TOWARDS IMPROVING THE NUTRITIONAL QUALITY OF PLANTS: EXPRESSING ARABIDOPSIS THALIANA PYRUVATE KINASE ISOZYMES IN E. COLI

Aurelia Leyva Castro

Dr. Thomas J. Savage, Faculty Mentor

ABSTRACT

Pyruvate kinase is an essential enzyme that regulates glycolysis. This research focused on establishing whether pyruvate kinases in plants have different kinetic and regulatory properties by characterizing highly-purified plant pyruvate kinases generated in E. coli. The intent was to obtain catalytically-active pyruvate kinase through either refolding of inclusion bodies or through expressing soluble protein with or without the presence of protease inhibitor and the antioxidant tris (hydroxypropyl) phosphine. Active protein was found only in the presence of tris(hydroxypropyl)phosphine and protease inhibitor. The ability to obtain catalytically-active protein offers an understanding of the metabolic role of plant pyruvate kinase isozymes, and ultimately will permit the engineering of glycolysis to increase levels of essential nutrients such as carotenoids, protein, or oils in plants.

The purpose of this study is to better understand the specific role that different pyruvate kinase (PK) isozymes, found in the cytosol of Arabidopsis thaliana, play in regulating glycolysis, based on their different kinetic values. PK is a protein that catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate in the presence of magnesium, potassium, and adenosine diphosphate (ADP) (Muñoz & Ponce, 2003). This is the terminal step of the consecutive sequence of reactions found in the glycolysis pathway. The purpose of the glycolysis pathway is to provide the energy source adenosine triphosphate (ATP) and metabolic starting materials (precursors) required for other essential pathways in cells (Voet, Voet, & Pratt, 2006).

LITERATURE REVIEW

In certain plants, such as the genetic model Arabidopsis thaliana, the glycolysis pathway is found in two different subcellular compartments: the cytosol and the plastid. The plastid PK provides precursors for carotenoid, starch, oil, and aromatic amino acid pathways. In the cytosol, the enzyme provides precursors required for the biosynthesis of the amino acids asparagine, alanine, leucine, and valine (Andre, Froehlich, & Benning, 2007).
In addition, PK exists in many forms (isozymes) in plants. For example, there are nine PK isozymes in Arabidopsis thaliana; six are found in the cytosol and three are targeted to the plastid (Savage, 2004).

To analyze the kinetics of each PK isozyme, first enough pure PK must be produced to measure the enzymatic activity and observe the kinetic behavior. For this research, the pET E.coli expression host system was used to produce PK protein more efficiently (Sorensen & Mortensen, 2004). The pET system consists of a circular DNA (plasmid) that contains the PK gene of interest fused to DNA encoding a histidine-tag, which allows the expressed protein to be purified by metal ion affinity chromatography. The pET plasmid is inserted into an E.coli bacteria expression host, which produces the protein of interest. The PK protein can be produced either as insoluble inactive protein (inclusion bodies) or soluble protein (active protein), which is folded and therefore is expected to have enzymatic activity (Novagen, 2003). To date, efforts to produce plant PK in E. coli have only produced inactive inclusion bodies. This present study attempts to obtain active plant PK from an E. coli expression system by refolding the insoluble inclusion bodies, or by producing soluble protein in large-scale cultures with or without a protease inhibitor and an antioxidant. The ability to obtain catalytically-active protein offers an understanding of the metabolic role of plant pyruvate kinase isozymes, and will lead ultimately to altering glycolysis and increasing levels of essential nutrients, such as carotenoids, tocopherol, protein or oils in plants.

**METHODOLOGY**

The following techniques were used to express cytosolic PK isozymes as refolded inclusion bodies or as soluble protein from large scale cultures, and to evaluate the enzymatic activity of the expressed protein.

