DEVELOPMENT OF A CHEMICALLY DEFINED MEDIUM TO ASSAY THE METABOLISM OF LACTIC ACID BACTERIA

A Thesis

by

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ABSTRACT

The purpose of this study was to develop a chemically-defined medium that is capable of supporting and detecting the metabolism of nutritionally-fastidious Lactic Acid Bacteria (LAB). The resultant Kim Menon Sturino (KMS) medium is comprised of only twelve chemically-defined ingredients. In order to increase the bioavailability of hydrophobic chemical additives, such as the isoflavone genistein (4',5,7-trihydroxyisoflavone), tween 80 was incorporated into the final medium at high levels (10% v/v). Furthermore, a commercial redox indicator was supplemented to the final formulation in order to facilitate the indirect assessment of cellular metabolism through the direct measurement of medium coloration. As proof of concept, the KMS medium was used to identify LAB with specific enzyme activities of interest, specifically β-glycoside hydrolase activity. In this model system, LAB were screened for their capacity to catabolize genistin (Gin^+), a glucoside (glycone) of genistein (genistein 7-*O*-β-D-glucoside) or its complementary aglycone, genistein (Gen^+). Of the fifteen strains screened, none were Gen^+ while *L. pentosus* was reproducibly Gin^+. As a result of this work, we conclude that *L. pentosus* expresses one or more β-glycoside hydrolases capable of hydrolyzing the glucose associated with genistin and fermenting it.

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NOMENCLATURE

APT	All Purpose Tween 80
ER	Estrogen receptor
ERα	Estrogen receptor α
ERβ	Estrogen receptor β
Gen^+	Genistein
Gin^+	Genistin
h	Hours
KMS	Kim Menon Sturino
LAB	Lactic Acid Bacteria
min	Minutes
MRS	De Man Rogosa Sharpe
MS	Menon Sturino
OD	Optical density

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CHAPTER I

INTRODUCTION

LITERATURE REVIEW

Estrogen

Estrogens are steroidal hormones. The three major forms of estrogen are: estrone, estradiol, and estriol (**Figure 1**). Estradiol contains two hydroxyl groups at positions 3 and 17. In esteron, the hydroxyl group at position 17 in estradiol is oxidized to a ketone group, while an additional hydroxyl group at position 16 is present in estriol. These subtle differences in chemical composition afford different affinities for their protein-based estrogen receptors (ER). For instance, estrone and estriol have lower affinities for estrogen receptors than estradiol (Vander Kuur et al., 1993). Because it typically elicits the greatest estrogenic effect *in vivo*, this thesis will primarily focus on 17β-estradiol as a model estrogen.

Estrogen receptors

In health and disease, the physiological effects of estrogen and estrogen-like compounds are mediated through the action of specific nuclear factors, called estrogen receptors (ER). Estrogens can bind to two subtypes of estrogen receptors, specifically estrogen receptor α (ERα) and estrogen receptor β (ERβ). As mentioned previously, estrone, estradiol, and estriol exhibit different affinities for estrogen receptors. For instance, estrone and estriol decreased the affinity of estrogen receptor and the receptor complexes of half-life of comparing to estradiol (Vander Kuur et al., 1993). When

estrogen binds to estrogen receptor, it evokes a conformational changing in the receptor that allows it to interact with DNA and act as transcription factor (Kuiper et al., 1998).

Physiological effects of estrogen

Steroidal hormones, including estrogen, play many important roles in the structure and function of the human body. For instance, steroid hormones influence cell growth (Kuiper et al., 1998), skeletal homeostasis (Pajamäki et al., 2008), low-density lipoprotein (LDL) cholesterol levels, high-density lipoprotein (HDL) cholesterol levels (Erdman, 2000), and cell differentiation (Kuiper et al., 1998). As an example, estrogens affect osteogenesis and modulate mechanosensation in bone cells (Pajamäki et al., 2008). Postmenopausal hormone-replacement therapy decreases remnant lipoprotein, increases HDL cholesterol and decreases LDL cholesterol (Lamon-Fava et al., 2010). Estrogen is also highly expressed in the uterus, prostate, ovary, and testis, where it also affects the function of the endocrine and reproductive systems (Kuiper et al., 1998). As an example, follicle-stimulating hormone stimulates ovarian granulosa cells into convert testosterone or androstenedione to estradiol (Zachow and Magoffin, 1997).

ERα is associated with cellular proliferation, while ERβ is associated with anti-proliferation (MacDonald and Wagner, 2012). Estradiol is necessary for the uterine growth and follicular phase during the first phase of the menstrual cycle (Zachow and Magoffin, 1997). In addition to its role in human health, estrogens also affect the progression of certain hormone-responsive diseases (Herman, et al., 1995). In contrast, ERβ is the dominant estrogen receptor in the intestinal tract and it is highly expressed in

the colon. Reduced expression of ERβ leads to impaired cells and increasing the risk of colon cancer in adult (MacDonald and Wagner, 2012). In addition, the loss of estrogen receptor β decreases apoptosis that is related to colon cancer (MacDonald and Wagner, 2012). As an example, estrogen receptor β-knockout mice exhibit reduced apoptosis and increased tumor cell proliferation in the colon (Armstrong et al., 2003). As a result, intestinal estrogen receptor β may suppress tumor growth through an anti-proliferative, pro-apoptotic pathway (MacDonald and Wagner, 2012). Research conducted by Menon et al., (2013) suggests that the gastrointestinal microbiota may also play an important role in these processes.

