 Renneting



6 Cutting

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Cheesemaking under containment conditions.



7 Scalding



8 Pitching



9 Whey Run



9 Whey Run



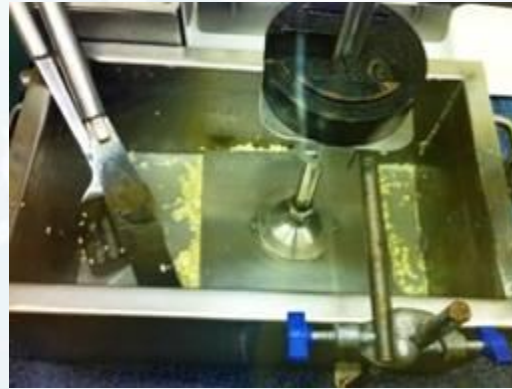
9 Finish draining

Behaviour of *M. bovis* during raw milk cheesemaking

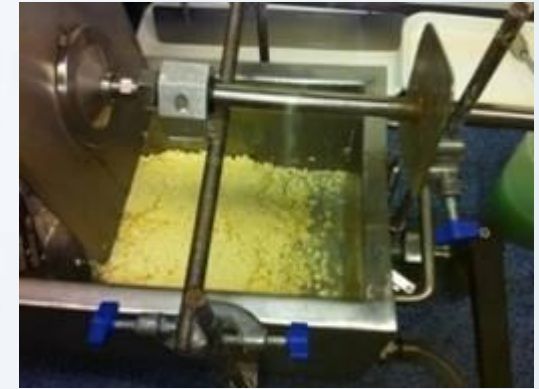
Cheesemaking under containment conditions - Cheddar.



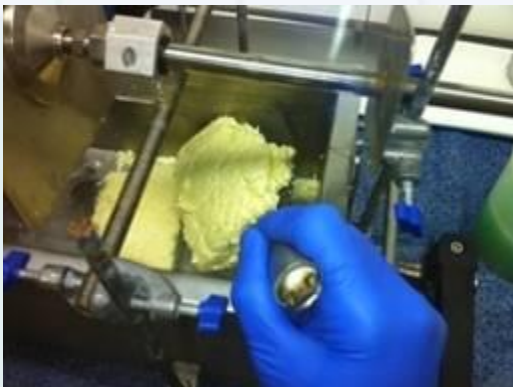
10 Channel



11 Mass on Plate 2 kg



12 Remove Mass



13 Cut & Stack Curd



14 Mass on Plate 8 kg



15 Cut & Stack Curd

Behaviour of *M. bovis* during raw milk cheesemaking

Cheesemaking under containment conditions - Cheddar.

Cheddaring has developed curd texture



Behaviour of *M. bovis* during raw milk cheesemaking

Cheesemaking under containment conditions - Cheddar.



16 Milling



16 Milling



17 Salting



18 Mould Filling



18 Mould Filling



19 Fix cheese Cloth

Behaviour of *M. bovis* during raw milk cheesemaking

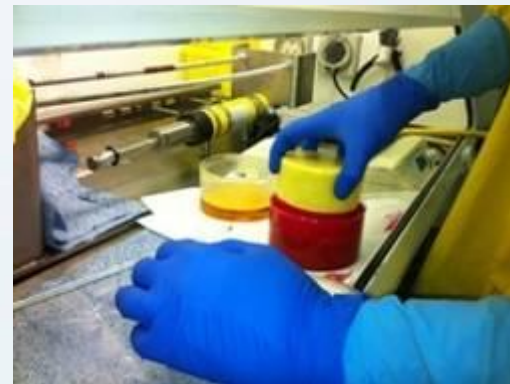
Cheesemaking under containment conditions - Cheddar.