Protein Expression in E. coli
Bacteria containing pET expression host system were plated and grown in the presence of antibiotic (kanamycin). Liquid cultures (2 mL) were inoculated from the plates then grown overnight and used to inoculate a 500mL culture. The larger culture was grown to late log phase, at which time 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce protein production. The culture was incubated overnight at 37°C, before harvesting the bacteria cells by centrifugation at 10,000 x g. Cells were lysed by incubation with nonionic detergent containing lysozyme. Insoluble inclusion bodies were isolated by centrifugation at 10,000 x g for 20 minutes, and were separated from cellular debris by several subsequent washings of the pellet with nonionic detergent, followed by centrifugation to recover the purified inclusion bodies. The soluble protein fraction was isolated by collecting the supernatant from the first centrifugation following cell lysis.

Inclusion Body Resolubilization
Inclusion bodies containing three different pyruvate kinase isozymes were harvested by centrifugation after expression in E. coli and cell lysis. Inclusion bodies were solubilized with 0.3% N-lauroylsarcosine (detergent) in TRIS buffer (pH 8.0), followed by dialysis into TRIS buffer (pH 7.5), and MOPS buffer (pH 7.0) for detergent removal.

Purification of Soluble Protein
Soluble lysate was loaded onto a 5 mL Ni-agarose affinity column. After washing the column with buffer containing 50 mM imidazole, the soluble, his-tagged protein was eluted with 1 M imidazole (Figure 1). Soluble protein was assayed for activity after dialysis to remove the imidazole.

Production and Purification of Soluble Protein with THP and Protease Inhibitor
Cell pellets were lysed in the presence of the antioxidant tris(hydroxypropyl)phosphine (THP) and protease inhibitor. 100mM THP was added to the binding, wash, and elution buffer. The elution buffer containing 50mM imidazole, the soluble his-tagged protein was eluted with 100mM THP in addition to 1M imidazole. Soluble protein was assayed in the presence of 100mM THP for activity after dialysis to remove the imidazole.

SDS-PAGE Analysis
Purified soluble protein was visibly observed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), where the PK was...
visualized as a broad band at 60kDa, compared to the molecular weight marker (MW). The 8% gel (PIERGE biotechnology) at 100 volts and Coomassie blue staining was used for observing protein bands.

**Enzyme Assay**

PK activity was measured using a lactate dehydrogenase-coupled assay whereby the loss of NADH is spectrophotometrically monitored as pyruvate kinase-generated pyruvate is converted to lactate, recording the absorbance at 340nm (Figure 2). The enzyme assay consists of a cocktail made of 40mM phosphoenolpyruvate (PEP), 4mM nicotinamide adenine dinucleotide (NADH), 20mM adenosine diphosphate (ADP), 5 units of lactate dehydrogenase (LDH), assay buffer, and the refolded or purified PK. Positive and negative controls were used before testing the refolded and purified soluble PK. The negative control contained no enzyme, and the positive control contained commercially available rabbit muscle PK.

![Chemical diagram](image)

**RESULTS**

To establish whether protein folded in vivo may have enzymatic activity, PK assays were performed on three different Arabidopsis PK isozymes that were expressed in E. coli and resolubilized from inclusion bodies. PK activity was not detected for any of the three resolubilized enzymes, whereas activity was observed when assaying commercially available rabbit muscle pyruvate kinase, indicating that assay conditions were capable of detecting activity, if any of the resolubilized proteins had been active (Figure 3). Because plastidial PK have been demonstrated to be heteromeric (multiple isozymes are required for enzymatic activity) (Andre et al., 2007), assays were also performed with mixtures of all three refolded isozymes and again, no activity was detected. Thus, the focus on this work was directed towards isolating soluble protein from the E. coli expression system.
Enzymatic Activity of Soluble Protein
To obtain sufficient amounts of soluble protein for measuring enzyme activity, induction experiments were performed with large scale (>500 mL) cultures, and highly-purified soluble protein was isolated by Ni-ion affinity chromatography (see Figure 1 on page X). However, assay of the purified soluble pyruvate kinase had no catalytic activity (activity with the rabbit muscle pyruvate kinase positive control was again positive). Each of the three isozymes, alone and in all possible combinations, were assayed and no combination demonstrated positive activity (Figure 4).
The upper left panel in Figure 4 represents the change of absorbance due to the conversion of NADH to NAD⁺ when the assay was performed with commercially available rabbit muscle pyruvate kinase (positive control), the upper right panel represents the change of absorbance when the assay was performed without addition of enzyme (negative control), and the lower panel represents the change of absorbance when the assay was performed with the refolded inclusion bodies.