Phytoestrogens and phytoestrogenic isoflavones

Phytoestrogens have recently been shown to be chemoprotective against select hormone-dependent diseases, including colon cancer. Phytoestrogens are diphenolic compounds with molecular structures that are similar to 17β-estradiol (Steer et al., 2003). Phytoestrogens include broad classifications of compounds, including kaempferol, lignans and the isoflavones (Kuiper et al., 1998). Although phytoestrogens elicit lower estrogenic activity than estradiol, phytoestrogenic isoflavones closely mimic estrogen in the human body (Erdman, 2000; Herman et al., 1995).

Isoflavones are well-known phytoestrogens that are found in soybeans and soy-based products (Pyo et al., 2005). In soy foods, isoflavones primarily exist in conjugated (glucoside) forms, such as genistin (5,7,4'- trihydroxyisolavone-7-*O*-glucoside) (Hur et al., 2000). The structures of isoflavones are similar to those of estrogen (**Figure 2**) (Pyo

et al., 2005). As an example, genistein can compete with estradiol to bind ERβ and its affinity for ERβ is 20- to 30- fold higher than its affinity for ERα (Kuiper et al., 1998). Many studies have demonstrated the health benefits and nutritional effects of isoflavones (Pyo et al., 2005). Isoflavone glucosides have higher molecular weights and hydrophilicity (Pyo et al., 2005) when compared to their cognate aglycones. As a result, isoflavone glucosides are poorly absorbed in the small intestine when compared to their cognate aglycones; thus, the glycone and aglycone chemical derivatives have markedly different estrogenic properties *in vivo*.

Lactic Acid Bacteria (LAB)

Lactic Acid Bacteria are Gram-positive bacteria that produce lactic acid as a primary product of carbohydrate metabolism. LAB play many important roles in human health, but are perhaps best characterized in their roles in bioprocessing and biopreservation. For instance, LAB impart many of the beneficial characteristics associated with the organoleptic quality and safety of fermented foods, including cheese, yogurt, pickles, wine, and soy-based foods (Axelsson and Ahrné, 2000). LAB are beneficial species in the gut, where their relative abundance is generally correlated with health and might reduce the risk of colon cancer decreasing apoptosis in the colon (MacDonald and Wagner, 2012). Many *Lactobacillus* spp. are found in the intestinal tract and *Lactobacillus* spp. are also well-studied probiotics (Axelsson and Ahrné, 2000). Colonic microbiota influences to the colonic environment and immune system by competing for nutrients and communicating with mucosa (MacDonald and Wagner, 2012). As an example, the intestinal microbiota have availability to consume

phytoestrogenic isoflavone, genistin and genistein, which have high estrogenic activities and decrease the inflammation of gastrointestinal tract. As such, the catabolism of phytoestrogenic compounds by intestinal microorganisms may act to co-regulate the bioactivity of these important compounds (Menon et al., 2013).

Soy-based product fermentation by LAB

Isoflavones in soybeans and soy-based products are found in two chemical forms, specifically, their glucosides (e.g., daidzin, genistin and glycitin) and their aglycones (e.g., daidzien, genistein and glycitein) (Izumi et al., 2000). In soybeans, the two primary isoflavones glucosides are genistin and daidzin; these isoflavones are predominantly found in their glucoside forms (Hur et al., 2000). Izumi et al., (2000) indicated that the isoflavone aglycones can be absorbed faster and greater than their glucosides in the gastrointestinal tract. For instance, when genistein (aglycone) and its cognate glucosides (genistin) were ingested at low doses (0.11 mM), the plasma concentration of the aglycone was twice that of the glycone after 2 and 4 h (Izumi et al., 2000).

Isoflavone glucosides can be hydrolyzed into isoflavone aglycones by intestinal bacteria that increase absorption and bioavailability (**Figure 3**) (Chun at el., 2007). Some microorganisms, including some intestinal bacteria such as lactobacilli (Choi et al., 1999) and bifidobacteria (Jeon and Hwang, 2002) are known to encode β-glycoside hydrolase activity. In addition, β-glycoside hydrolase activity is needed to liberate the sugar moiety from its aglycone backbone and, thus, facilitate the absorption and increase

the bioavailability of the aglycone (Chun et al., 2007) (**Figure 3**). Chun et al., (2007) used soymilk to identify conversion of isoflavone glucosides into aglycones by fermentation with Lactic Acid Bacteria. In this study, *Lactobacillus paraplantarum* KM, *Enterococcus durans* KH, *Streptococcus salivarius* HM and *Weissella confusa* JY were inoculated into soymilk. The soymilk was rapidly fermented and the isoflavone glucoside (genistin) was efficiently converted to genistein by *Lactobacillus paraplantarum* KM. In addition, *Lactobacillus paraplantarum* KM expressed the highest acid production and showed the highest growth rates during fermentation of soymilk.

Pyo et al., (2004) demonstrated that *Lactobacillus* spp. were highly proliferative in soymilk and rapidly hydrolyzed the glucoside isoflavones, whereas *Bifidobacterium* spp. were less effective. In this study, *Lactobacillus* spp. (*Lactobacillus plantarum* KFRI 00144, and *Lactobacillus delbrueckii* subsp. *lactis* KFRI 01181) and *Bifidobacterium* spp. (*Bifidobacterium breve* K-101, and *Bifidobacterium thermophilum* KFRI 00748) were screened for their ability to generate bioactive aglycones (specifically genistein and daidzein) via a non-specific β-glucosidase activity. In this study *Lactobacillus plantarum* KFRI 00144 showed the highest β-glucosidase activity in soymilk after 24h cultivation at 37°C. In addition, the bioconversion rate of isoflavone aglycones (genistein and daidzein) was higher in *Lactobacillus* spp. (*Lactobacillus plantarum* KFRI 00144, and *Lactobacillus delbrueckii* subsp. *lactis* KFRI 01181) after 24 h in fermented soymilk than for *Bifidobacterium* spp. (Pyo et al., 2005).

