

## Determination of Yeasts Antimicrobial Activity in Milk and Meat Products

<sup>1</sup>L.B. Roostita, <sup>2</sup>G.H. Fleet, <sup>1</sup>S.P. Wendry, <sup>3</sup>Z.M. Apon and <sup>1</sup>L.U. Gemilang

<sup>1</sup>Faculty of Animal Husbandry Padjadjaran University, Bandung-West Java, Indonesia

<sup>2</sup>School of Chemical Engineering, University of New South Wales-Sydney, Australia

<sup>3</sup>The Indonesian Institute of Sciences, Cibinong-West Java, Indonesia

**Abstract:** The research was arranged to isolate yeasts from livestock products and then the yeasts antimicrobial activity was tested towards putrefaction and pathogenic bacteria. Yeasts isolated from livestock products using Malt Extract Agar (MEA), the total yeasts population counted with using total plate count method, antimicrobial activity tested using diffusion methods against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* and then the chosen isolate identified with using 18s RNA method. The results have shown that the total yeasts population on pasteurized cow's milk were  $1.2 \times 10^6$  cfu/g, fruit yoghurt  $5.4 \times 10^6$  cfu/g, lamb meat  $1 \times 10^5$  cfu/g, beef  $1 \times 10^5$  cfu/g and beef sausages  $1 \times 10^6$  cfu/g total yeasts population. Fruit yoghurt isolate shown the best antimicrobial activity with 35 mm clear zone diameter against *Pseudomonas aeruginosa*, 8 mm clear zone diameter against *Staphylococcus aureus* and 10 mm clear zone diameter against *Escherichia coli*. The 18 s RNA test shown that fruit yoghurt isolate was 100% (FR3-F primer) and 99% (FR3-R primer) identical with *Candida parapsilosis*.

**Key words:** Antimicrobial activity, milk and meat products, yeasts

### INTRODUCTION

Yeasts are one of microorganisms found in livestock products such as milk and meat. Beside it has grown on raw materials, yeasts also found in processed products such as pasteurized milk, yoghurt, and sausages. Yeasts have potential antimicrobial activities that could inhibit the growth of putrefaction bacteria or mould. In addition, some kinds of yeasts are producing extracellular protease that also has antimicrobial ability thereby food have long shelf life.

The activity of extracellular proteases was found in some yeast species such as *Candida lipolytica* (Šalomskienė and Macioniene, 2009). Extracellular protease produced by yeast were also has antimicrobial characteristics (Roostita and Fleet, 1996). Another interesting development is the engineering of recombinant strains of yeasts such as *S. cerevisiae* with genes encoding the production of bacteriocins and lysozyme that are active against spoilage bacteria (du Toit and Pretorius, 2000). On the other hand, yeasts ability in producing sulphur dioxide gives interesting inhibitory action on the growth of spoilage lactic acid bacteria (*Lactobacillus hilgardii*, *Lactobacillus brevis*, *Leuconostoc mesenteroides*) (Fleet, 2003).

The extracellular proteases and antimicrobial activity found in yeasts, enabling them to be exploited as novel agents in the bio-control of food spoilage (Fleet, 2006). The use of antimicrobial ingredients is one of the widely used methods to maintain microbiological safety and prolong the shelf-life of food products (Blngöl and

Bostan, 2007). Therefore, the research arranged to characterize and optimize the potential of yeast isolate for extracellular protease production and antimicrobial compound that furthermore can be use as food biopreservation agent.

