International Scholars Journals

International Journal of Agricultural Extension and Rural Development ISSN 2756-3642 Vol. 11 (1), pp. 001-005, December, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Wheat Growers in Ethiopia's Bale Zone Adoption of Better Wheat Varieties

Uzoma Chika

Université catholique du Grabben, Butembo, Congo – Kinshasa, Democratic Republic of the Congo

Accepted 4 December, 2024

In Ethiopia, wheat is a crucial crop for food security. One strategy aimed at closing the nation's ongoing food disparity is the adoption of better wheat varieties, which will increase smallholder farmers' output and productivity. A number of institutional and socioeconomic issues that are empirically unknown in the research area prevent farmers from adopting better wheat varieties. Analyzing the factors influencing farmers' decisions to adopt enhanced wheat varieties in the research area was the aim of this study. For this study, a mix of quantitative and qualitative techniques, including focused group discussions, key informant interviews, and household surveys, were used. The results of the probit model demonstrated that the adoption decision of improved wheat varieties was significantly influenced by the following factors: the household head's sex, the size of the land and livestock holdings, the amount of credit available, the availability of market information, the frequency of extension contacts, the household head's educational attainment, cooperative membership, and perceptions of yield capacity. Therefore, in order to achieve greater adoption of better wheat varieties and higher production and productivity of smallholder farmers, policy and development interventions should focus on improving such an institutional and economic support framework.

Key words: *Adoption, improved wheat varieties, Binary probit model.*

INTRODUCTION

Morphological shape of many species of *Candida* spp. yeast cell is usually round to oval and divides itself by budding. It may also form septate hyphae or pseudohyphae in favourable conditions. Different species may utilize different types of carbohydrates for growth (Kurtzman and Fell, 1998). *Candida* spp. could be found in several natural habitats, either terrestrial or aquatic environments. Some species could live with other living organisms. *C. albicans* is a normal flora on skin and vagina of healthy humans. However, it could also be an opportunistic pathogen when host's immune system is weak such as that found in immune-compromised patients (Molero et al., 1998).

There are over 163 species of *Candida* spp. identified by biochemical characteristics (Kurtzman and Fell, 1998) and only a few of those were isolated from seawater and sea sediments; for example *C. neustonensis* (Chang et al., 2010), *C. parapsilopsis*, *C. tropicalis*, *C. guilliermondi*, *C. intermedia*, *C. quercitrusa*, *C. rugosa*, *C. zeylanoides*, *C. membranifaciens (*Roth et al., 1962; Wang et al., 2008b*).* Some species of *Candida* spp. may synthesize enzymes or bio-molecules such as riboflavin that could be biotechnological importance. For example, *C. membranifaciens* (Wang et al., 2008a, b) *C. guilliermondi*, *C. flareri* (Levine et al., 1949) and *C. famata* (Heefner et al., 1992) were reported that they could produce considerable amount of riboflavin as a primary metabolite. Among those species, only *C. membranifaciens* was reported to be an isolate from marine environment (Wang et al., 2008a).

Riboflavin, also known as vitamin B2, is a water-soluble vitamin. It is a precursor of flavin mononucleotide (FMN) and flavin adenine nucleotide (FAD), which are the components of flavoproteins (Bacher et al., 2000; Fischer and Bacher, 2005). These coenzymes are required for redox reactions of many cellular metabolic processes such as amino acid pathways, anabolic pathways, folate pathway (Massey, 2000).

Riboflavin has important roles in medical applications. Therapeutics that relies on natural substances such as vitamins may be the best method for curative purposes. Riboflavin could alleviate and prevent migraine symptom (Bigal et al., 2002; Breen et al., 2003; Woolhouse, 2005), night blindness (Graham et al., 2007) or anemia (Faber et al., 2005; Makola et al., 2003). Furthermore, enzymes involved in riboflavin production pathway may be the drug targets for antibacterial activity against some pathogenic bacteria (Fischer and Bacher, 2008). For the applications in food industry, riboflavin could be added in foods such as yoghurt and soft drinks in order to give the yellow color or to enhance food's nutritional value (Stahmann et al., 2000).