Defined media for LAB

These studies were conducted in complex food systems, which complicate the analysis. A variety of complex media (e.g., MRS; De Man, Rogosa and Sharpe, 1960), semidefined media (e.g. MS; Menon et al., 2013). All purpose Tween 80 medium (APT) is designed for cultivation of LAB, including *Lactobacillus plantarum* (Archibald, 1983). Unfortunately, these media contains undefined sources such as yeast extract (Elli et al., 2000), which complicates functional genomic analyses. In order to address this, a variety of chemically defined media such as CMD (Teusink et al., 2006), and PMM (Wegkamp, 2010) have been developed in order to facilitate the metabolic study of LAB. Since the lactobacilli are fastidious microorganisms, they require numerous nutritional factors, such as amino acids, sugar sources and vitamins to grow (Axelsson and Ahrné, 2000). For instance Elli et al. (2000) developed chemically defined media to support growth of *Lactobacillus* spp.: DM1 and DM2 (**Table 1**). Genetic heterogeneity of the *Lactobacillus* group is reflected by their ability to proliferate in these media. For instance, *Lactobacillus johnsonii* ATCC 332 failed to grow in DM1, which includes guanine, thymine, cytidine, 2'-deoxyadenosine, and 2'-deoxyuridine but grew in DM2. DM2 contains inosine and uracil, but not guanine, thymine, cytidine, 2'-deoxyadenosine, and 2'-deoxyuridine (**Table 1**). Even a single factor can affect the growth of these strains. For insance, *Lactobacillus johnsonii* ATCC 332 showed lower biomass accumulations when it was cultivated in avoid ferrous sulfate of DM2. In addition, *Lactobacillus plantarum* NCDO 1193 can grow in both DM1 and DM2, but the growth was higher in DM2 medium.

Moreover, *Lactobacillus plantarum* NCDO 119 showed similar biomass accumulation in DM2 with or without ferrous sulfate. As a result, Elli et al., (2000) suggested that inosine and uracil were essential to grow in *Lactobacillus* spp., and iron plays an important role in purine and pyrimidine metabolism in *Lactobacillus* spp..

Tetrazolium redox dye

A variety of chemical indicator dyes are routinely incorporated into bacteriological media. As an example, tetrazolium redox dye produces a color change in response to cellular respiration rather than cell growth (Bochner, 2003). During this process, cells produce NADH, which acts to reduce the tetrazolium dye, which then produces a produce purple color (Bochner, 2009).

Hypothesis

Based on a review of the literature, there is a distinct need to develop a new chemically-defined medium to facility the study of β-glycoside metabolism by LAB, specifically *L. plantarum.* From a practical perspective, the resultant chemically defined medium should use as few chemical ingredients as possible. The purpose of this study was to address this gap and to develop a chemically-defined medium that is capable of supporting and detecting the metabolism of nutritionally-fastidious Lactic Acid Bacteria. In addition, we hypothesize that the resultant chemically-defined medium (KMS medium) can be used to identify *Lactobacillus* spp. with β-glycoside hydrolase activity that are capable of catabolizing phytoestrogenic isoflavones, specifically genistein and genistin.

CHAPTER II

THE B-GLUCOSIDE HYDROLASE ACTIVITY OF LACTOBACILLI

METERIALS AND METHODS

Reagents

Genistein (4',5,7-trihydroxyisoflavone) was purchased from US Biological (Swampscott, MA), while genistin (5,7,4'- trihydroxyisolavone-7-*O*-glucoside) was purchased from LC Laboratories (Woburn, MA). Sodium acetate, L-cysteine hydrochloride, $MgSO_4$, KH_2PO_4, ammonium citrate, K_2HPO_4, nicotinic acid, pyridoxine hydrochloride, and pantothenic acid were purchased from Sigma-Aldrich (St. Louis, MO). $MnSO_4$ was purchased from Amresco (Solon, Ohio) and synthetic complete supplementary mixture (SC) was purchased from Sunrise Scientific Product (San Diego, CA). Lactobacilli MRS broth was purchased from VWR (Wayne, PA). Redox Dye mix G was purchased from Biolog (Hayward, CA) and was added at KMS medium for colorimetric analysis.

Bacterial strains and cultivation conditions

The composition of the optimized KMS medium is found in **Table 2**. The strains used in this study are described in **Table 3**. Frozen cultures were passed in MRS broth and incubated statically at 37˚C for 24 h. Overnight cultures were then centrifuged at 4˚C and 796 × g for 5 min. MRS broth was aspirated from the cell pellet, and cells were resuspended in sterilized water (1 mL). Cells were washed three times to remove residual MRS broth, as described previously (Menon et al., 2013).

Optimization of KMS, a nutrient-defined medium

Strain *L. fermentum* ATCC 14931 ^{T} was used during the initial optimization of KMS; thereafter, the strains in **Table 3** were inoculated into KMS base medium, KMS+glycone, KMS+aglycone, and KMS+glucose. One percent (v/v) Biolog Redox Dye mix G was added in order to measure the metabolic activity of these strains (Biolog Inc., Hayward, CA, USA). The pH was adjusted to 6.5 (±0.3) and filter sterilized using a 0.2µm filter (VWR).

Solubility of genistin and genistein in KMS

Different concentrations of Tween 80 were used to dissolve genistein and genistin in MS media. Tween 80 was added at 0% (v/v), 5% (v/v), 10% (v/v), and 20% (v/v) was added to MS and incubated the sealed glass tubes in the shaker at 320 rpm for 3 h (**Figure 4**). The solvation of genistein and genistin was assessed visually.

