

Expression of the Gene of Predicted Zymogen Granule Protein (G3MZ19) homologue in the Bali cattle (*Bos javanicus*) Saliva

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Main Research:

1. Crops/rice
2. Bali cattle (large ruminants)
3. Sea weed and pearls (marine biology)
4. Tropical diseases



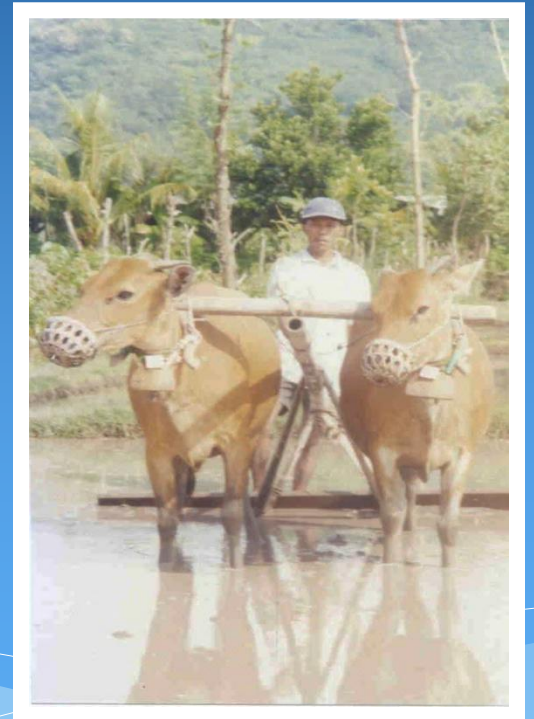


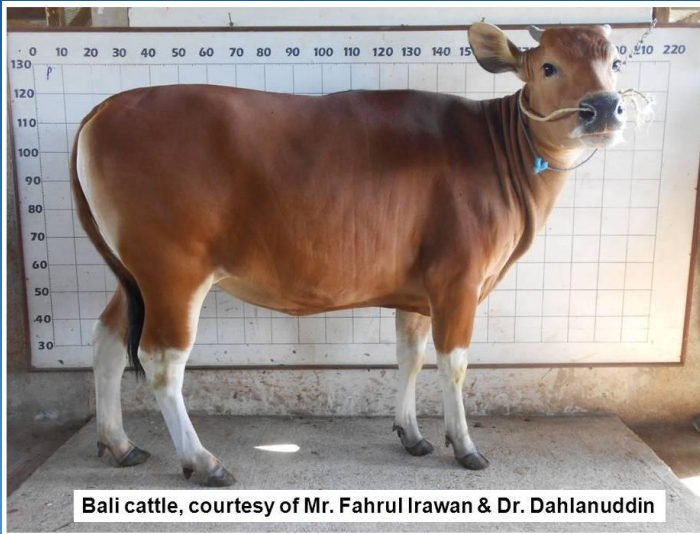
Photo by Prof. Soekardono



The main purposes of large ruminants:

1. Mainly for meat
2. as a source of labor to plow the land in rural areas





Bali cattle, courtesy of Mr. Fahrul Irawan & Dr. Dahlanuddin



The “story” behind the studies on Bali cattle saliva

- Is it possible to develop noninvasive test methods for ruminants?
 - Effects of mimosine (toxin of *Leucaena leucocephala*)
- Do Bali cattle have “specific bioactive” compounds?



Project 1. Isolation and characterization of saliva compounds

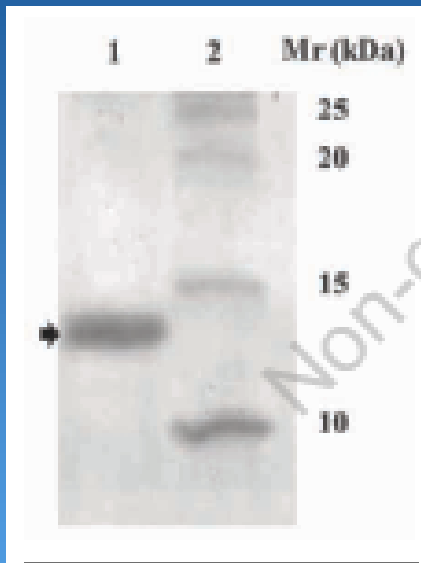
- Compound Mr ~ 14 kDa: Able to inhibit the growth of *Staphylococcus aureus*
- **Predicted: Lysozyme** (Depamede et al., 2012)



Project 2.