Because proteins can suffer oxidative or proteolytic damage during purification, the expression and purification of one of the three isozymes was repeated in the presence of the antioxidant THP and a protease inhibitor. In this case, assay of the purified protein revealed significant activity (Figure 5).
**DISCUSSION**

Whether the protein is folded in vitro or in the presence of other isozymes does not seem to influence the lack of enzymatic activity of the expressed protein. However, the presence of the antioxidant and protease inhibitor did have an effect on enzymatic activity.

None of the pyruvate kinase isozymes resolubilized from inclusion bodies were catalytically active. Because some PKs have been demonstrated to be heteromeric, all possible combinations of the three isozymes also were assayed for catalytic activity, with negative results. It is now established with the experiments reported here that the soluble protein was undergoing oxidation or reducing reactions and protein degradation throughout the isolation, purification, or enzyme assay reactions. To establish whether the antioxidant or protease inhibitor had greater effect on enzymatic activity, further research needs to be done.

Efforts to obtain enzymatic activity are now focusing on repeating this experiment with and without the remaining active PK isozymes, to further analyze the inhibiting factors of soluble protein to remove other protein factors in the extract that may be inhibiting the activity.

**LIMITATIONS**

Although one cytosolic PK isozyme was found to be active, this experiment needs to be repeated with different isozymes that are all active. Additionally, other cytosolic isozymes need to be tested to further compare their difference in kinetic activity. Once kinetic variations are observed, it is possible to further analyze how each isozyme influences glycolysis differently to supply precursors in making nutritionally-important compounds, such as carotenoids, tocopherol, protein or oils in plants.

**CONCLUSION**

The current study tested for methods to obtain highly-purified, enzymatically active, Arabidopsis thaliana PK. The results indicate that soluble PK protein is only enzymatically active when purified from E. coli in the presence of the antioxidant THP and protease inhibitor. This protocol for expressing highly purified active PK isozymes can now be applied to analyze the differences in the kinetics of the cytosolic PK isozymes and understand the role each isozyme plays in regulating glycolysis. This understanding ultimately may allow the engineering of glycolysis to increase the levels of metabolic precursors to carotenoids, tocopherol, protein or oils to make nutritionally rich foods.
REFERENCES


PERCEPTIONS OF STUDENT ACCESS AND SUCCESS IN THE FIELD OF SCIENCE: EXPLORING EDUCATIONAL EXPERIENCES OF MEXICAN DESCENT COLLEGE STUDENTS IN RELATION TO NON-MEXICAN DESCENT FACULTY IN THE SCIENCES

Alma E. López Flores

Dr. Julie Lopez Figueroa, Faculty Mentor

ABSTRACT

The enrollment of Mexican descent students has significantly increased in higher education. Unfortunately, they continue to remain underrepresented, particularly in the area of science. The primary aim of this two-fold, qualitative study is to identify the possible social and academic factors that impact the representation of these students in the sciences. Through semi-structured interviews, this study examines the undergraduate experiences of current and former Mexican decent students and those of non-Mexican descent faculty, at a four-year institution in northern California. Ultimately, this paper proposes strategies and approaches that can increase the enrollment and completion rates of this particular ethnic-minority group of students in the field of science.

Research has demonstrated a direct correlation between understudied health issues specific to minority communities and the underrepresentation of ethnic minority professionals in these fields. Minority scientists are more likely to study minority issues than their majority counterparts (Nichols, 1997). They also have a general knowledge and understanding of their communities that facilitates the resolution of population-specific health problems (Committee for the Assessment of NIH Minority Research Training Programs, 2005). As a way to recruit minority students in science disciplines, Wilson (2007) suggests that top research universities hire more minority scientists to serve as role models to increase student persistence in pursuing a degree in the fields. More specifically, Lopatto (2007) believes the likelihood of minority students pursuing graduate school increases through participation in undergraduate research. Undergraduate research improves knowledge and understanding in science, students develop technical,
problem-solving, and presentation skills as well as the development of a professional self-confidence (Lopatto, 2004; Mabrouk & Peters, 2000).

