Hermansyah, Proceeding/2.3_Proceeding_Growth Retardation of Saccharomyces cerevisiae by Noni Fruit (Morinda citrifolia) Extract Occurred in G1 to S Transition of the Cell Cycle.pdf

By Hermansyah Hermansyah

Journal of Physics: Conference Series

PAPER • OPEN ACCESS

Growth Retardation of *Saccharomyces cerevisiae* by Noni Fruit (*Morinda citrifolia*) Extract Occurred in G1 to S Transition of the Cell Cycle

To cite this article: Hermansyah *et al* 2018 *J. Phys.: Conf. Ser.* **1095** 012019

IOP Conf. Series: Journal of Physics: Conf. Series **1095** (2018) 012019 doi:10.1088/1742-6596/1095/1/012019

Growth Retardation of *Saccharomyces cerevisiae* by Noni Fruit (*Morinda citrifolia*) Extract Occurred in G1 to S Transition of the Cell Cycle

Hermansyah[a)*)], Susilawati[b], and Dyah Subositi[c]

a Chemistry Dept, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Jalan Raya Palembang-Prabumulih KM 32 Indralaya, Ogan Ilir, South Sumatera, Indonesia 30662. Phone (+62) -711-580269
b Faculty of Medicine, Sriwijaya Univerity, Jalan Dr.Moh. Ali Komp. RSMH Palembang, South Sumatera, Indonesia, 30126. Phone . (+62). 711-352342
c Research and Development of Medicinal Plants and Traditional Medicinal Center, Karanganyar-Tawamangu, Jawa Tengah, Indonesia.

*email. hermansyah@unsri.ac.id

Abstract : We previously reported that methanolic extract of noni fruit (*Morinda citrifolia*) can inhibit growth of *Saccharomyces cerevisiae*. This finding suggested that noni fruit has anti-proliferative effect. Previous data based upon FACS (Fluorescence Activated Cell Sorting) showed that growth of *S.cerevisiae* inhibited in G1 to S transition phase in the presence of 1% methanolic extract of noni fruit. Further analysis to explore genes involved in this slow transition step, we conducted real time PCR. Expression some genes changed in the presence of 1% methanolic extract of noni fruit. Transcriptional level of gene *CDC28* (cyclin dependent kinase-28), *CLN2*, *CLN3*, dan *SWI6* changed 2.25, 2.79, 4.87 and 7.21 folds, respectively. The data suggested that over transcription of those gene inhibit cell cycle progression. *CDC28* is master regulator of mitotic and meiotic cell cycles, *CLN2* and *CLN3* are G1 cyclin which play an important role in regulation of cell cycle, and SWI6 is transcription cofactor where forms complexes with *SWI4* and *MBP1* in regulation of transcription at G1 to S phase transition.

Keywords : *Morinda citrifolia, Saccharomyces cerevisiae*, G1 phase, Antiproliferative

1. Introduction

Noni plant (*Morindra ciytrifolia L*) is a small tropical tree that well known as volk medicine for some diseases. Almost all parts of this plant such as fruit, leaf, bark, root, flower, and seed are widely used in traditional medicine including diabetes, hypertension, arthritis, infections, gastric ulcers, depression, senility, menstrual difficulties, headaches, sprains, muscles aches, colds, cancer, poor digestion, atherosclerosis, blood vessels problems, drug addiction, antibacterial, antiviral, anti-tumor, antihelmin, analgesic, hypotensive, antiinflammatory, immune enhancing properties [1-2]. Noni plant grows widely in Polynesia. Unfortunately, many of these property claims are still needed scientific experiment. We reported antiproliferative effect of methanolic extract of noni fruit using *Saccharomyces cerevisiae* cell organism model [3].

Antiproliferative property of noni fruit is an essential property to inhibit growth of cells. Many antiproliferative mechanism involve genetics and epigenetics alteration, change in cell cycle progression mechanism, DNA replication, response of genomic integrity stress. Therefore *Saccharomyces cerevsiae* has a potent as organism model in screening medicine candidate for anti cancer and elucidating genes function cellular mechanism for antiproliferative property [4].