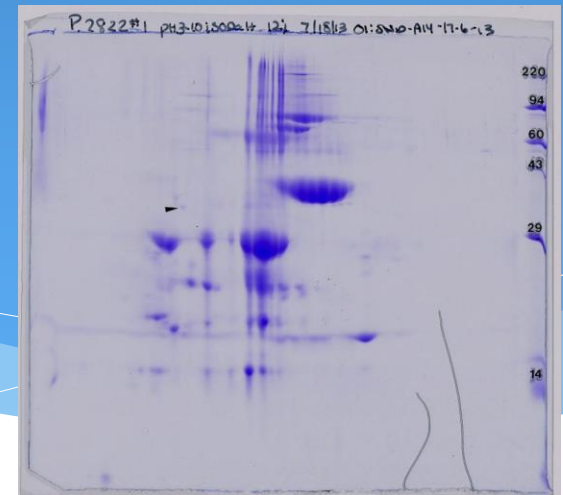
1D & 2D-SDS-PAGE + MALDITOF of 14 kDa compounds

- Proved was not Lysozyme: MBL/Jacalin-like superfamily



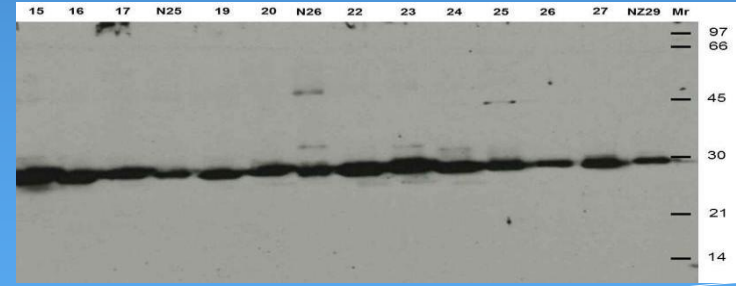
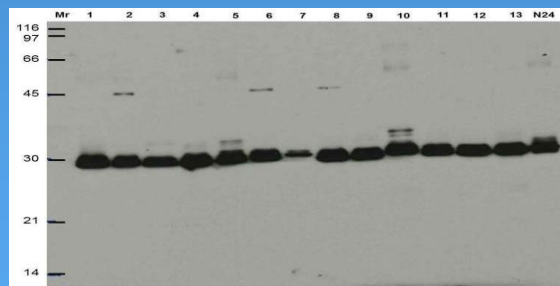
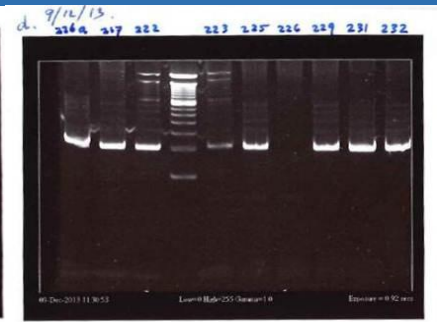
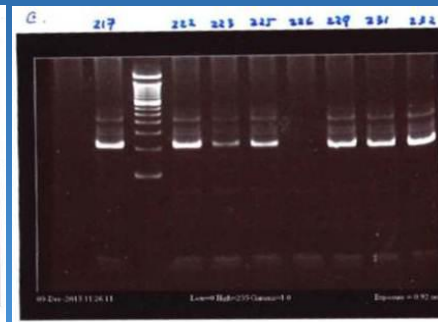
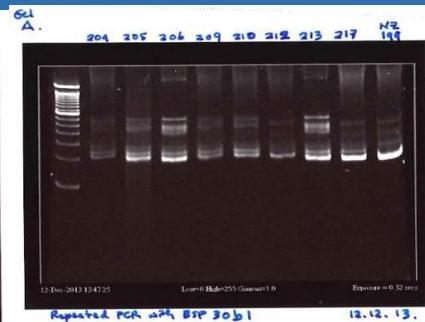
G3MZ19	Predicted zymogen granule protein 16 homologue
F1N1Z8	Pancreatic adenocarcinoma up regulated factor-like
F1MCV8	Prolactin-inducible protein homologue precursor