20 Pressing



21 Clear Wax



22 Red wax



Behaviour of *M. bovis* during raw milk cheesemaking

Media development

Two main media types

Egg Based Media

1. Stonebrinks
2. Lowenstein Jensen

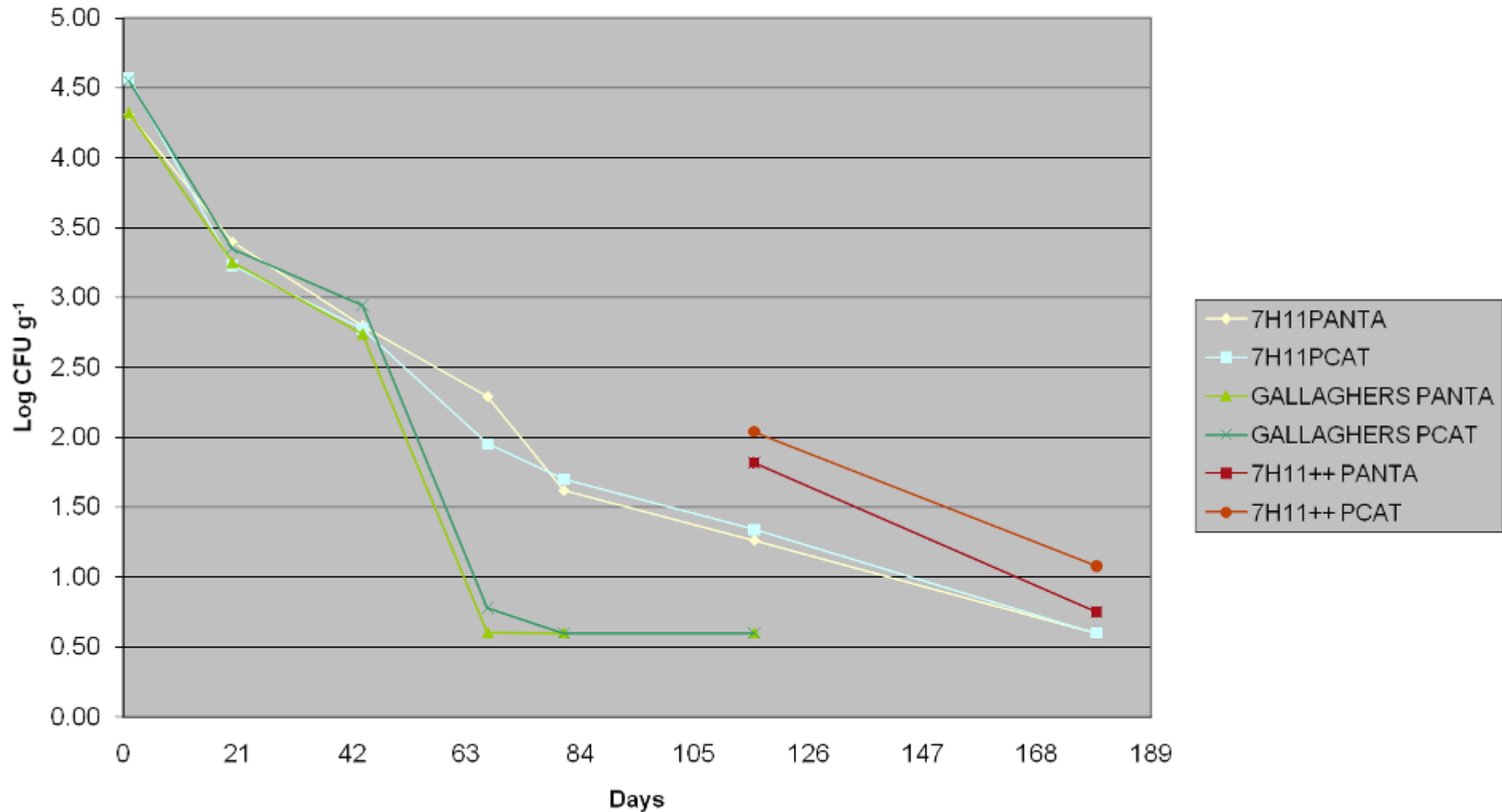