SC (synthetic complete supplement) titration experiment

After solubility test of Gen^{+} and Gin^{+}, Tween 80 was constantly added at 10% (v/v) to MS medium. Filtered MS medium (0.2µm filter) without synthetic complete supplement was divided into different tubes: 1) MS + 0.5g/L SC – glucose, 2) MS – SC – glucose, 3) MS + 0.5g/L SC + 500µm glucose, and 4) MS – SC + 500µm glucose. Washed *L. fermentum* ATCC 14931 ^{T} and Biolog Redox Dye mix G were both added at 1% (v/v) to each tube (**Figure 5**). After inoculated all the tubes at 37°C for 24 h, color change were checked.

Screening bacteria activity in the different condition of media

The strains in **Table 3** were inoculated into KMS base, which is devoid of carbohydrates and undefined ingredients, KMS supplemented with 500µM genistin (KMS+glycone), KMS supplemented with 500µM genistien (KMS+aglycone), and KMS supplemented with 500µM D-glucose (KMS+glucose). In order to dissolve the genistin and genistein tubes were incubated at room temperature on the shaker at 320 rpm for 3 h. Washed cells and Biolog Redox Dye mix G were both added at 1% (v/v). The glass tubes were sealed with para film and kept in the incubator at a temperature 37°C for 24 h. Optical density value was read at 460 nm and 600 nm for 24 h. After 24 h, the final OD value, pH difference, and color change were checked.

Final cell count

Inoculated cells (100 µl) into KMS base medium, KMS+glycone, KMS+aglycone, and KMS+glucose were diluted in to sterilized water (900 µl) and the resultant cell suspension was serially decimally diluted to 10^{-7}. The diluted samples were spread-plated onto MRS agar and incubated at 37°C incubator. The numbers of colony forming units (CFUs) were enumerated after 48 h.

RESULTS

Composition of the development of a chemically defined base medium: KMS

Carbohydrate replacement

In this study, 500µM of D-glucose and 500µM of genistin (glycone) were used as a carbohydrate source in the KMS medium for cultivation of LAB. In addition, 500µM of genistein (aglycone) was used to compare with glycone results. Moreover,

KMS base medium, which was devoid of carbohydrate sources and undefined ingredients, was used as control medium. As a result, media selected were as KMS base medium, KMS+glycone KMS+aglycone and KMS+glucose.

Adjust of synthetic complete supplement

After *L. fermentum* ATCC 14931 ^{T} (v/v) and Biolog Redox Dye mix G were both added at 1% (v/v) to each tube from 1 to 4 (**Figure 5**), tube 1 (MS + 0.5g/L SC – glucose) and 2 (MS – SC – glucose) were indicated clear color after 24 h incubation at 37°C. However, tube 3(MS + 0.5g/L SC + 500µm glucose) and 4 (MS – SC + 500µm glucose), which include glucose as positive control for tube 1 and 2, were indicated violet color after 24h incubation at 37°C. As a result, no matter what present or absent of SC, reduced amount of SC (0.5g/L) didn't affect to color change in the MS medium. In this result, *L. fermentum* ATCC 14931 ^{T} only catabolized glucose as growth source.

Use of KMS-based bioassay to identify LAB with β-glucoside hydrolase activity

Genistein (aglycone) was perfectly dissolved in MS with10% of Tween (v/v) and un-dissolved genistin (glycone) was observed in MS with 10% of Tween (v/v) (**Table 4**). To increase solubility of genistin (glycone) and genistein (aglycone), 10% (v/v) of Tween 80 was selected in the basal medium KMS. In addition, synthetic complete supplement, which is a source of nucleobases, vitamins and amino acids, was reduced to 75% (from 2 g/L to 0.5 g/L) in the KMS medium (**Table 2**). The purpose of the development of a chemically defined base KMS medium is to limit amino acid sources and reduce carbohydrate sources that can be growth sources for the LAB. In addition,

KMS medium was used to increase solubility of genistein (aglycone). In this research, KMS base medium was used as control medium to assay utilization of genistin and genistein by the β-glucosidase activity from LAB.

Colorimetric reporter system

All KMS-based media were colorless (i.e., KMS, KMS+aglycone, KMS+glycone, and KMS+glucose) (**Table 5**). After cultivation at 37°C for 24 h, colorless media turned purple when the supplemented substrate was catabolized. For instance, *Lactobacillus pentosus* ATCC 8014^{T} converted the medium from colorless (−) to deep violet (+++) after 48 h at 37°C (**Figure 6**). Interestingly, *Lactobacillus pentosus* ATCC 8014^{T} was the only strain that showed activity against genistin (Gin^{+}).

Final cell count of L. pentosus

L. pentosus ATCC 8014^{T} was selected for final cell count (n = 3) that indicates strong violet color in KMS basal medium with concentration of 500 µM of glycone and 500µM of glucose. For visible cell count, diluted *L. pentosus* were plated on MRS agar and counted after 48 h at 37°C incubation. These results are listed in **Table 6**.

CHAPTER III

CONCLUSION

No undefined ingredients, such as proteose peptone, beef extract, and yeast extract, were used in the optimized KMS medium from MS medium (Menon et al., 2013). Also, the amounts of defined synthetic complete supplement and Tween were changed. In the previous study (Menon et al., 2013), they confirmed the carbohydrate-supplemented semidefined base medium (MS) to against non-LAB, which is alternative to MRS. In this study, a chemically defined base medium (KMS) was developed as basal medium that is eliminated carbohydrate sources and limited amino acid sources from MS (Menon et al., 2013). Developed the KMS medium was used to analysis the consumption of phytoestrogenic isoflavone by *Lactobacillus* spp. that have β-glycoside hydrolase activity. As a result, *L. pentosus* indicated β-glucoside hydrolase activity that actually utilized genistin (glycone) as a growth source in the KMS medium and it indicated dark violet color in KMS+glycone as similar to KMS+glucose media after 48 h cultivation at 37°C. Thus, both media the MS and KMS are useful for colorimetric reporter system, but we confirm that the chemically defined KMS base medium can be used to identify metabolism of LAB with β-glucoside hydrolase activity.