Through role modeling and mentorship, Seymour and Hewitt (1997) conclude that minority students can better align, if not negotiate, important personal and cultural values of the family and community without disregarding them in order to succeed in science, math, and engineering careers. Research-related activities, positive faculty-student interaction and mentorship can increase students’ academic achievement, educational aspirations, student self-concept and persistence (Astin, 1997; Chickering, 1969; Kuh & Hu, 2001; Pascarella, 1985; Spady, 1970).

Consequently, if this minority force can play a significant role in this country’s competitiveness, especially for its increasing number in population, then we need to ask; What are the factors that limit and impede their advancement to keep pace with society’s demands? Nationally, the numbers of Latina/os obtaining degrees in higher education do not reflect their overall numbers in the population. Latina/os are expected to comprise one-fourth of all students in each educational level, by 2025 (White House Initiative, 2001). From the year 1990 to 2000, the Latina/o population increased 57.93% in the United States, making Mexican descent the largest sub-group, as it encompassed 52.94% of this increase (US Bureau of Census, 2001). Moreover, Ramirez and de la Cruz (2002) reported that in 2002 there was a total of 37.4 million Latina/os in the civilian, noninstitutional population of the United States, representing 13.3 percent of the total; among that Latina/o population 66.9 percent are of Mexican descent. The disproportionate gap of Latina/o student participation in higher education is evident when comparing the percentage of population increase and the percentage of higher education enrollment. The same degree of underrepresentation holds true in the area of science. Chapa and de la Rosa (2006) describe the higher education pipeline as a pipette. “Pipette” is a term used to characterize the decreasing rate of science degree attainment by Latina/o students, across the continuum of advanced degrees (e.g., bachelors, masters, and Ph.D.) in higher education.

The primary aim of this study is to identify possible social and academic factors accountable for today’s low number of Mexican descent college students graduating with biology and chemistry degrees at a northern California campus. More specifically, this study compares the academic experiences of current and former Mexican descent undergraduate biology majors to those of non-Mexican descent faculty in the sciences. For the purpose of this study “Mexican descent” is used as an inclusive term to refer to all people born in México or the United States, but whose parents or grandparents were born in México, [italics added] and are permanently living.
in the United States (Allensworth, 1997), irrespective of their immigration generation. “Hispanic” and “Latina/o” are terms used interchangeably in this paper, as they are often used by other scholars in their work to refer to people from Latin-America are from of Spanish-speaking communities. Although these terms do not recognize the diverse ethnic groups within the American continent; the term “Latina/o” is meant to include all persons of Latin American origin or descent, irrespective of language, race or culture (Hayes-Bautista & Chapa, 1987; Laden, 2004).

Undergraduate Student and Faculty Profile in CSUS
California State University, Sacramento (CSUS) is composed of African Americans, American Indians, Asians, Filipina/os, Pacific Islanders, Southeast Asians, Hispanics, Euro-Americans, international students, and those classified as other or unreported. To emphasize the purpose of this study, Table 1 provides figures on the undergraduate student profile of Hispanics, Euro-Americans, and students in the other or unreported category. It is important to note that many Latina/o students prefer to mark “other/unreported” because they refuse to identify themselves as Hispanic.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall</td>
<td>Spring</td>
<td>Fall</td>
<td>Spring</td>
<td>Fall</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3104</td>
<td>2902</td>
<td>3214</td>
<td>3135</td>
<td>3548</td>
</tr>
<tr>
<td></td>
<td>3113</td>
<td>3125</td>
<td>3126</td>
<td>3325</td>
<td>3327</td>
</tr>
<tr>
<td>Euro-American</td>
<td>9639</td>
<td>9166</td>
<td>9567</td>
<td>9391</td>
<td>9803</td>
</tr>
<tr>
<td></td>
<td>9315</td>
<td>9873</td>
<td>9288</td>
<td>9480</td>
<td></td>
</tr>
<tr>
<td>Other/Unreported</td>
<td>3446</td>
<td>3368</td>
<td>3601</td>
<td>3398</td>
<td>3370</td>
</tr>
<tr>
<td></td>
<td>3366</td>
<td>3531</td>
<td>3614</td>
<td>4265</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22582</td>
<td>21253</td>
<td>22555</td>
<td>21870</td>
<td>23029</td>
</tr>
<tr>
<td></td>
<td>21665</td>
<td>23615</td>
<td>22447</td>
<td>23724</td>
<td></td>
</tr>
</tbody>
</table>