Agar Albumin Based Media

1. Middlebrook 7H10
2. Middlebrook 7H11
3. Gallagher's Media

Behaviour of *M. bovis* during raw milk cheesemaking

Media development

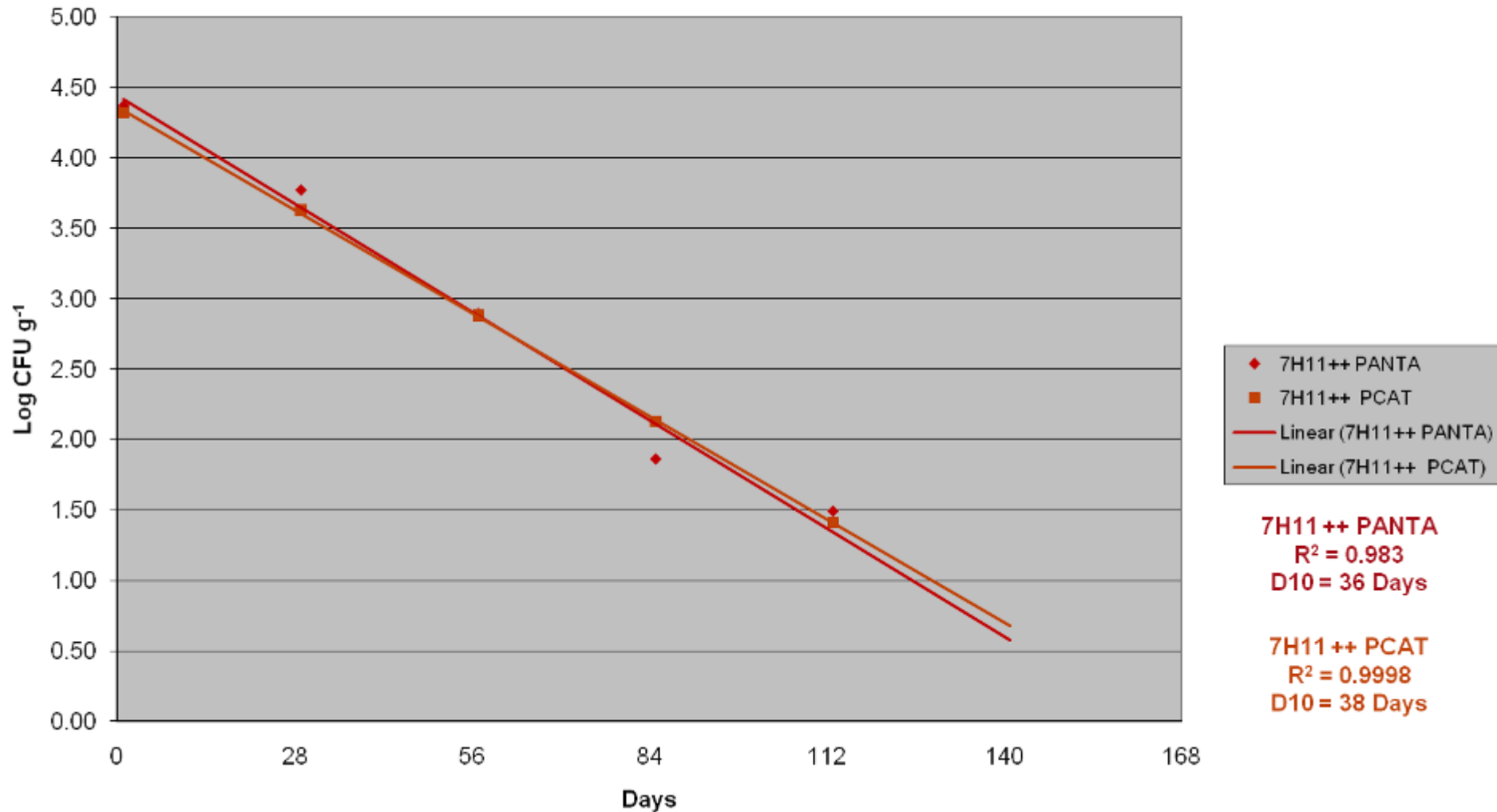
Growth *M. bovis* AF2122 Cheddar Media Trial