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APPENDIX I

FIGURES

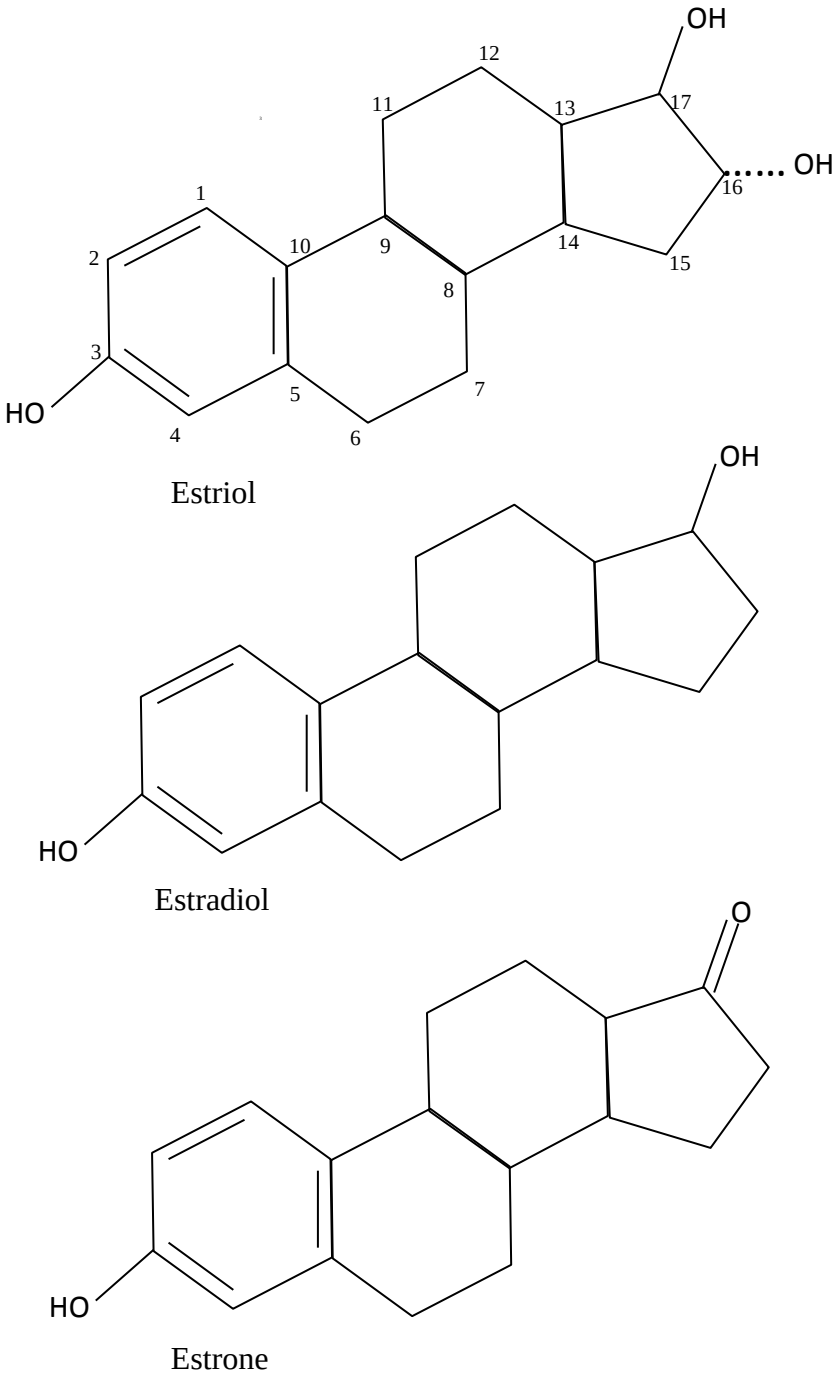
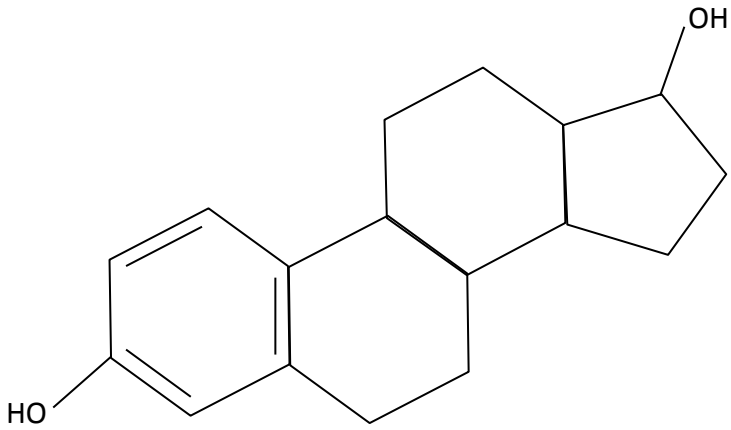
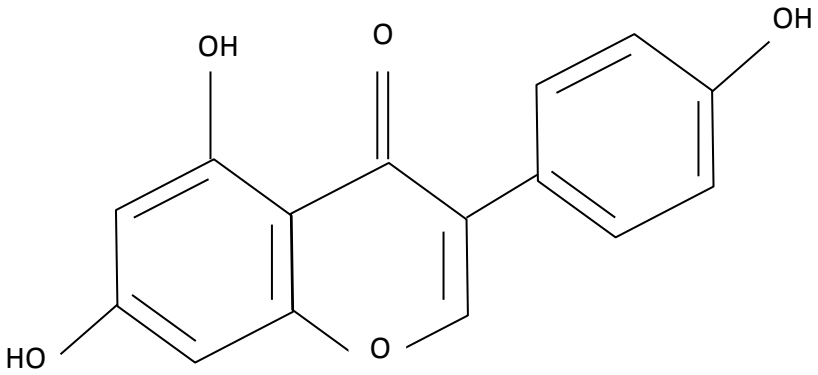