Source: California State University, Sacramento, FactBook

Figure 1 portrays the number of students graduating with a biology and chemistry degrees, from 2002 to 2005. Vividly, it illustrates the Latina/o student underrepresentation in this institution’s divisions of science, as these degree numbers are compared to the overall CSUS Latina/o student population.
Theoretical Framework Guiding the Study
Social capital plays a major role in the success of minority students because connections, networking, mentoring, and peer relationships provide greater access to academic information and opportunities within institutions of higher education. Yosso (2006) noted that there is an assumption that students of color lack such capital for success, placing them at a disadvantage. Institutional agents, academic advisors, faculty, and student affair administrators may facilitate or hinder a student’s access to opportunities because they are most likely to assess student’s potential and refer them to opportunities accordingly (Stanton-Salazar & Dornbush, 1995). Historically, social capital has been utilized by minorities to maneuver through the system and to turn around and give the information and resources gained through the navigation process to social networks of people and community resources (Yosso, 2006). Given this framework, previous research on the encounters Latina/os face in the sciences will be provided, followed by a brief discussion on the aspect of multicultural education and Hispanic-serving institutions, and the importance of having faculty of color as mentors and advisors. To understand the significance of Mexican descent student representation in the area of science, a set of case studies is provided with students’ and non-Mexican descent faculty’s narratives and encounters. Finally this study concludes by discussing strategies that can create equitable conditions for teaching and learning in higher education.

LITERATURE REVIEW
Institutional barriers mediate minorities’ quality of academic success; such constraints include inadequate financial aid, lack of institutional resources, and hostile campus climate (Gándara & Maxwell-Jolly, 1999; Hurtado &
Carter, 1997; Loo & Rolinson, 1986). Smedley, Myers, and Harrell (1993) discuss the different levels of stress that minority students experience in higher education. Social climate stresses occur when there are few students and not enough professors of the students’ same ethnicity in classes, and when few courses involve issues relevant to student’s ethnic group. Poor academic performance expectations from students of an ethnic minority by white students and faculty, as well as the lack of concern and support for the needs of minority students by the university, also cause social climate stresses. Interracial stresses occur when minority students have negative relationships between different ethnic groups at the university, when the culture of their campus is white-oriented, when there is a lack of unity and support among members of the same race/ethnicity, and when they try to maintain their ethnic identity in their university. Another way to frame this issue is “acting-white,” meaning that students internalize behaviors corresponding to a majority group at the cost of losing their cultural identity (Gonzalez-Espada, 2004; Fordham & Ogbu, 1986). Racism and discrimination stresses are experienced when minority students are treated rudely, unfairly, or stereotypically because of their ethnicity. Minority students also have to prove their abilities to others by working twice as hard, which is the case for most minority students due to the lack of a strong math and science foundation from their high school (Loo & Rolinson, 1986).

Compared to second generation students, first generation and transfer students from community colleges to four-year institutions face a higher number of institutional obstacles. These obstacles include bureaucratic hurdles, confusing choices, student-initiated guidance, limited counselor availability, poor advice from staff, delayed detection of costly mistakes, and poor handling of conflicting demands (Laden, 2004). Loo and Rolison (1986) also noted that minority students rely on people of their own ethnicity for social support as a result of the distrust between Euro-American students and faculty and minority students. Such distrust further intensifies the student’s alienation. In some ways, the presence of stressors expose the lack of capacity institutions have to address diverse academic needs that are well beyond cultural congruency and more about cultural and academic responsiveness.