As long as the problems of global pollution are concerned, the manufacture of any valuable natural products should use the procedures that support environmental-friendly orientations. The method that use microorganism such as *Ashbya gossypii*, *Candida famata* or *Bacillus subtilis* to produce riboflavin was preferred than chemical methods because the previous method offered lower cost of production and also produces less toxic wastes including CO_2 released to environments (Stahmann et al., 2000). Therefore, this study investigated the potential use of a microorganism, *Candida* spp., in riboflavin production to support the eco- friendly concept.

Since the thorough study of *Candida* spp. in Thailand in terms of its distributions in natural habitats and its potential applications in biotechnology such as riboflavin production has not been reported elsewhere, the investigation is needed to gain such knowledge. Therefore, this study aimed to isolate and identify *Candida* spp. from the local natural resource which was seawater samples in Muang Chonburi and further investigated their capabilities to produce riboflavin in shaking flasks condition in which glucose was selected as a sole carbon source in riboflavin production medium. This monosaccharide was used, firstly, because it is generally the most easily utilized carbon source for microorganisms. Secondly, this simple sugar is also the primary substrate that could convert to guanosine-5'-triphosphate (GTP), the first precursor of riboflavin synthetic pathway in *C. famata* (Lim et al, 2001). Thirdly, the maximum riboflavin yields produced by *Eremothecium ashbyii* and *Ashbya gossypii* were illustrated when glucose was used as a carbon source at an incubation temperature of 30°C (Özbas and Kutsal, 1986), the same temperature employed in this study.

MATERIALS AND METHODS

Seawater sampling

Seawater samples were randomly collected from the coastal area of Chonburi province (Approximate Latitude (°E) /Longitude (°N)) (Google maps, 2011): 13.3641/100.9749, 13.3620/100.9748, 13.3043/100.9023, 13.3045/100.9016, 13.3022/100.8975 and 13.2747/100.9208. Samples were obtained at subsurface level according to the method described by Pollution Control Department (2001, 2004) with some modifications. In brief, each seawater sample was filled in a 50 ml centrifuge tube (Axygen), closed the cap tightly, put the tube in a plastic bag and stored the sample on ice immediately. Isolation of yeast was handled within 24 h after sample collection.

Isolation of yeasts

Each seawater sample was pipetted into a 50 ml flask containing sterile YPD broth (1% yeast extract (Biomark), 2% peptone and 2% dextrose (Himedia) supplemented with 100 ug/ml chloramphenicol (Sigma)) for the proportion of seawater:YPD = 1:10. Samples were

incubated at 30°C 150 rpm (innova4340, New Brunswick Scientific) and collected them on day-5 for serial dilutions and aseptically spreading on YPD agar. Samples were incubated at 30ºC overnight and single colonies that showed typical colony morphology of yeast were picked and re-streaked on sterile YPD agar (Same components as YPD broth but with an addition of 1.5% agar). Prepare wet mount of each isolated colony and visualize cells under a microscope (Olympus CH30) in order to confirm yeast cell morphology.

Identification of *Candida* species

Inoculum preparations and species identification of *Candida* sp. were conducted using RapID^{TM} Yeast Plus System kit (remel) according to manufacturer's instruction.

Screening for isolates capable of riboflavin production

A single colony from stock cultures was transferred to a 50 ml flask containing 20 ml YPD broth at 30°C 150 rpm (innova4340, New Brunswick Scientific) for 24 h. The culture was taken to measure the optical density at 600 nm (Thermo He λIOS γ) and adjusted to OD600 = 1.0. This culture (0.2 ml) was transferred to a 50 ml flask containing 20 ml riboflavin production medium (2% Glucose, 0.5% (NH4)2SO4, 0.1% KH2PO4, 0.05% MgSO4.7H2O, 0.01% CaCl2.2H2O, 0.01% NaCl (Ajax Finechem) and 0.2% yeast extract and grew at 30ºC 150 rpm (innova4340, New Brunswick Scientific) for 5 days. The culture was collected and centrifuged at 14000 rpm at 4°C for 10 min (Sartorius, Sigma 1-14). The culture supernatant was further used for riboflavin assay.