Figure 1. Structure of primary estrogens

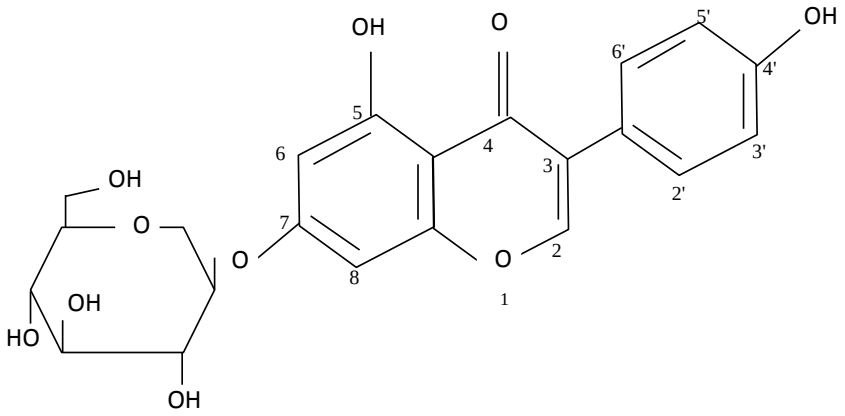


Estradiol



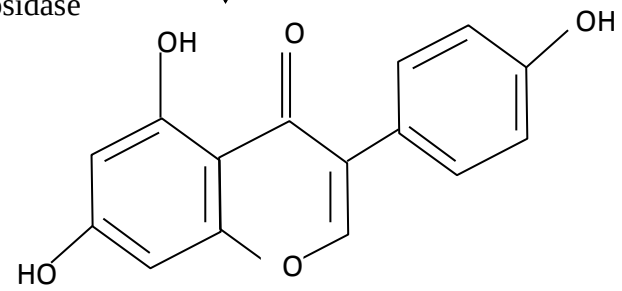
Genistein (5,7,4'-trihydroxyisoflavone)

Figure 2. Structure of estradiol and genistein



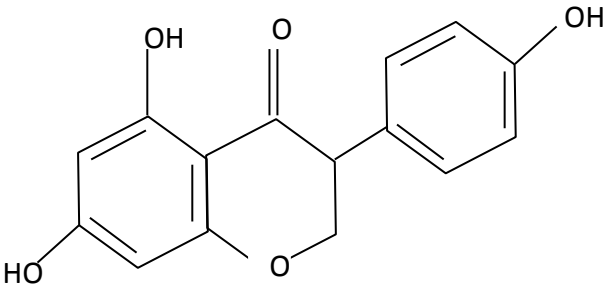
Genistin (5,7,4'- trihydroxyisolavone-7-*O*-glucoside)

Hydrolysis by glucosidase



Genistein (5,7,4'-trihydroxyisoflavone)

Reduction



Dihydrogenistein

Figure 3. Metabolism of genistin

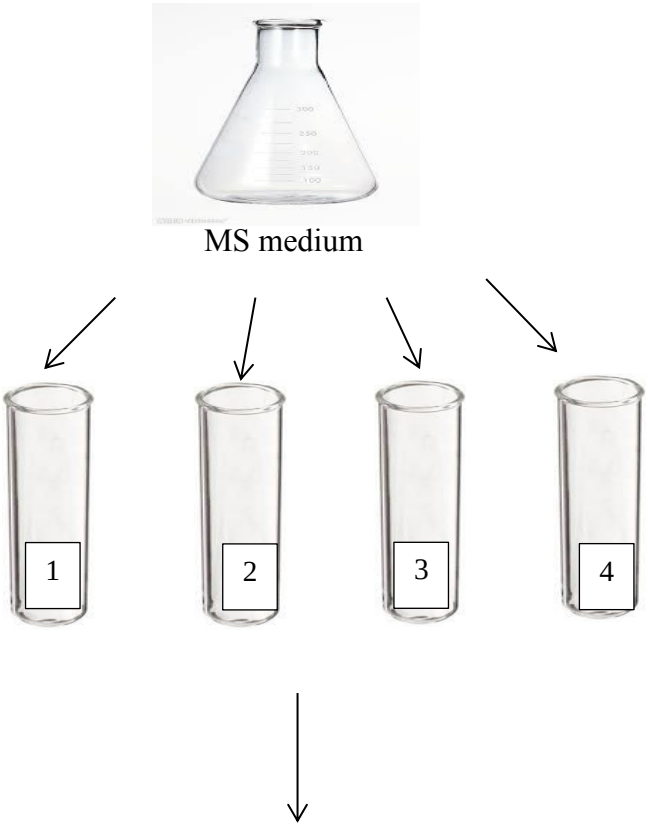


Figure 4. Solubility of genistein (Gin) or genistein (Gen^{+}) in MS medium

Tube 1, Tween10% (v/v) + 500 µM Gen or Gin; Tube 2, Tween 5% (v/v) + 500 µM Gen or Gin; Tube 3, Tween 1% (v/v) + 500 µM Gen or Gin; Tube 4, Tween 0% (v/v) + 500 µM Gen or Gin.