Cell cycle is the series of events to produce two daughter cells divided in four phases in the following order G1, S, G2, and M phases. DNA replication occurs in S phase, while replicated DNA molecule are segregated to daughter cell in M phase. During G1 phase, the cell grows and prepares itself for S phase, while G2 phase is the gap between the end of replication and the beginning of mitosis. These cycles progression have to run properly and control tightly [5-6]. The regulatory subunits the cyclin-dependent kinases (CDKs) or cyclins, that regulate progression through the cell ceycle, and cyclin D1 overexpression is found in more than 50% of human breast cancers and causes mammary cancer in transgenic mice [7].

This study aimed to explore genes involved in anti-prolifertaive effect of noni fruit to *S.cerevisiae* as organism model, *S.cerevisiae* wild type strain was used as an organism model. Our recently research based upon FACS data resulted that in the presence of 1% extract methanol of noni fruit inhibited growth of *S.cerevisiae* wildtype strain FY833 slightly inhibited in G1 phase. mRNA level some genes were determined using quantitative PCR. From our study indicated genes *CDC28*, *CLN2*, *CLN3*, and *SWI6* involved in arrested in G1 phase.

2. Materials and Methods

2.1 Materials

Isolates, strains, and media *S.cerevisiae* used in this experiment was wild type strain FY833 with genotype *Mata his3Δ200 ura3–52 leu2Δ1 lys2Δ202 trp1Δ6*. YPDA broth medium comprised of 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 400 mg/L adenine, while 20 g/L bacto agar was added to prepare YPDA agar. Extract methanol of noni fruit was mixtured into autoclaved YPDA media.

2.2. Experimental procedure

2.2.1 Culture

Routine cultivation of yeast strain was conducted by streaking yeast on YPDA agar media plate incubated at 30°C for 1-2 day.

2.2.2 Spot culture

A 5 mL cells grown on YPDA broth culture until reach OD_{660} 1.0, cells were then collected using centrifuge 5000 rpm, and resuspended cell with a sterile water adjusted until getting cells with concentration ten fold dilution series started from highest concentration 10^8 cells/mL, and 5 µL cell suspensions were spotted on YPAD agar media plate with and without 1% methanolic extract of noni fruit, Incubated cells at 30°C for 1-2 day.

2.2.3 Synchronization of cell cycle

Synchronization of cell cycle were performed according to manufacture's protocol with slight modification. FY833 strain cells were grown in YPAD to mid exponential phase, and washed with a 30 ml sterile water twice by centrifugation 2000 rpm 5 minutes to remove a protease (Bar1p) produced *MAT***a** cells that destroyes the α-factor, then were arrested in G1 phase using 6 µg/ml α-factor (Sigma) incubated with shaking three hours, after which cells were washed five times with fresh YPAD and transferred into fresh medium in the presence and absence of noni fruit methanol extract.

2.2.4 FACS (Fluorescent-Activated Cell Sorting) analysis.

Cells were grown in YPAD medium overnight and transferred into YPAD fresh medium to reach exponential phase culture (OD_{660} = 1.0 or 10^7 cells/ml) at 30°C. It then was prepared to performed

FACS analysis according to [8] with slight modification. After washed three times with 30 ml sterile water, the cells were released by resuspending them into the same volume of fresh YPAD broth media with and without 1% (w/v) extract methanol of noni fruit. Sample culture was taken and transferred into 2 ml eppendorf tube every 15 or 20 minutes. Cells were collected by centrifugation for 1 min at 12,500 rpm and resuspend in 300 µl of 0.2 M Tris-HCl pH 7.5, and then 700 µl of cold ethanol (- 20°C) were added gradually with vortexing, and stored it at -20°C until using fixed cells (> 12 h). Cells samples were washed twice with 0.2 M Tris-HCl pH 7.5, sonicated with minimum output (set the level "1") for 5 second (2 second x 2 times with interval. Cells were harvested by centrifugation for 5 min at 15,000 rpm and resuspended in 100µl of RNase solution (1 mg/ml RnaseA in 0.2 M Tris-HCl pH 7.5) at 30°C for 3-4h. Afterwards samples were collected by centrifugation for 5 min at 15,000 rpm and resuspended in 100 µl of PI solution (Trisodium citrate dehydrate 20 mg, NaCl 11.6mg, NP-40 20 µl, PI 1 mg, H_2O 20 ml) on ice for 15 min added 400 µl of 0.2 M Tris-HCl pH 7.5 and chill on ice until measuring DNA contents. Suspension cells were filtered using 5 ml polystyrene round bottom tube within cell stainer cap. Finally, treated samples were analyzed using FACS (Fluorescent-Activated Cell Sorting).