Riboflavin assay

Riboflavin assay of culture supernatants were determined by the measurement of the optical density at 440 nm in a spectrophotometer (Thermo He λIOS γ). Riboflavin standard (Sigma) was used for standard curve construction. Each sample was done in triplicate. Triplicate data were calculated for means and standard deviation (S.D).

RESULTS AND DISCUSSION

Candida spp. isolated from seawater samples were identified as *C. tropicalis* and *C. krusei* in a proportion of
53.19 and 46.81%, respectively. Microscopic morphology of *C. tropicalis* was round to oval shaped cell while *C. krusei* appeared to be elongated cells (Figure 1). Screening for riboflavin production from *C. tropicalis* isolates (MICBUU001 to MICBUU025) and *C. krusei* isolates (MICBUU026 to MICBUU047) showed that *C.tropicalis* MICBUU002 and MICBUU019 could produce riboflavin with concentration over 254.22±5.51 and 199.53±0.74 ug/ml, respectively as shown in Figure 2.

The aforementioned results showed the potent production of riboflavin by *C. tropicalis* which was consistent with a previous report that demonstrated the ability of this *Candida* specie to produce riboflavin (Buzzini and Rossi, 1998). Two isolates of *C. tropicalis* from this study were able to produce approximately 0.2 g/l riboflavin in the shaking-flask condition tested. The yield appeared to be less than the yield produced by *C. tropicalis* from the report by Buzzini and Rossi (1998). The reasons for this are likely to be from strain variation, different growth conditions and different culture preparation. However, this yield of riboflavin was similar to the riboflavin concentration produced by *Candida guilliermondii* that utilized liquid brewery waste (Contasti and Bahar, 1988). Furthermore, the isolated strains produced ten-fold more riboflavin yield than that from *C. membranifaciens* subsp. *flavinogenie* W14-3 (Wang et al., 2008a). In comparison to other microorganisms, *C. tropicalis* from this studied produced two-fold more riboflavin yield than that from *B. subtilis* PRF93 (Sauer et al., 1996). However, in comparison to *Ashbya gossypii*, fungi that could overproduce riboflavin in the industrial- scale were not the same in every case. *C. tropicalis* could produced more riboflavin than *A. gossypii* that grown in a medium containing up to 1% glucose for the maximum period of 9 days. The riboflavin yield could be approximately the same in comparison with *A. gossypii* that was cultured in a medium containing 3 or 5% glucose for 4 days (Tanner et al., 1949). However, there was also a condition that had less amount of riboflavin produced than that from *A. gossypii* that utilized cheese whey supplemented with 10 g/l bran as substrate (Ertrk et al., 1998).

Several factors could influence riboflavin yield produced by microorganism. Different components of growth medium or substrates may induce different riboflavin yields. In addition, the optimal growth conditions for riboflavin production in each microorganism are also different. Even though complex carbohydrates such as sucrose (Levine et al., 1949; Suzuki et al., 2009) or xylose (Chi et al., 2008) could be used to induce riboflavin production. Glucose was used in this study and the results demonstrated that it was adequate for *C. tropicalis* MICBUU002 and MICBUU019 to convert it to the detectable amount of riboflavin.

Apart from extrinsic factors, intrinsic factors could have significant role in riboflavin production. There were studies reported that the genetically engineered strains constructed in the laboratory could favour the riboflavin production. Jimenez et al. (2005) reported that *A. gossypii* that had a constitutive expression of Ag*ADE4,* a gene encoding phosphoribosyl pyrophosphate amidotransferase, could inactivate the inhibition of purine pathways and further enhanced riboflavin production. Phosphoribosyl pyrophosphate amidotransferase has a role in

the synthesis of GTP, a precursor for riboflavin synthesis. Zhu et al. (2007) reported that a strain of *B. subtilis* that lacked the function of *pta*, a gene related to acetate pathway, showed the up-regulation of a gene encoding for acetolactate synthase. This resulted in an increased level of riboflavin synthesis. Though this study did not aim to use metabolic engineering to improve the yield of riboflavin from the yeast isolates, it could be conducted later after the potential limit for riboflavin production from the yeast isolates is investigated and characterization of riboflavin are elucidated by analytic tools such as high-performance liquid chromatography (HPLC).