### MATERIALS AND METHODS

**Preparation of samples and isolation of yeasts:** Five kind livestock products such as pasteurized cow's milk, fruit yoghurt, lamb meat, beef and beef sausages were bought from random sellers in Bandung city and kept in sterile beakers separately. Each samples were taken 1 g and diluted with 9 mL physiological NaCl, and the procedure was continued to obtain a final dilution of  $10^6$ . About 0.1 mL of  $10^5$  and  $10^6$  dilutions spread into each Malt Extract Agar (MEA/Oxoid Ltd., pH of 5.4) that contained 100 mg per ml oxytetracycline (Sigma Chemical Co.) to inhibit bacterial growth and then incubated at 37°C for 48 h (Roostita, 1993). To determine the total yeasts populations, the colonies formed were counted and averaged. Separate colony taken with osse and streaked into MEA and incubated at 27°C for 48 h and stored at 5°C for stock.

**Antimicrobial activity test:** Yeast colony from stock sub cultured into 15 mL of Glucose Yeasts Extract Broth (GYEB) and incubated at 27°C for 48 h. Sweep aseptically the incubated broth into separates MEA using sterile swab until covered then incubated at 27°C for 48 h. *Staphylococcus aureus*, *Escherichia coli* and

*Pseudomonas aeruginosa* sub cultured into 15 mL of Nutrient Broth (NB) and incubated at 37°C for 24 h. Nutrient Agar (NA) plates aseptically sweep until covered with *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* broth using sterile swabs. Plug aseptically yeast agar plate, use a sterile forceps or needle to carefully pick up the plug and place them onto each NA plates. Incubate the NA plates at 37°C for 24 h then diameter of the clear zones were measured.

**Identification of yeast isolate:** Chosen isolate identified with using 18s RNA method with the primer used for amplification genes were FR3-F (ATTGGA GGGCAA GTCTGGTG) and FR3-R (CCGATCCCT AGTCGGC ATAG) (Loeffler *et al.*, 2000). Identity and similarity of the sequence were tested with using BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### RESULTS AND DISCUSSION

The results shown the total yeasts population on pasteurized cow's milk were  $1.2 \times 10^6$  cfu/g, fruit yoghurt  $5.4 \times 10^6$  cfu/g, lamb meat  $1 \times 10^5$  cfu/g, beef  $1 \times 10^5$  cfu/g and beef sausages  $1 \times 10^6$  cfu/g. Highest total yeasts populations were given by milk products such as pasteurized cow's milk and fruit yoghurt. Milk has a good nutrition for yeasts to grow, yeasts could ferment lactose, metabolize the lactates, and producing volatile acids and carbonyl compounds (Fleet, 1992). Moreover, cultured milk products such as yoghurt have favorable conditions for yeasts growth (Viljoen *et al.*, 2003). The addition of fruit was also gives more yeasts population on fruit yoghurt products and during the storage, yeasts count increased about 3.3-3.4 times (Šalomskienė and Mačionienė, 2009).

Table 1: Yeasts antimicrobial activity that taken from milk and meat products

Samples	Inhibition (mm)		
	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
Pasteurized cow's milk	30	11	8
Lamb meat	31	8	9
Beef	29	10	0
Beef sausages	0	9	9
Fruit yoghurt	35	8	10

In meat products, the presences of yeasts are objectionable. They could grow at a wide range of temperature and pH values then resulting in spoilage of the product (Pitt and Hocking, 1997). Yeast was a part of the natural microflora of meat. Their counts are used as an index of storability and sanitary quality of the products (Ahmed and Ismail, 2010).

Some isolates showed various antimicrobial activities towards pathogen bacteria such as *Escherichia coli*, dan *Staphylococcus aureus*, and then spoilage bacteria such as *Pseudomonas aeruginosa*. The result shows that every isolate has a different characteristic in generating antimicrobial activity. Table 1 shown fruit yoghurt isolate gives the best antimicrobial activity with 35 mm clear zone diameter against *Pseudomonas aeruginosa*, 8 mm clear zone diameter against *Staphylococcus aureus* and 10 mm clear zone diameter against *Escherichia coli*. Other isolate were also gives great inhibition towards *Pseudomonas aeruginosa* except beef sausages isolate, it's mean the yeasts isolate that taken from pasteurized cow's milk, lamb meat and beef were also potential in bioactives compound production which useful for food and feed biopreservatives. Moreover, all the isolates also give inhibition towards pathogen bacteria such as *Escherichia coli*, dan *Staphylococcus aureus* except beef isolates that didn't shows any inhibition towards *E. coli*.