2.2.5 Determination of mRNA level using quantitative PCR (RT-PCR

RNA was isolated by the hot phenol method [9] with some modification. Briefly, cells grown in independent cultures to mid-log phase at 30°C in YPAD in the presence or absence of 1% (w/v) noni fruit methanol extract were harvested by centrugation 2000 rpm 5 minute. Total RNA was isolated by sequential treatment of TES/ distilled water saturated phenol, Trizol-LS (BRL), chloroform, and isopropanol prior to precipitation with sodium acetate buffer (pH 5.2) and 99.9% Ethanol mixture. The resulting RNA was dissolved in pre-chilled distilled sterile water.

First-strand cDNA was synthesized using a High Capacity cDNA Archive kit (Applied Biosystems) and used as the template for quantitative RT PCR with a 7300 Real Time PCR system with SYBR green PCR Master Mix (Applied Biosystems). The RT-PCR primers used in this study were designated using Primer Express (Applied Biosystems). The primers sequence are shown in the Table 1:

Table 1. Primer pairs used in this study

No	Primer	Nucletide Sequence	Sequence Amount
1	KfRT-*ACT1*	5'- TGGTATGTGTAAAGCCGGTTTTG -3'	(23)
	KrRT-*ACT1*	5'- CATGATACCTTGGTGTCTTGGTCTA -3'	(25)
2	KfRT-*CDC28*	5'-TGGGAACGCCGAATGAAG-3'	(18)
	KrRT-*CDC28*	5'-GAGGAAAGCTTGGCTTGAAATC-3'	(20)
3	KfRT-*CLN1*	5'-AATGGTCCTGTAAGAGAAAGTCACAA-3'	(26)
	KrRT-*CLN1*	5'-TTTGCGGTGAACTACTGATATGTTC-3'	(25)
4	KfRT-*CLN2*	5'-TTACGGGACCAAGCCAAATT-3'	(20)
	KrRT-*CLN2*	5'-TTACAACCGCCCCAAGTTTT-3'	(20)
5	KfRT-*CLN3*	5'-CGGCAACATTCGACTCCTA-3'	(19)
	KrRT-*CLN3*	5'-GGACAACGCCAGGCGATA-3'	(18)
6	KfRT-*SWI6*	5'- TGAAGGCGATATTTCCAAACTATTAG-3'	(26)
	KrRT-*SWI6*	5'- CTGCGGGAAGTTGATTTCGT-3'	(20)

7	KfRT-*WHI5*	5'- GGCCCATCCGCGAAAT-3'	(16)
	KrRT-*WHI5*	5'- TGCAGCTTGACTAACGCGTAA-3'	(21)

3. Results and Discussions

3.1 Phenotypic Assay

Anti-proliferative effect induces cell cycle arrest. Recently, we reported that the growth of *S.cerevisiae* wild type strain FY833 was strongly inhibited in the presence of 3% noni fruit methanol extract, and cell cycle completely inhibited at G1 to S transition progression [3]. This indicated that cell arrested either before or after the establishment of DNA synthesis.

In this article, we report that in the presence of 1% noni fruit methanol extract on YPAD media, the growth of *S.cerevisiae* cell slightly slow down. The growth was observed slower comparing with cells cultivated in the absence of extract and incubated at 30°C for 1 day as shown in Figure 1.

Figure 1. Spot analysis growth of yeast *S.cerevisiae* strain FY833 in YPAD agar media in the presence of 1% methanolic extract of noni fruit.

3.2 Yeast cells synchronization with α-factor pheromone

This experiment aimed to obtain cells with unbudding cells form because cell cycle delayed in G1 to S phase. After 6μg/mL α-factor pheromone was added into **MAT**a cells cultured in the YPAD medium. Based upon morphological observation stated that 80% cells in the unbudding form and 20% cells in the budding indicated that cells were inhibited in G1 to S progression (Figure 2a). Oppositely, in the absence of α-factor pheromone, 40% cells in the unbudding form and 60% cells in the budding indicated that mostly cells were in G2 to M phase (Figure 2a).

The α-factor pheromone effect synchronized cells into one form at G1 phase and arrested of cell cycle progression. Morphological observation showed that α-factor pheromone was inducible responses in unbudding, especially the shmoo form. Response of **MATa** yeast cells to the α-factor pheromone also induce the arrest of cell cycle progression. The pheromone response is a suitable production phase, and that it may be useful for informing synthetic biology design [10].