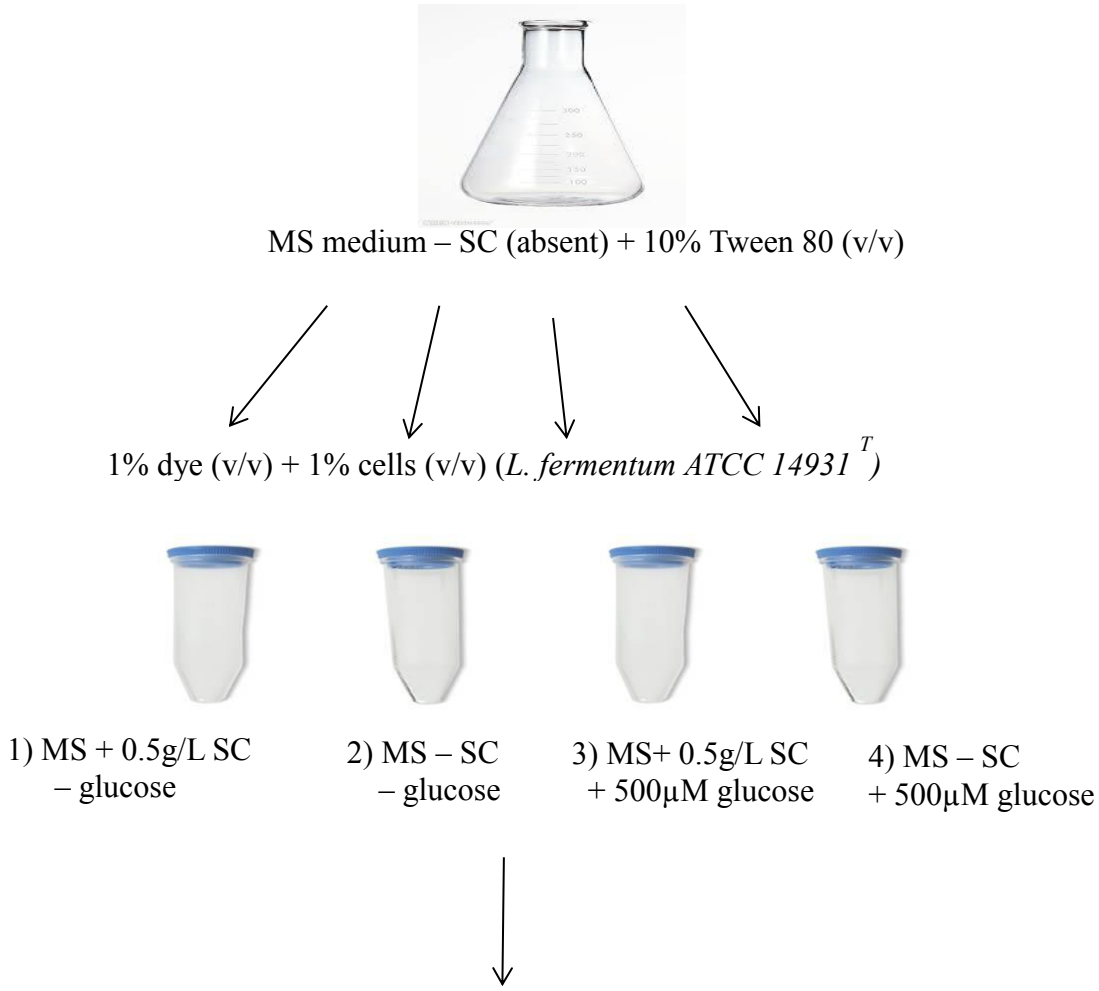
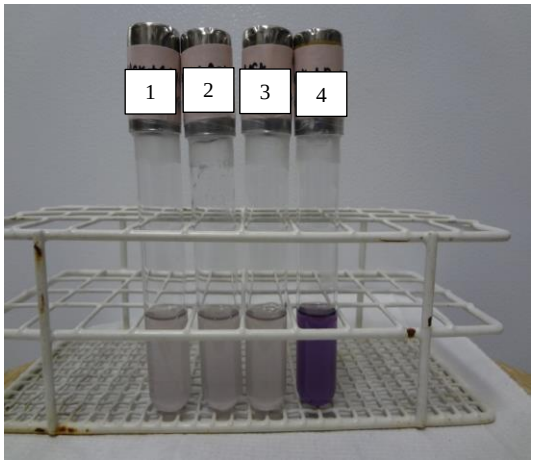
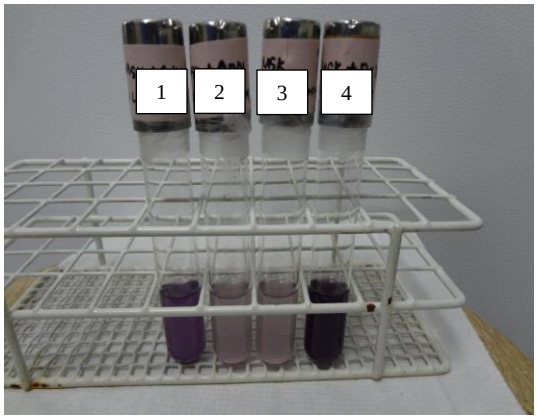