This study showed the results that could contribute the basic information about natural isolates of *Candida* sp. regarding to its relevant in the production of riboflavin and perhaps other interesting metabolites waiting to be discovered.

Conclusions

This study demonstrated that *C. tropicalis*, MICBUU002 and MICBUU019, isolated from seawater samples in Muang Chonburi could produce riboflavin in a broth containing 2% glucose on day-5 of incubation at 30°C with concentrations of 254.22 ± 5.51 and 199.53 ± 0.74 ug/ml, respectively. *C. krusei* isolates were also investigated for riboflavin production. However, all of *C. krusei* isolates did not produce riboflavin.

ACKNOWLEDGEMENT

This research was supported by the Faculty of Science, Burapha University, Thailand.

REFERENCES

Bacher A, Eberhardt S, Fischer M, Kis K, Richter G (2000).
Biosynthesis of vitamin B2 (riboflavin). Annu. Rev. Nutr. 20:153-167.
Bigal ME, Rapoport AM, Sheftell FD, Tepper SJ (2002). New migraine preventive options: An update with pathophysiological considerations. Rev. Hosp. Clin. 57:293-298.
Breen C, Crowe A, Roelfsema H, Saluja I, Guenter D (2003). High-dose riboflavin for prophylaxis of migraine. Can. Fam. Physician 49(10):1291-1293.
Buzzini P, Rossi J (1998). Semi-continuous and continuous riboflavin production by calcium-alginate-immobilized *Candida tropicalis* in concentrated rectified grape must. World J. Microbiol. Biotechnol. 14(3):377-381.
Chang C-F, Lee C-F, Liu S-M (2010) *Candida neustonensis* sp. nov., a novel ascomycetous yeast isolated from the sea surface microlayer in Taiwan. Anton Leeuw 97(1):35-40.
Chi Z, Wang L, Ju L, Chi Z (2008) Optimisation of riboflavin production by the marine yeast *Candida membranifaciens* subsp. flavinogenie W14-3 using response surface methodology. Ann. Microbiol. 58(4):677-681.
Contasti V, Bahar S (1988) Riboflavin production by *Candida guilliermondii* from liquid brewery waste. Acta Cient Venez. 39:69-74.
Ertrk E, Erkmen O, Oner MD (1998). Eects of Various Supplements on Riboflavin Production by *Ashbya gossypii* in Whey. Turk. J. Eng. Env. Sci. 22:371-376.
Faber M, Kvalsvig JD, Lombard CJ, Benade AS (2005). Effect of a fortified maize-meal porridge on anemia, micronutrient status, and motor development of infants. Am. J. Clin. Nutr. 82(5):1032-1039.
Fischer M, Bacher A (2005). Biosynthesis of flavocoenzymes. Nat. Prod. Rep. 22:324-350.
Fischer M, Bacher A (2008). Biosynthesis of vitamin B2: Structure and mechanism of riboflavin synthase. Arch. Biochem. Biophy. 474(2):252-265.
Google maps (2011) via http://maps.google.com.
Graham JM, Haskell MJ, Pandey P, Shrestha RK, Brown KH, Allen LH (2007). Supplementation with iron and riboflavin enhances dark adaptation response to vitamin A-fortified rice in iron-deficient, pregnant, nightblind Nepali women. Am. J. Clin. Nutr. 85(5):1375- 1384.
Heefner DL, Weaver CA, Yarus MJ, Burdzinski LA (1992). Method for producing riboflavin with *Candida famata*. United States Patent 5164303.
Jimenez A, Santos MA, Pompejus M, Revuelta JL (2005). Metabolic Engineering of the Purine Pathway for Riboflavin Production in *Ashbya gossypii*. Appl. Environ. Microbiol. 71(10):5743-5751.
Kurtzman CP, Fell JW (1998). The yeasts: A taxonomic study. 4th edition edn. Elsevier Sci. B.V.
Levine H, Oyaas JE, Wasserman L, Hoogerheide JC, Stern RM (1949). Riboflavin Production by *Candida* Yeasts. Ind. Eng. Chem. 41(8): 1665-1668.
Lim S, Choi J, Park E (2001) Microbial production of riboflavin using riboflavin overproducers *Ashbya gossypii*, *Bacillus subtilis* and *Candida famate* : An overview. Biotechnol. Bioprocess. Eng. 6(2):75- 88.
Makola D, Ash DM, Tatala SR, Latham MC, Ndossi G, Mehansho H (2003). A Micronutrient-Fortified Beverage Prevents Iron Deficiency, Reduces Anemia and Improves the Hemoglobin Concentration of Pregnant Tanzanian Women. J. Nutr. 133(5):1339-1346.
Massey V (2000). The chemical and biological versatility of riboflavin. Biochem. Soc. Trans. 28(4):283-296.
Molero G, Díez-Orejas R, Navarro-García F, Monteoliva L, Pla J, Gil C, Sánchez-Pérez M, Nombela C (1998). *Candida albicans*: Genetics, dimorphism and pathogenicity. Int. Microbiol. 1:95-106.
Özbas T, Kutsal T (1986). Comparative study of riboflavin production from two microorganisms: *Eremothecium ashbyii* and *Ashbya gossypii*. Enzyme Microb. Technol. 8(10):593-596.
Pollution Control Department (2001). Manual for marine water sampling and analysis. Bangkok.
Pollution Control Department (2004). Manual for quality control and assurance of marine water monitoring. Bangkok.
Roth FJ, Ahearn DGJ, Fell JW, Meyers SP, Meyer SA