More often than not, colleges and universities fail to shift to a multicultural perspective from the monocultural framework and the Eurocentric cultural norm that frames its traditions and practices. A student of color may feel perceived as unworthy in a predominantly white classroom, marginalized, and even invisible, when faculty and student interactions remain unquestioned. Examining this relationship is fundamental in understanding minority students’ level of integration, thus their representation in the sciences. One
approach to examine this relationship comes from the critical multicultural theory through which class, race, gender characteristics, and power are analyzed (Kincheloe, 1996).

**Integrating a Multicultural Perspective in Higher Education**
Multicultural education calls for all aspects of education to be continuously examined, critiqued, reconsidered, and transformed, in order to teach all students, regardless of their background (Gorski, 2002). Bensimon and Tierney’s (1993) definition of multiculturalism is a way of understanding the commonalities and differences between diverse groups and using those characteristics to create alliances, within those groups. Rendon (1999) explains that this philosophy is humanistic and has faculty, administrators, and staff who themselves represent different cultures; hence, they would be able to give the students a safe and welcoming environment that also contributes to academic success. Multicultural education strives to provide all students with equity education, curriculum, and instrumental strategies so that each student’s background, learning style, experiences, skills, talents, and language proficiency are purposefully considered (Banks & McGee-Banks, 1997; Bennett, 2001).

Sadly, undergraduate science courses are popularly known for ineffective teaching styles and methods, oversized classes, grading on a curve, and for focusing more on memorization rather than developing critical thinking (Romer, 2002; Seymour & Hewitt, 1997). Seymour and Hewitt also point out that students are “filtered out” in introductory science courses, also known as “gatekeeper courses”. A study conducted by the American Association for the Advancement of Science, in 1991, suggested that the biggest problem for underrepresented groups was the lack of a nurturing environment, where the prevailing mentality is dog-eat-dog and faculty members worry more about grants than grading (Larsen, 1995). The reconstruction of an educational setting that reflects the democratic ideals of equality, justice, and freedom and along with nurturing educational inclusiveness is highly encouraged by critical multiculturalists. With this approach of critically teaching and learning, minority students overcome obstacles and barriers that aid them to succeed in the field of science.

**Hispanic-Serving Institutions**
Hispanic-serving Institutions (HSIs) play a major role in educating and increasing the numbers of Latina/o students in higher education (Laden, 2004). Generally, HSIs are two- and four-year colleges and universities that serve a large number of students who self-identify as Hispanic or Latina/o; these colleges provide access and degree attainment, particularly at the community college level. HSIs award larger percentages of associate,
bachelors, masters, first professional, and doctoral degrees to Latina/o students than non-HSIs; in 1999, 30.7% associate, 30.9% bachelors, 19.8% master’s, 16.8% first-professional, and 12.9% doctoral degrees, were awarded to Latina/os enrolled in HSIs than those enrolled in non-HSIs (Stearns, Watanabe, & Snyder, 2002). HSIs offer a variety of academic and student support programs and holistic approaches that are specifically designed to raise Latina/o students’ aspirations and increase their retention rates.

Table 2 illustrates that, despite higher numbers of enrollment and graduation rates in HSI than in non-HSI, particularly in biological sciences, the participation and completion rates of Latina/os continue to lag behind the dominant student group (Laden, 2004); thus, greater work must be done to exam the educational pathways of Mexican descent students in the sciences.

Most of the students attending HSI were first-generation college students and from either low- or lower-middle class families. This factor implies that in order to provide access to these students, there needs to be a sense of inclusiveness and a responsive, comprehensive curriculum, as well as quality transfer programs and articulation agreement (Laden, 2004).