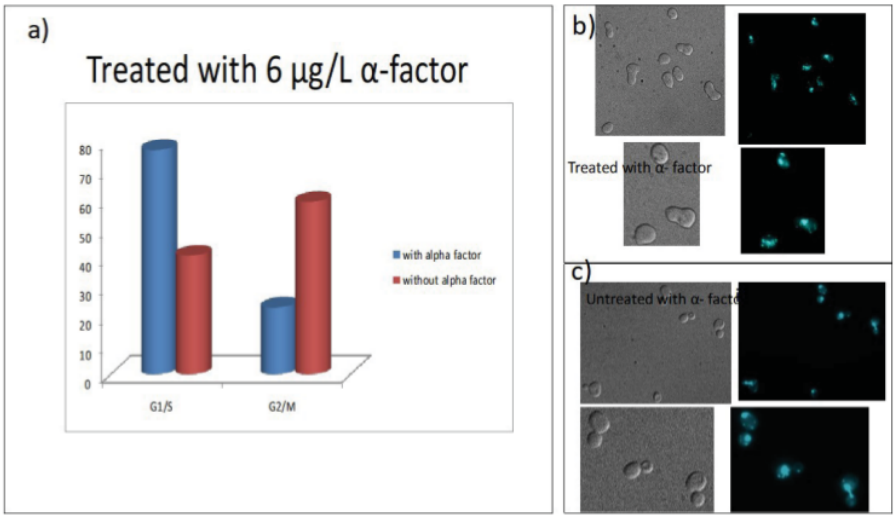

Figure 2. Yeast cells synchronization with α-factor pheromone. Cells treated with α-factor arrested at G1 to S transition where those cells were higher at G1/S phase than at G2/M2 phase (Figure a). Cells treated with α-factor generated unbudding with shmoo cells form .

3.3 FACS analysis of cells gown in YPDA medium with methanolic extract of noni fruit

Further experiment to verify whether growth inhibition or sensitive phenotype of yeast cell when grown in YPDA containing 1% methanolic extract of noni fruit, we conducted Fluorescence Activated Cell Sorting (FACS) analysis.

Cells synchronized with α factor, then cells were released in YPDA medium with this noni fruit extract. FACS analysis data resulted that in the presence of 1% methanolic extract noni fruit, cells were difficulty to form budding or enter into G2 to M phases of cell cycle (Figure 3a and Figure 3b). Oppositely, when α factor-synchronized cells ware release in YPDA media without noni fruit extract, cells progress to form budding or enter into G2 to M phase properly (Figure 3c and Figure 3d). These resulted indicated that methanolic extract of noni fruit inhibited G1 to S transition progression.

Noni ethylacetate extract caused an increase in the proportion of cell in sub G1 phase, which reflects the induction of apoptosis [11].

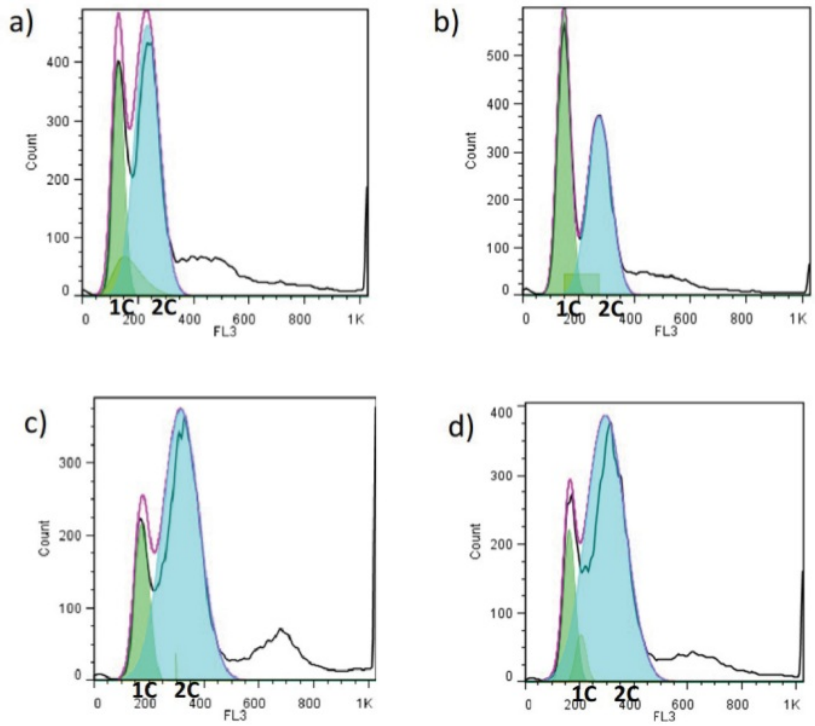

Figure 3. FACS analysis data. The α-factor-treated cells were analysed using FACS, cells after synchronization (a) and(c); and synchronized cells were released in YPAD media containing methanolic extract (b) compared with without noni extract (d).