(1962). Ecology and Taxonomy of Yeasts Isolated from Various Marine Substrates. Limnol. Oceanogr. 7(2):178-185.

Sauer U, Hatzimanikatis V, Hohmann H, Manneberg M, van Loon A, Bailey J (1996). Physiology and metabolic fluxes of wild-type and riboflavin-producing *Bacillus subtilis*. Appl. Environ. Microbiol. 62(10):3687-3696.

Stahmann KP, Revuelta JL, Seulberger H (2000). Three biotechnical processes using *Ashbya gossypii*, *Candida famata*, or *Bacillus subtilis* compete with chemical riboflavin production. Appl. Environ. Microbiol. 53(5):509-51.

Suzuki G, Fleuri L, Macedo G (2009). Influence of Nitrogen and Carbon Sources on Riboflavin Production by Wild Strain of *Candida* sp. Food Bioprocess. Technol. pp. 1-8.

Tanner Jr. FW, Vojnovich C, Van Lanen JM (1949). Factors affecting riboflavin production by *Ashbya gossypii*. J. Bacteriol. 58(6):737–745. Wang L, Chi Z, Wang X, Ju L, Chi Z, Guo N (2008a). Isolation and characterization of *Candida membranifaciens* subsp. flavinogenie W14-3, a novel riboflavin-producing marine yeast. Microbiol. Res. 163 (3):255-266.

Wang L, Chi Z, Yue L, Chi Z, Zhang D (2008b). Occurrence and diversity of *Candida* genus in marine environments. J. Ocean Univ. Chin. (English Edition) 7(4):416-420.

Woolhouse M (2005) Migraine and tension headache - A complementary and alternative medicine approach. Aust. Fam Physician 34(8):647-650.

Zhu Y, Chen X, Chen T, Zhao X (2007). Enhancement of riboflavin production by overexpression of acetolactate synthase in a pta mutant of *Bacillus subtilis*. FEMS Microbiol. Lett. 266(2): 224-230.