Application of Metagenomics to the Valorization of Kenyan Traditional Spontaneously Fermented Milk Products

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Introduction: Fermentation

- Fermentation is the metabolic process in which carbohydrates and related compounds are partially oxidised, with the release of energy, in the absence of any external electron acceptors.
- The final electron acceptors are organic compounds produced directly from the breakdown of carbohydrates.
- With fermentation, incomplete breakdown of the parent compound occurs and only a small amount of energy is released during the process.
- The products of fermentation consist of some organic compounds that are more reduced than others.

Fermented Foods

- Foods that have been subjected to the action of microorganisms or enzymes, in order to bring about a desirable change.

- Numerous food products owe their production and characteristics to the fermentative activities of microorganisms.

- Fermented foods originated many thousands of years ago when presumably micro organisms contaminated local foods and comprise about 1/3 of the worldwide consumed food.

Benefits of Food Fermentation

Benefit	Raw material	Fermented food
Preservation	Milk (Most materials)	Yoghurt, cheese
Enhancement of safety		
Acid production	Fruit	Vinegar
Acid and alcohol production	Barley	Beer
	Grapes	Wine
Production of bacteriocins	Meat	Salami
Removal of toxic components	Cassava	Gari, polviho azedo
	Soybean	Soy sauce
Enhancement of nutritional value		
Improved digestibility	Wheat	
Retention of micronutrients	Leafy veges.	
Increased fibre content	Coconut	
Synthesis of probiotic compounds	Milk	

Spontaneously Fermented Milk Products

Kule naoto (Maasai)

Mursik (Kalenjin)

Bongo (Uganda)

Amabere Amaruranu (Kisii)

Suusac (Suusa) (N.E. Pastoralists)

Emasi (Swaziland)

Fermentation for the production of these products is uncontrolled and dependent on microorganisms from the environment

Controlled Vs Uncontrolled Fermentation

Uncontrolled (Spontaneous fermentation)

- Inefficient production
- Safety & quality not assured,
- Limited shelf life,
- Narrow acceptance

Controlled fermentation

- Efficient production
- Safety & quality are assured,
- Extended shelf life,
- Broader acceptance

Our Study Objectives

Spontaneously fermented milk products are integral to human diet and play a central role in enhancing food security and income generation. Some of these products have demonstrated therapeutic and probiotic effects although recent reports have linked some to death, biotoxin infections, and esophageal cancer (Daily Nation, 2015; Nieminen *et al*., 2013).

Aim of the study: To develop methodologies for valorization of traditional spontaneously fermented milk products by using starter cultures and probiotic strains in controlled fermentation processes targeting the efficient production of safe, broadly acceptable, and high quality fermented milk products.

Specifically;

- To characterize the microbial diversity and dynamics and the chemical profile of *Mursik* and *Amabere amaruranu*.
- To isolate, identify, and characterize potential starter cultures and probiotic strains from *Mursik* and *Amabere amaruranu*.
- To establish optimum physical fermentation parameters (temperature, agitation & aeration speeds) for the production of fermented milk products using starter cultures and/or probiotic strains isolated from *Mursik & Amabere amaruranu*.
- To develop & test products made using starter cultures and/or probiotic strains isolated from *Mursik* & *Amabere amaruranu*.
- To promote, conduct market viability assessment & explore commercialization of products developed using starter cultures or probiotic strains isolated from *Mursik* & *Amabere amaruranu*.

Methodology

1. Characterization of microbial diversity and identification of starter cultures and probiotic strains

Microorganisms in *Amabere amaruranu: Culturing*

Organism	Range
Lactic acid bacteria	$1.0\times10^{6} – 1.0\times10^{9}$
Coliforms	$1.0\times10^{4} – 1.0\times10^{7}$
Yeasts and molds	$1.0\times10^{4} – 1.0\times10^{6}$

Samples from Kisii County: pH 3.7–5.2

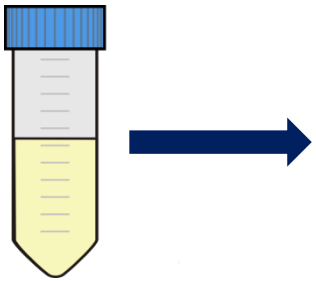
- Previous studies: Employed classical microbiological culturing techniques (Culture-dependent), which are inadequate in identifying & characterizing microbial consortia (Lore *et al*., 2005; Mathara *et al*., 2004; Nieminen *et al*., 2013; Nyambane *et al*., 2014)

Application of Metagenomics

- Cultivability of the microbes in the lab using synthetic media is inaccurately presumed & the absence of prior knowledge of their presence, selectivity of culture media & microbial interdependence, can result to utterly lopsided conclusions & misleading information (van-Hijum *et al*., 2013).

- Metagenomics: The application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species (Chen & Patcher, 2005).

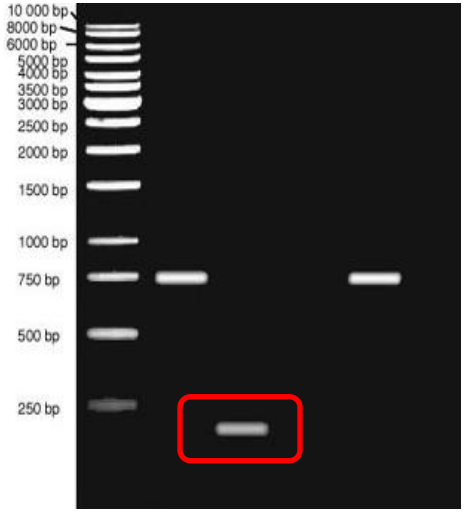
Metagenomics in the Study of *Amabere amaruranu*