(Depamede, 2013)



Project 3.(collaborative research with Ruakura RC, Hamilton AgRes, NZ)

- **Studies on Phenotypes and genotypes of BSP30 in Bali cattle**



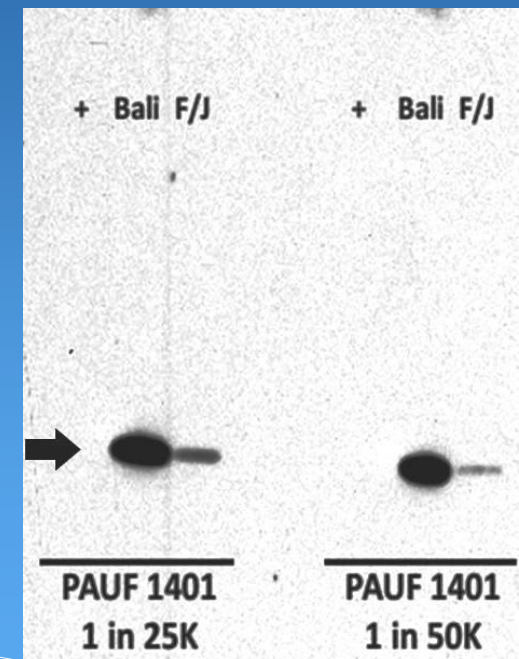
Project 4

(collaborative research with Hamilton AgRes, NZ)

- **Raising Ab against Pancreatic adenocarcinoma up-regulated factor (PAUF) in the saliva of Bali cattle.**

MALDI-TOF/TOF results

```
1  MLLWLTALL  WSPTCWAQQK  YGPGGGTYFS  TSRDFQNDIT  GIRVFIGPLG
51  LIKSIQVRF  SSWSEKYGAP  GGTPQEVILL  PEEHITGIYG  SYKNFLRHLV
101 IYTDRGRLFP  FGKEDGNTFI  AFPDESQKVL  IGVCGHYKLL  GITSIGFEWG
    YPSFLKK
151
```



Project 5

- **G3MZ19 gene expression based on MALDI-TOF/TOF.**

G3MZ19	Predicted zymogen granule protein 16 homologue
F1N1Z8	Pancreatic adenocarcinoma up regulated factor-like
F1MCV8	Prolactin-inducible protein homologue precursor

Project 5

- G3MZ19 gene expression based on MALDI-TOF/TOF.

Materials and Methods

Animal samples

- non-pregnant and non-lactating Bali cows (*Bos javanicus*), housed in a barn with individual pen, fed with King grass (*Pennisetum purpureum*) and water was given *ad libitum*.
- Collections of saliva were carried out in the barn using disposable plastic pipettes



Project 5

- **G3MZ19 gene expression based on MALDI-TOF/TOF.**

Materials and Methods

DNA extraction

- Total genomic DNA was extracted from the whole blood samples of the Bali cattle collected aseptically through the jugular vein.
- The extractions were carried out using a commercially available kit (NucleoSpin[®], Macherey-Nagel, Germany) following the manufacturer's instructions.
- The isolated DNA then subjected a standard PCR analysis.



Project 5

- **G3MZ19 gene expression based on MALDI-TOF/TOF.**

Materials and Methods

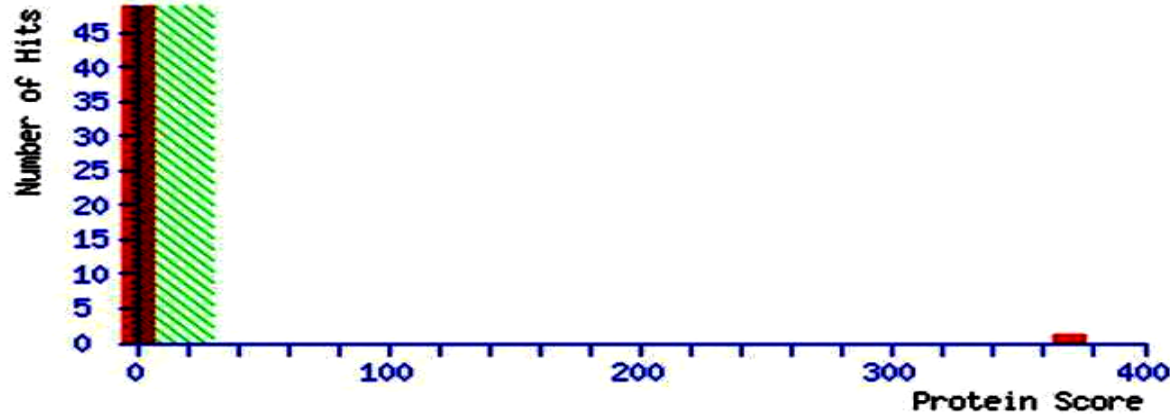
Detection of G3MZ19 in Bali cattle genomic DNA