**Latina/o Faculty Representation**

Beyond HSI institutions Latina/o students receive little exposure to faculty members from their own cultural backgrounds. Statistics reported by the National Science Foundation and the Alfred P. Sloan Foundation reveal a gap between the number of minority students earning undergraduate and doctoral degrees in science versus the number of minority professors holding faculty jobs in that area of study (Wilson, 2007). In 1996, Latina/os nationally represented 10.3% of all undergraduates, and Latina/o faculty constituted only 2.6% of all higher education faculty (National Center for Education Statistics, 1999). Recruiting Hispanic faculty trained in biomedical sciences and basic and clinical research as educators in medicine and other health professions is a key concern among a group of HSI presidents, chancellors, and CEOs according to De Los Santos (2003). This is a concern because Latina/o and other faculty of color are more likely to offer emotional support, encouragement, raise students’ aspirations, and are also more willing to serve as formal and informal advisors, mentors, and sponsors (Laden & Hagedorn, 2000; Turner & Myers, 2000). The difficulty
in recruiting credentialed minority faculty, particularly Latinas, to teach in the areas of mathematics, science, and engineering is due to limited resources and budget demands within higher education institutions, not excluding HSIs.

**Methodology**

A qualitative approach was utilized to conduct one-hour, in depth, open-ended, semi-structured interviews that provided narratives of the experiences that Mexican descent students encounter as science majors in higher education. The participants were recruited using purposive sampling and nominations from a four-year institution in northern California. Faculty in the chemistry department were also recruited and interviewed in the same manner as the student participants with the intention of obtaining their perspective as undergraduate science students themselves, and as current professors teaching multi-ethnic student populations. The researcher’s objective is to use both the students’ and faculty’s perspectives to identify the possible social and academic factors that impact the representation of these students in the field of science with the ultimate goal of proposing possible solutions to better understand the needs of Mexican descent students and facilitate their road to a successful career in science.

**Participants**

There were a total of 12 participants in this study; eight female, Mexican descent, students and four male, Euro-American, chemistry professors. The student participants included two former and six current undergraduate biology majors. The former students switched their area of study to ethnic studies with a minor in sociology and to government with a minor in English. A total of 13 professors were contacted; five pertaining to biology and eight to chemistry. Nine professors replied; six chemistry and three biology professors. Out of the six chemistry professors only two agreed to participate in in-person interviews, two other agreed to answer a set of questions via email, and the remaining two chemistry professors were not available for either an interview or email questionnaire. The three biology professors stated their ability to participate in the future. All identities were kept confidential.

**Procedure**

At the beginning of the study, the purpose and importance of this study was explained to the participants; they were also informed that their participation was entirely voluntary and that they would be able to withdraw at any point during the study without penalty. There was no form of inducement or incentive offered for their participation. All participants were provided written informed consent forms outlining the ethical guidelines.
and procedures of the study, and were told that signing the form signified that they agreed to be part of the study. To protect the participants’ right to privacy, their names were not disclosed in any written or verbal context; instead, pseudonyms were assigned to each participant. Direct quotes, from the participants who gave their written consent, were used from the written and audio-taped interviews, in which careful notes and observations were taken throughout the interview, including observed facial expressions and body movements.

Participants were encouraged to freely answer the questions and to skip any that they did not feel comfortable answering. The researcher agreed to return or destroy all participants’ audio tapes used in their interviews following the final submission of the paper or in the case that the participant decided to withdraw from the study. The place and the time of the interviews were left to the convenience of the participants.

FINDINGS

The lack of access to information in degree attainment in relation to careers in medicine directed the student participants to declare biology as their major. Some of the students began their undergraduate studies as nursing majors, later switching to biology, not knowing that they could major in a non-science field and still apply to medical school, as long as they met the pre-requisites. This information was exposed to them three or four semesters after being in the university, as it was in Melin’s case, who would have majored in English if she had known this information earlier in her academic career.