Behaviour of *M. bovis* during raw milk cheesemaking

Challenge Test Work

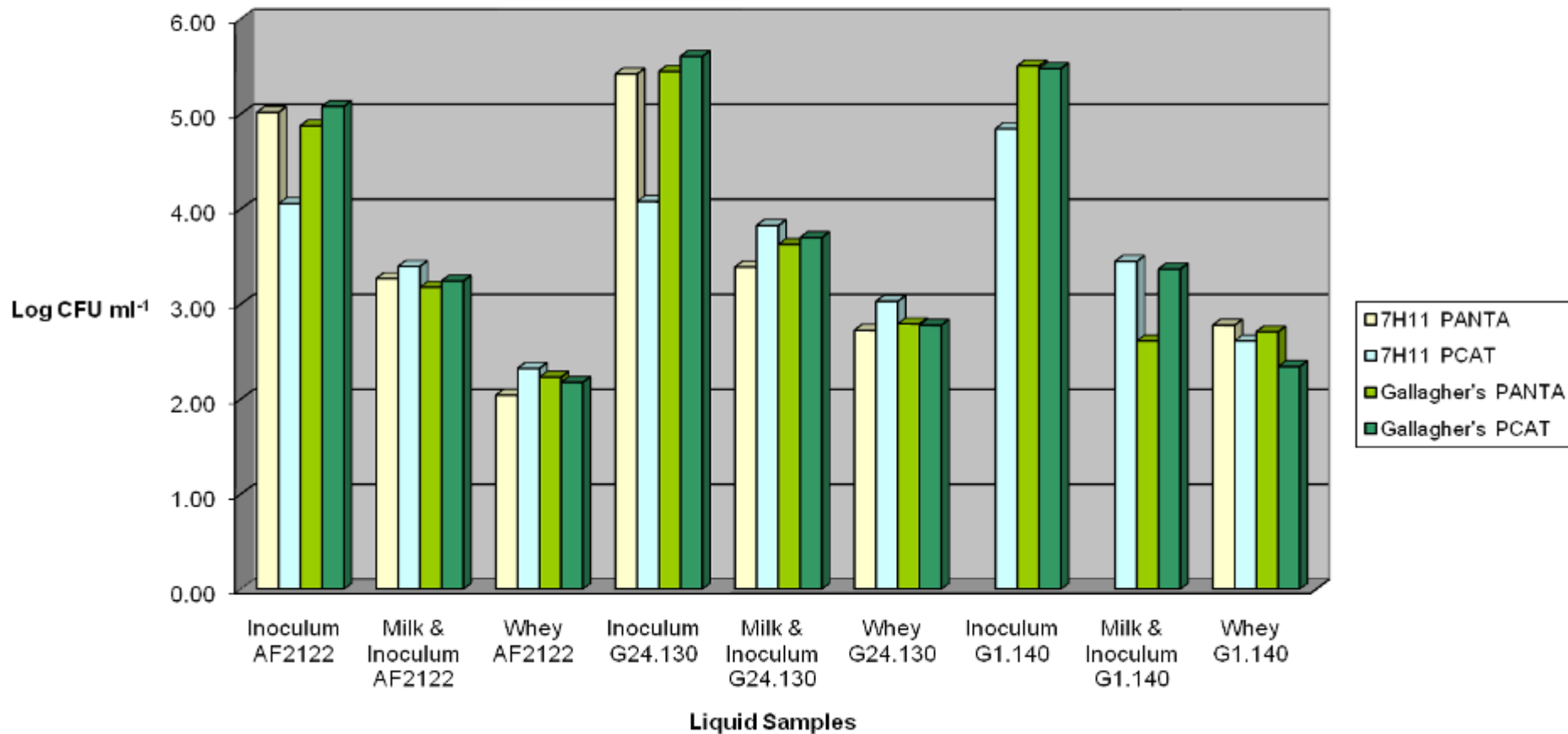
Inactivation *M. bovis* AF2122 in Cheddar 1