- Genomic DNA (20 ng) was subjected to be amplified using KAPA2G Fast Ready MIX PCR Kit (KAPA Biosystem, USA)
- PCR program: predenaturation for 5 min at 94°C, 40 cycles of 40s at 94°C, 40s at 56°C, 15s at 72°C, followed by a final extension of 5 min at 72°C.



Project 5

- G3MZ19 gene expression based on MALDI-TOF/TOF.

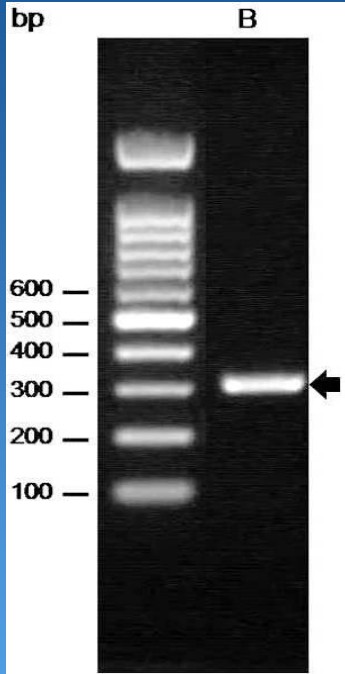


G3MZ19 Mass: 17594 Score: 370 Matches: 10(10) Sequences:
10(10)
tr|G3MZ19|LOC100295741 Uncharacterized protein Tax_Id=9913
[Bostaurus]

Representation of Mascot search result on G3MZ19 in Bali cattle saliva.

Project 5

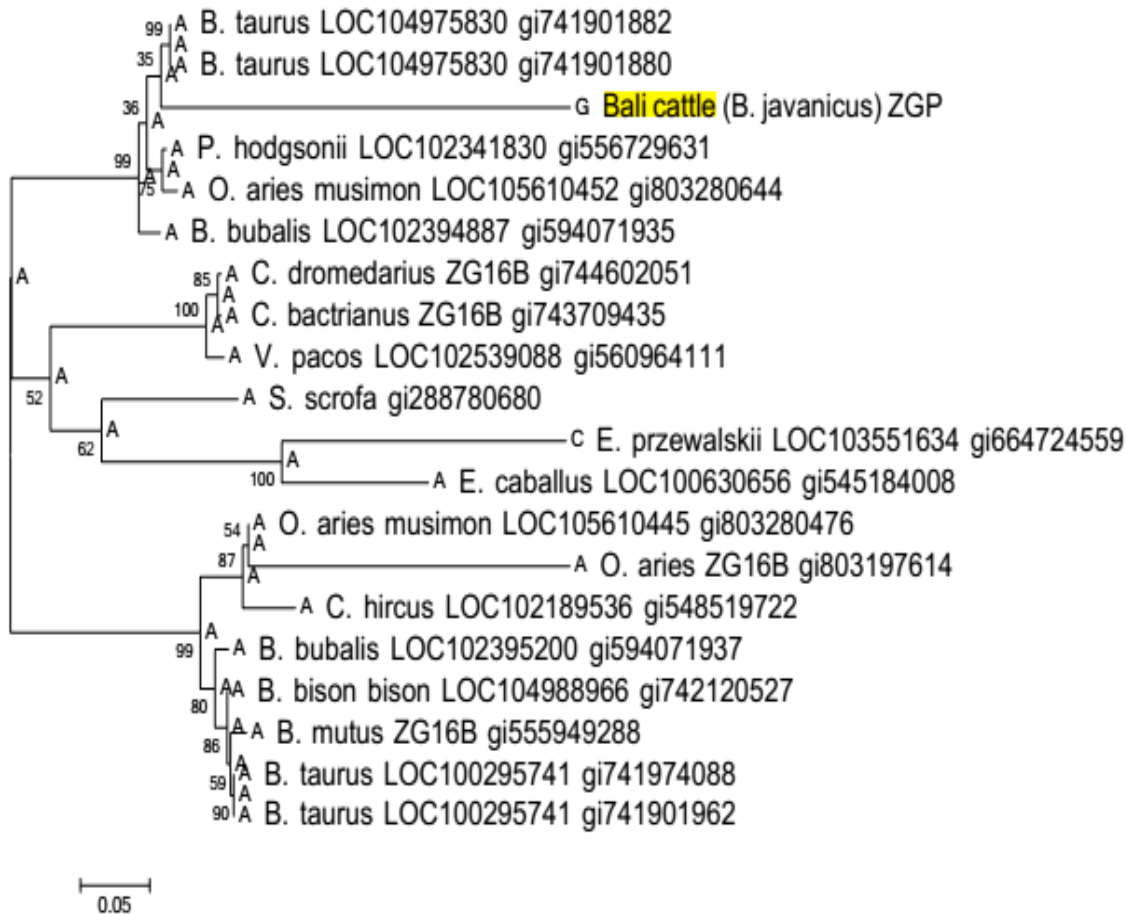
- **G3MZ19 gene expression based on MALDI-TOF/TOF.**