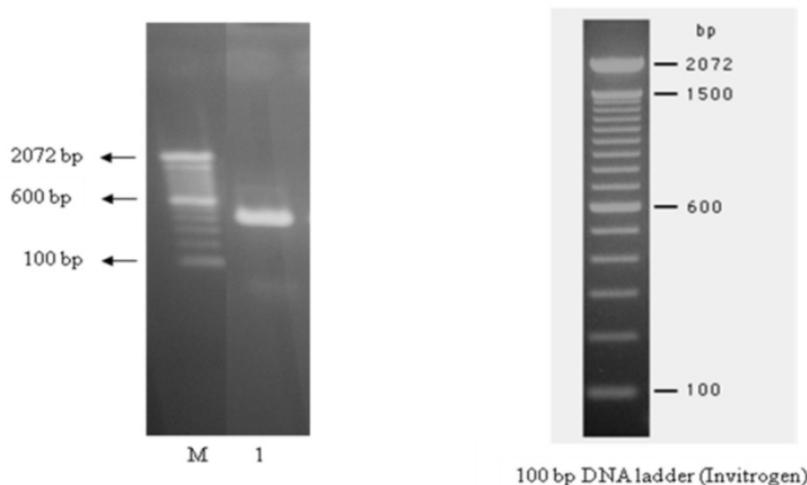


Fig. 1: The results of PCR amplification from fruit yoghurt isolate

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>1st_BASE_334238_8_FR3F
NNNNNNNNNGGGNAANNAGCTCCAAAGCGNANNNNAAGNTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGCTTGGCTGGCC
GGTCCATCTTTTTGATGCGTACTGGACCCAGCCGAGCCTTTCCTTGGCTAGCCTTTTGGCGAACCCAGGACTTTTACTTTGAAAAAAT
TAGAGTGTCAAAGCAGGCCCTTCTCGAATATATTAGCATGGAATAATAGAATAGGACGTTATGGTTCTATTTTGGTTTCTAGGACCA
TCGTAATGATTAATAGGGACGGTCGGGGGTATCAGTATTCAGTAGTCAGAGGTGAAATCTTGGATTACTGAAGACTAACTACTGCGAAA
GCATTTCAAGGACGTTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGATGATCAGATACCCTGCTAGTCTTAACCATAACTATG
CNCAANNNGGATCGGANATCGTCTATGGATCAGACTANACGTCTCTGATCATCTCATCTAACCTTTGTTCTTGATGAAGAAAAGTCTTGGTA
AATGCTTTANNNTTGTCTTCTNAAATCANAATTTCTCTGACTCAGAACGATNCCCGAGCTCTATAAATCNANATGTCTGNAACACAA
ATANAACATAACGTCATTCTATGAGTCNGCTAATATATNNANAAGCNGCTNGNACATCTAATTTTTTCAAGTAAAANNCNGGNTTGCCA
AAAGCTATCGAAAGAAAAGCTCGTGGGTCANCTANNACAAAAAGATGNCGCNCNCCNNNTACTACANCTNTTACTCACACNNGTAN
ANGNCCNNNNNCTGGANNNNNNNCTCNGCAGANTTCCNNNNNNNNNA
```

100% identical with *Candida parapsilosis*

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>1st_BASE_334239_8_FR3R
NNNNNNNNAATCNCGGTATNTGNCATCTNCGATCCCCTAACTTTCGTTCTTGATTAATGAAAACGCTCTGGTAAATGCTTTCGCGAGT
AGTTAGTCTTCAGTAAATCCAAGAATTCACCTCTGACTACTGAATACTGATACCCCGACCGTCCCTATAATCATTACGATGGTCTAGA
AACCAACAAAATAGAACCAATACGTCCTATTCTATTAATCCATGCTAATATATTCGAGCAAAGGCCTGCTTGAACACTCTAATTTTTCAA
AGTAAAAGTCTGGTTTCGCCAAAAAGGCTAGCCAGAAGGAAAGGCTCGGCTGGGTCAGTACGCATCAAAAAAGATGGACCGGCCAGC
CAAGCCCAAGGTTCAACTACGAGCTTTTAACTGCAACAACCTTAATATACGCTTTTGGAGCTGGAATACCGCGGCTGCTGGCACCAGAT
GNCGCNCAAAGNCCANNTGCTATCCGNNGGCGGCCGTGGGGGACCGGGGTCAGAGTTTTTCGGAG