Behaviour of *M. bovis* during raw milk cheesemaking

Media development

Initial Levels *M. bovis* Media Trial - Cheddar



Behaviour of *M. bovis* during raw milk cheesemaking

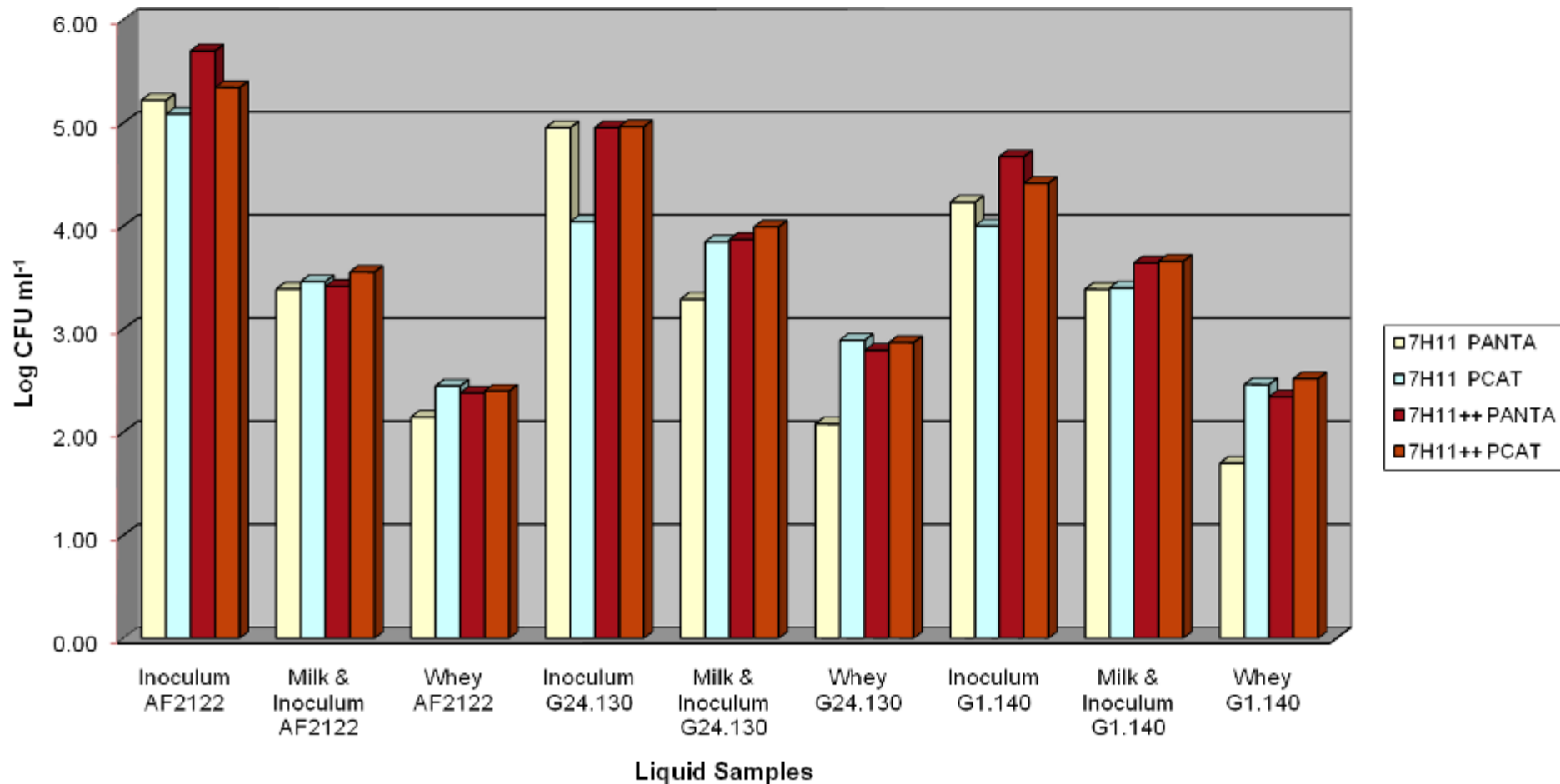
Challenge Test Work

Cheddar D10 Values (Days)			
	Cheddar 1	Cheddar 2	Cheddar 3
AF2122	37	33	42
G24.130	37	43	47
G1.140	51	81	66
Grand Mean		48	