Representation of purified PCR products of Bali cattle G3MZ19 gene of 306 bp (B, arrow).

The primers used for the reactions were ACGTACTATTGGATAATCCCAG (Fwd) and AGTATCCAGGTGAGGTTTGG (Rev), derived from bovine (*Bos Taurus*, G3MZ19|LOC100295741, Tax_Id=9913, LudwigNR database and UniProt) and were designed using the Primer Quest program of Integrated DNA Technologies (IDT®, USA).

Project 5 G₃MZ₁₉ gene expression based on MALDI-TOF/TOF.



Phylogenetic tree constructed by maximum likelihood-ancestor method based on the nucleotide sequence of maximum score of zymogen granule protein genes explored in NCBI BLAST using Mega 6.

Bootstrap values are pointed to the major nodes as percentage of the data obtained from 1,000 resamplings. Zymogen granule protein fragment gene (ZPG) of Bali cattle is highlighted.

Conclusion

- saliva is a potential candidate media not only for noninvasive disease diagnostics and monitoring but also for evolution study of ruminants
- Various methods/techniques can be applied – proteomics-genomics





Feed efficiency ?

Diagnostics?

Future plans



Immune systems?

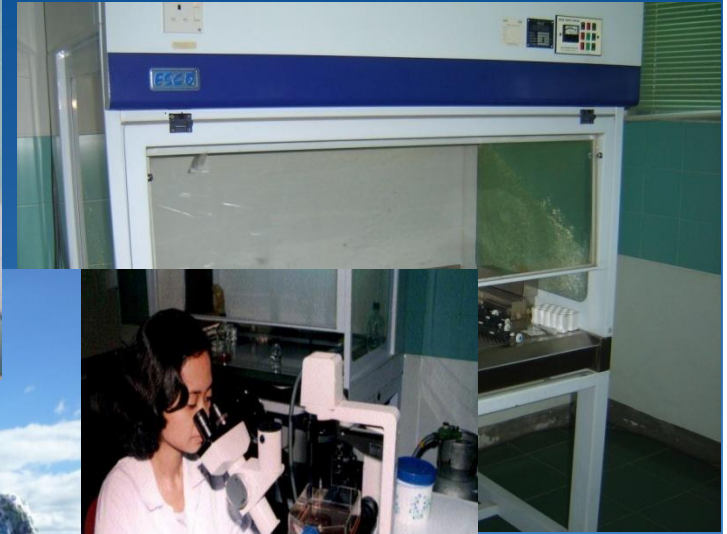
Bioactive/
cancer?

Acknowledgments:

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Thank you