3.4 Alteration of some genes involved in cell cycle, CDC28, CLN2, CLN3, WHI5, and SWI6

To better understand anti-proliferative activity from noni fruit extract the cultured cells with lower concentration (1% w/v) was then analyzed the expression level of genes cyclin dependent kinase Cdc28 which is crucial genes for cell cycle progression and related genes cyclins (*CLN1*, *CLN2*, *CLN3*), *WHI5* and *SWI6*.

Based upon this RT-PCR data, expression level of *CDC28*, *CLN2*, *CLN3*, and *SWI6* increase 2.2, 2.8, 4.9, and7.2, respectively while expression level of *WHI5* was similiar between presence and absence of extract methanol of noni fruit (Figure 4). These resuts indicated that genes *CDC28*, *CLN2*, *CLN3*, and *SWI6* involved in arrested in G1 phase.

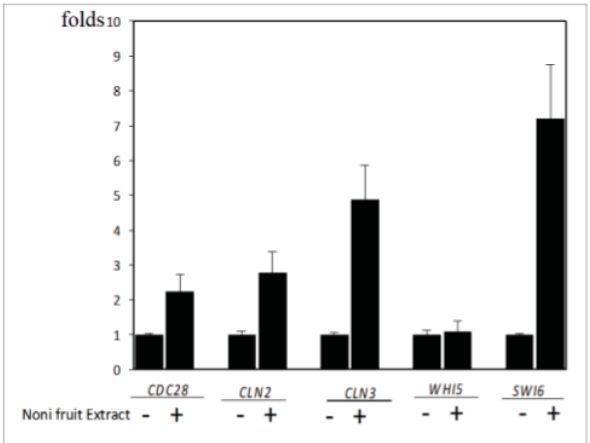

Figure 4. RT-PCR analysis genes involved in G1 to S transition cell cycle

Cyclin genes play an essential role in cell cycle progression. As described in www.yeastgenome.org stated that *CDC28* is Cyclin-dependent protein kinase; interacts with regulatory subunit Cyb1; determination of cell morphology during the cell cycle; phosphorylated mostly by Swe1 and phosphorylation is regulated by Hsl1; 5'-UTR intron; Spider biofilm repressed. *CLN2* is G1 cyclin involved in regulation of the cell cycle; activates Cdc28p kinase to promote the G1 to S phase transition; late G1 specific expression depends on transcription factor complexes, MBF (Swi6p-Mbp1p) and SBF (Swi6p-Swi4p). *CLN3* is G1 cyclin involved in cell cycle progression. *WHI5* is repressor of G1 transcription that binds to SCB binding factor (SBF) at SCB target promoters in early G1; phosphorylation of Whi5p by the CDK, Cln3p/Cdc28p. *SWI6* is Transcription cofactor that activates transcription by RNA polymerase II during heat stress and during the G1/S mitotic transition; also regulates meiotic recombination; component of both the MBF and SBF complexes. Other study using human liposarcoma SW872 cells were characterized by strong antiproliferative effect and modulating oxidative stress [12].

4. Conclusion

In this research described that expression some genes changed in the presence of 1% methanolic extract of noni fruit. Transcriptional level of gene *CDC28* (cyclin dependent kinase-28), *CLN2*, *CLN3*, dan *SWI6* changed 2.25, 2.79, 4.87 and 7.21 folds, respectively. This data suggested that over transcription of those gene might inhibit cell cycle progression.

Acknowledgment

The authors would like to thank Research and Development of Medicinal Plants and Traditional Medicinal Center B2P2TOOT for supporting through RISTOJA research grant. Special thank to Asc.Prof Minetaka Sugiyama for facilitating RT-PCR analysis.