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99% identical with *Candida parapsilosis*

Fig. 2: The sequences of yoghurt fruit isolate that 18s RNA tested

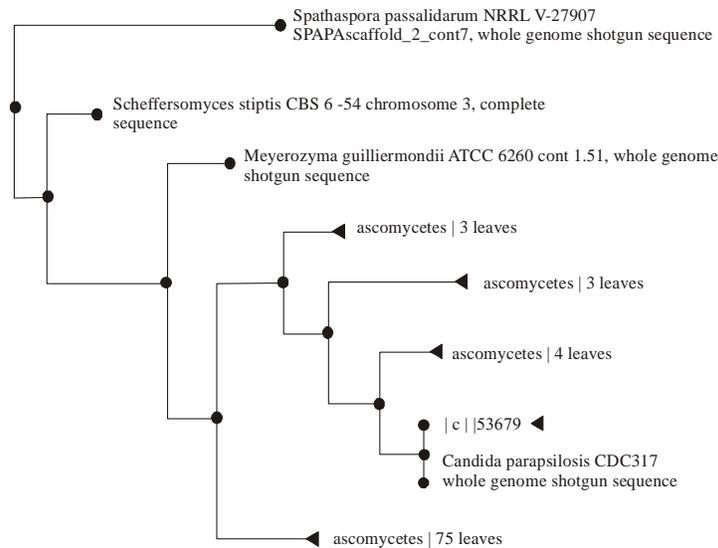


Fig. 3: Phylogenetic tree of analyzed sequence by using blast

Yeasts antimicrobial compounds were known as organic acids (heksanoat, oktanoat, and dekanooat) and protein that could inhibit the growth of bacteria and mold (Roostita, 2004). Antimicrobial proteins that act as an antimicrobial agent were found in *Sacharomyces cerevisiae* that produces several proteins that have little antimicrobial properties (Roostita, 2001). Moreover, yeasts also have ability in producing sulphur dioxide that could inhibit the growth of spoilage lactic acid bacteria (Fleet, 2003).

Fruit yoghurt isolate which gives the best antimicrobial activity were identified with 18 s RNA test and then identity and similarity of the sequences were tested with BLAST search (the PCR results was shown in

Fig. 1, the sequences shown at Fig. 2 and phylogenetic tree shown at Fig. 3. The result shown that fruit yoghurt isolate was 100% (FR3-F primer) and 99% (FR3-R primer) identical with *Candida parapsilosis*.

### CONCLUSION

Best antimicrobial activity shown by isolate that taken from fruit yoghurt with total yeasts population of  $5.4 \times 10^6$  cfu/g. The isolate gives inhibition of 35 mm clear zone diameter against *Pseudomonas aeruginosa*, 8 mm clear zone diameter against *Staphylococcus aureus* and 10 mm clear zone diameter against *Escherichia coli*. The identification with using 18s RNA test with FR3-F and

FR3-R primer shown that fruit yoghurt isolate was 100% (FR3-F primer) and 99% (FR3-R primer) identical with *Candida parapsilosis*.

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