Behaviour of *M. bovis* during raw milk cheesemaking

Media development

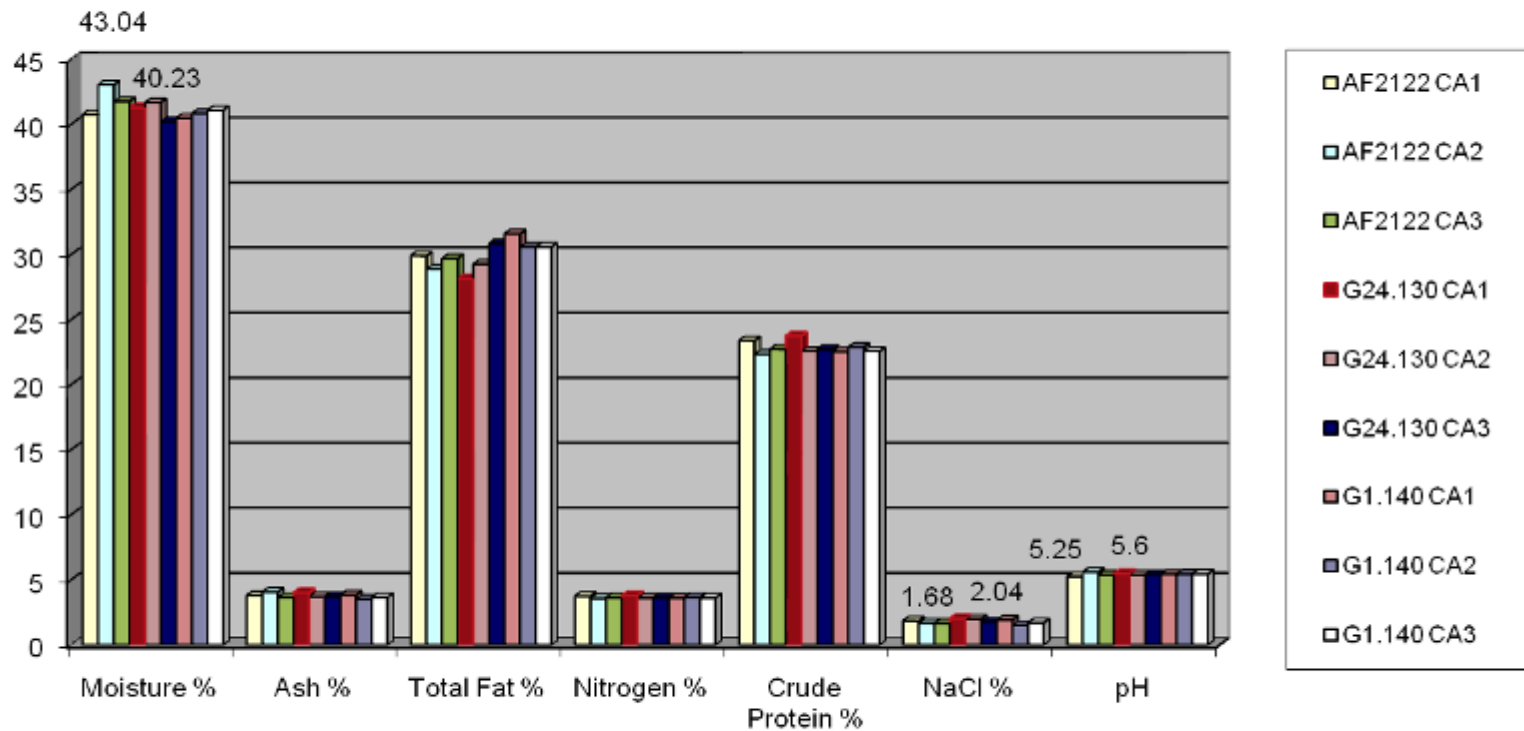
Initial Levels *M. bovis* Media Trial - Caerphilly



Behaviour of *M. bovis* during raw milk cheesemaking

Challenge Test Work

Caerphilly Day 1 Chemical Analysis



Behaviour of *M. bovis* during raw milk cheesemaking

Challenge Test Work

Caerphilly D10 Values (Days)			
	Caerphilly 1	Caerphilly 2	Caerphilly 3
AF2122	52	62	34
G24.130	131	35	27
G1.140	79	54	45
	Grand Mean	58	

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Challenge Test Work

Conclusions

1. Have developed a method which allows the production of cheese in CL3 Laboratory.
2. Have selective media which allow the isolation of *M. bovis* from raw milk cheeses without decontamination.
3. Performed challenge test work which has allowed the calculation of an overall average D_{10} for *M. bovis* in Cheddar of 48 days and in Caerphilly of 58 days.

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Challenge Test Work

Inactivation *M. bovis* G24.130 in Caerphilly 1