Figure 5. SC (synthetic complete supplement) titration experiment

24 h incubation at 37°C



48 h incubation at 37°C



1) KMS+glycone
2) KMS+aglycone
3) KMS
4) KMS+glucose

Figure 6. Colorimetric reporter system of *L. pentosus* ATCC 8041^{T}

APPENDIX II

TABLES

Table 1. Composition of a completely chemically-defined growth medium*

Ingredient	**Final concentration (g l^{-1})**	
	DM1	**DM2**
Glucose	10	10
Potassium hydrogen phosphate	3.1	3.1
di-ammonium hydrogen citrate	2	2
Potassium dihydrogen phosphate	1.5	1.5
Sodium chloride	0.02	0.02
Ascorbic acid	0.5	0.5
Potassium acetate	10	10
Tween-80	1	1
Heptahydrated magnesium sulphate	0.5	0.5
Hydrated manganese sulfate	0.02	0.02
Cobalt sulfate	0.5	0.5
Calcium lactate	1	1
DL-aminobutyric acid, L-phenylalanine, L-serine, L-threonine, L-cysteine, L-asparagine, L-isoleucine, L-methionine, L-tyrosine, L-tryptophan, L-valine	0.1	0.1
DL-alanine, glycine, L-histidine HCl, L-lysine HCl, L-proline, L-arginine, L-leucine	0.2	0.2
L-aspartic acid, L-glutamic acid	0.3	0.3
Nicotinic acid	10 mg	10 mg
Calcium pantothenate	10 mg	10 mg
Cyanocobalamin	0.02 mg	0.02 mg
Para-aminobenzoic acid	0.2 mg	0.2 mg
Myo-inositol	10 mg	10 mg
Pyridoxal HCl	10 mg	10 mg
Riboflavin	10 mg	10 mg
Biotin	1mg	1mg
Folic acid	0.2mg	0.2mg
Guanine	0.1	-
Thymine	0.1	-
Cytidine	0.1	-
2'-deoxyadenosine	0.1	-
2'-deoxyuridine	0.1	-
Inosine	-	Amount was not shown
Uracil	-	Amount was not shown

* Elli et al., 2000, DM2; guanine, thymine, cytidine, 2'-deoxyadenosine and 2'-deoxyuridine were replaced to inosine and uracil from DM1

Table 2. Composition of the chemically-defined KMS medium

Ingredient	Vendor	Amount (g l^{-1} or ml l^{-1})		
		bMRS	MS	KMS
Undefined				
Proteose peptone No. 3	Becton Dickinson	10	-	-
Beef extract	Becton Dickinson	10	5	-
Yeast extract	Becton Dickinson	5	2.5	-
Defined				
Ammonium citrate	Sigma-Aldrich	2	2	2
L-cysteine hydrochloride	Sigma-Aldrich	-	0.4	0.4
KH_2PO_4	Sigma-Aldrich	-	2	2
K_2HPO_4	Sigma-Aldrich	2	2	2
$MgSO_4$	Sigma-Aldrich	0.2	0.1	0.1
$MnSO_4$	Amresco	0.005	0.1	0.1
Nicotinic acid	Sigma-Aldrich	-	0.5	0.5
Pyridoxine hydrochloride	Sigma-Aldrich	-	0.1	0.1
Pantothenic acid	Sigma-Aldrich	-	0.5	0.5
Tween 80	Amresco	1*	1*	100*
Sodium acetate	Sigma-Aldrich	5	5	5
Synthetic complete	Sunrise Scientific Product	-	2	0.5

* ml l^{-1}

Table 3. The strains used in this study

Strain	Source
***Lactobacillus* spp.**	
L. buchneri ATCC 4005 ^{T}	ATCC
L. casei ATCC 393^{T}	ATCC
L. coryniformis ATCC 25602^{T}	ATCC
L. curvatus ATCC 25601^{T}	ATCC
L. delbrueckii ATCC 9649 ^{T}	ATCC
L. fermentum ATCC 14931 ^{T}	ATCC
L. helveticus ATCC 15009^{T}	ATCC
L. paraplantarum ATCC 700211^{T}	ATCC
L. pentosus ATCC 8041^{T}	ATCC
L. plantarum ATCC 14917^{T}	ATCC
L. rhamnosus ATCC 53103^{T}	ATCC
L. ruminis ATCC 27780^{T}	ATCC
L. salivarius ATCC 11741^{T}	ATCC
L. intermedius NRRL B-3692	ARSCC
***Pediococcus* spp.**	
P. acidilactici DSMZ 20284^{T}	DSMZ

T, Type or neotype strain for the species; ATCC, American Type Culture Collection; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

Table 4. Result of solubility test of genistin and genistein

MS medium	Tween 80	Total volume	Result	
			Genistin (gly)	Genistein (agly)
9 ml	1 ml (10%)	10 ml	Not dissolved	Dissolved
9.5 ml	500 µl (5%)	10 ml	Not dissolved	Not dissolved
9.9 ml	100 µl (1%)	10 ml	Not dissolved	Not dissolved
10 ml	0%	10 ml	Not dissolved	Not dissolved

500 µM of genistin and genistein were used.

Table 5. Qualitative assessment of strain metabolism (n = 3)

Strain	KMS		KMS +aglycone		KMS +glycone		KMS +glucose	
	24 h	48 h	24 h	48h	24 h	48 h	24 h	48 h
L. paraplantarum ATCC700211^T	−	−	−	−	−	−	+	+
L. pentosus ATCC 8041^T	+	+	+	+	+	++	+ +	+++
L. plantarum ATCC14917^T	−	−	−	−	−	−	+	+
P. acidilactici DSMZ 20284^T	−	−	−	−	−	−	+	+

(−) no color change; (+) pale pink to pink; (++) violet; (+++) deep violet;

Table 6. Endpoint viable plate cell count of *L. pentosus* ATCC 8041^{T} as a function of medium

Medium	**$\log_{10}$ CFU ml^{-1}**
KMS	8.30 ± 0.24^{a}
KMS+glucose	9.28 ± 0.04^{b}
KMS+glycone	8.70 ± 0.47^{a}
KMS+aglycone	8.70 ± 0.31^{a}

Cell count (n = 3) are presented as mean ± standard deviation (ANOVA)
Means with different letters are statistically different ($p < 0.05$)