References

[1] Wang MY, Su C 2000 *Annal New York Academy of Sciences*, 61
[2] Samoylenko V, Zhao J, Dunbar DC, Khan IA, Rushing JW MI Damnacanthal. J 2008 *Agric Food Chem* **54(17)** 6398
[3] Hermansyah, Susilawati 2017 *J Mol* **12(1)** 37
[4] Qaddouri B, Guaadaoui A, Bellirou A, Hamal A, Melhaoui A, Brown GW Bellaoui M 2011 *Evidence-Based Complementary and Alternative Medicine* **2011** 1
[5] Vermeulen,K.,Van Bockstaele,D.R.,Berneman ZN, Vermeulen K, Van Bockstaele DR, Berneman ZN 2003 *Cell Prolif.* **36(3)** 131
[6] Amigoni L, Colombo S, Belotti F, Alberghina L, Martegani E 2015 *Cell Cycle* **14(15)** 2429

[7] Casimiro MC, Crosariol M, Loro E, Li Z, Pestell RG 2013 *Genes and Cancer* 3(11-12) 649
[8] Schönthal AH, Pozarowski P, Darzynkiewicz Z. Analysis of Cell Cycle by Flow Cytometry 2004 *Checkp Control Cancer* **281** 301
[9] Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith J 2003 *Current Protocols in Molecular Biology* **1** 146
[10] Williams TC, Peng B, Vickers CE, Nielsen LK. 2016 Metab Eng Commun 6(3) 142
[11] Sharma K, Pachauri S, Khandelwal K, Ahmad H, Arya A, Biala P, 2015 *Drug Res* **66(3)** 141
[12] Ruhomally Z, Somanah J, Bahorun T, Neergheen-Bhujun VS 2016 *J Tradit Chinese Med Sci* **6(3)** 299

Hermansyah, Proceeding/2.3_Proceeding_Growth Retardation of Saccharomyces cerevisiae by Noni Fruit (Morinda citrifolia) Extract Occurred in G1 to S Transition of the Cell Cycle.pdf

ORIGINALITY REPORT

21%

SIMILARITY INDEX

PRIMARY SOURCES

1	tsukuba.repo.nii.ac.jp Internet	85 words — 3%
2	dspace.mit.edu Internet	77 words — 3%
3	scholar.lib.vt.edu Internet	48 words — 2%
4	Fulvia Ferrazzi, Paolo Magni, Lucia Sacchi, Angelo Nuzzo, Uroš Petrovič, Riccardo Bellazzi. "Inferring gene regulatory networks by integrating static and dynamic data", International Journal of Medical Informatics, 2007 Crossref	44 words — 2%
5	onlinelibrary.wiley.com Internet	42 words — 1%
6	dl.uctm.edu Internet	35 words — 1%
7	www.candidagenome.org Internet	33 words — 1%
8	chemogenomics.pharmacy.ubc.ca Internet	26 words — 1%
9	www.mbl.or.kr Internet	21 words — 1%

10	Sharma, K., S. Pachauri, K. Khandelwal, H. Ahmad, A. Arya, P. Biala, S. Agrawal, R. Pandey, A. Srivastava, A. Srivastav, J. Saxena, and A. Dwivedi. "Anticancer Effects of Extracts from the Fruit of Morinda Citrifolia (Noni) in Breast Cancer Cell Lines", Drug Research, 2015. Crossref	20 words — 1%
11	www.science.gov Internet	20 words — 1%
12	espace.library.uq.edu.au Internet	19 words — 1%
13	othes.univie.ac.at Internet	18 words — 1%
14	db.yeastgenome.org Internet	17 words — 1%
15	journals.plos.org Internet	15 words — 1%
16	www.frontiersin.org Internet	13 words — < 1%
17	jmolecularsignaling.biomedcentral.com Internet	11 words — < 1%
18	Hirasaki, M.. "Protein phosphatase Siw14 controls intracellular localization of Gln3 in cooperation with Npr1 kinase in Saccharomyces cerevisiae", Gene, 20080215 Crossref	11 words — < 1%
19	eprints.uthm.edu.my Internet	9 words — < 1%
20	link.springer.com Internet	8 words — < 1%
21	discovery.dundee.ac.uk Internet	8 words — < 1%

22	biit.cs.ut.ee Internet	8 words — < 1%
23	eprints.whiterose.ac.uk Internet	8 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 1%
EXCLUDE BIBLIOGRAPHY	ON		