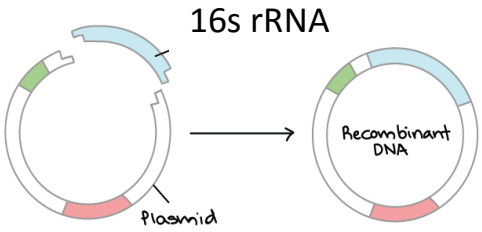
Sample:
Amabere amaruranu

DNA Extraction (Community DNA)

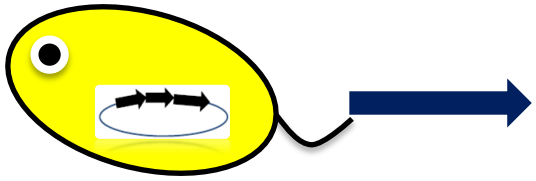
PCR Amplification of 16S rRNA of Lactic acid bacteria



Hypervariable section of 16S rRNA isolated

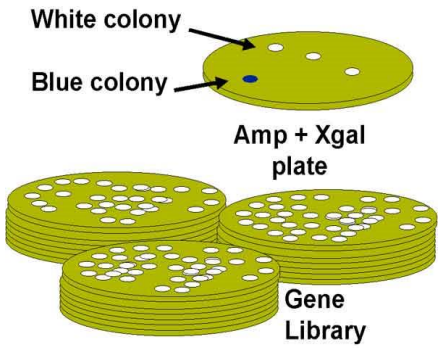


Gene cloning



E. coli

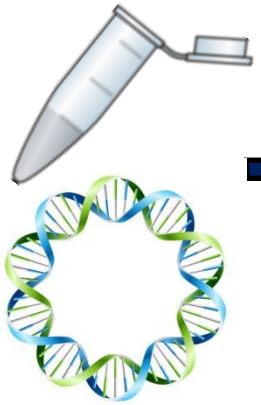
Transformation

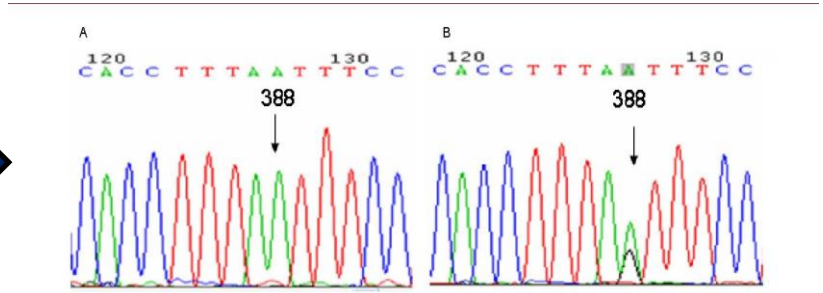


Transformant selection

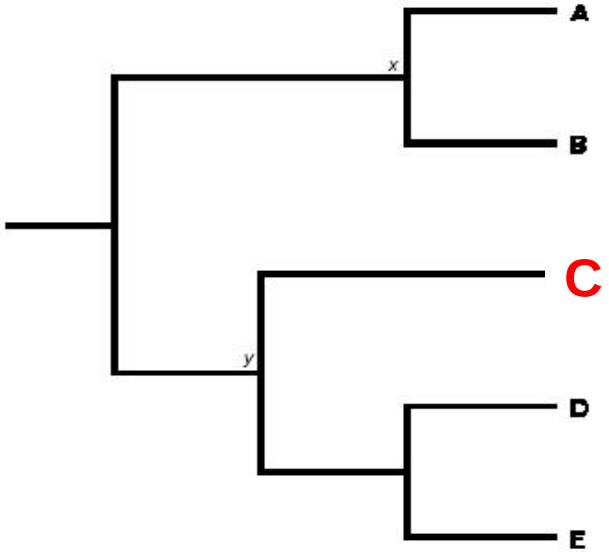
Metagenomics in the Study of *Amabere amaruranu*

Plasmid Extraction



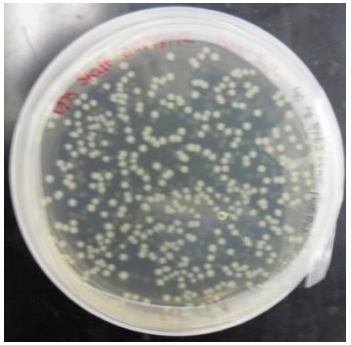


16S rDNA sequencing
(High throughput)



BLAST & Phylogenetic tree creation

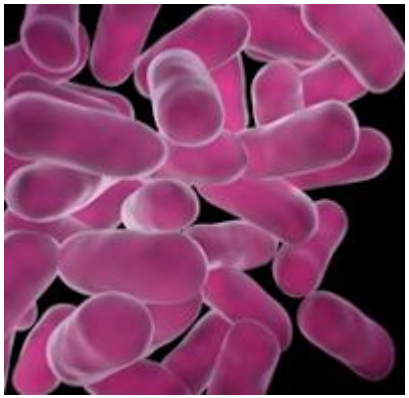
Abundant
Strain(s)



Strain Characterization
(Biochemical)

Application of Isolated Microorganisms

Isolated Starter culture/
Probiotic strain



Controlled fermentation

Product Development &
Promotion

The ultimate goal is to modernize the production of traditional fermented milk products

Conclusion

- The application of metagenomics for starter culture/probiotics design can allow the tailoring of starter cultures/probiotics to yield products with specific flavors / textures and/or health benefits.

- There is a growing consumer interest in attaining wellness through diet, necessitating the need for consumption of probiotic strains and the Kenyan fermented milks offer a vast source of starter cultures and probiotic strains, yet these opportunities have not been exploited.

- Starter culture/Probiotics design and bioreactor technology improvement for controlled fermentation processes: Achieved by June 2017.

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twas

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NBA

MSc students

Thank You!