Student-Professor Interaction

For the most part, the students took chemistry courses with the same professors, but in different semesters. The student participants concurred that they felt intimidated by their chemistry professors, thus limiting their confidence in seeking help because they did not feel comfortable approaching them. The students believe that the professors were qualified in terms of being experts in their area of study, but were unable to teach the material to all students. They also found them inaccessible, even during their office hours. Some students, like Cuca, preferred to email her professor rather than ask him directly for help, but even emails became an issue because he would not respond to them. Melin recalls her organic chemistry professor favoring those students who knew the material very well, mainly Euro-American students, and those who had high grades in his class. Melin, who was a B/C average student, was belittled and felt “dumb,” due to the body language and facial expressions of her professor when she asked him for help. This experience was enough for Melin to look for help elsewhere.
Student participants rated the biology courses somewhat better; the professors were more approachable than those in chemistry, with the exception of a couple. In Angela’s cancer biology class, the professor made it clear to his students that if they had questions, those questions better be important. He demanded that the students have a good idea of what they wanted to talk about, especially if it was in regards to a question on an exam, and expected the students to research their arguments before seeing him. Although, the professor was supposed to be a resource for the students, his standpoint closed the doors for Angela; she could not approach him due to his intimidating persona. Alondra also encountered this same professor but as an ineffective advisor. He advised Alondra in taking the necessary and required courses to graduate, but never counseled her with relevant information for the potential career she wanted to pursue. Alondra also found her general biology professor to not be culturally sensitive, “she had a lot of prejudice against people of color,” states Alondra. A perfect example Alondra shared was when the professor advised the students to vote, one of the students commented that some students could not vote because they were not citizens of this country. The professor’s response was, “Well, that’s too bad, that is why you come to America, otherwise you shouldn’t be here.” Alondra took this unnecessary comment very personally because she was not a citizen of this country. Alondra’s perception of the professor was that if she knew her immigration status, it would put her at a disadvantage in the class.

True and Pseudo Help and Support
Several student participants took a pre-chemistry course and found the instructor to be extremely helpful. Pati mentions how he would sacrifice his weekends to help the students out in understanding the material. The general chemistry laboratories were taught by teaching assistants (TAs) and the adjunct classes were taught by other undergraduate students. This did not, in any way, better the situation for this minority sub-group. Erika, for instance, found the TA to be extremely unfair and, in a sense, discriminatory towards her. He failed to acknowledge her every time she would raise her hand to ask a question, but would quickly help other students who were for the most part were Euro-American and Asian. Erika was afraid to confront him because she believed he would do anything in his power to fail her from the class; she did not believe it would make a difference if she said or did something about it, except get her in more trouble. Erika decided to switch her major after her second year as a biology student, specifically after her experience in the laboratory class.
In terms of the adjuncts classes, Erika, Alondra, and Melin agreed about receiving little or no help because there was not much being taught by the facilitators. Euro-American students in the class would form small cliques, just as they did in the laboratory sections; they were very hesitant and gave limited and unclear responses when they were asked for help. Angela perceives this as a class issue, “Those in the upper class are more competitive and they see only for themselves; they have a mentality of being better than anyone else, versus the middle class, whom tend to be more ‘cool.’”

Science Educational Equity (SEE) Program
The Science Educational Equity (SEE) Program was designed in 1986, to serve a population of minority students that face social, economic, and educational barriers in their science career pathways. SEE aims to create a learning community and improve the accessibility to resources and information to students as they share their knowledge and provide personal as well as moral support to each other. The program offers a study room with a couple of computers and free printing for SEE students only. Due to the limited space, the study room was always crowded and noisy, thus, Sandra Maria, Alondra, Cuca, and Pati only made use of the printing resources because their study skills required a quiet environment.

Even though the program offers great opportunities, such as conferences, adjunct classes, and major advising, it also has a couple of drawbacks. The first drawback is the ineffective outreach to students; students in this present study did not hear about the SEE program until they were in their third semester in college. In Angela’s case, she did not hear about the program until after her third year and a half. All of the student participants formed part of the program, except for Angela and Erika. Erika saw no point in applying because she had decided to switch to a different area of study. Although the staff in the SEE office was friendly, Angela and Sandra Maria felt unwelcome by other students in the SEE study room.

Student Challenges
Every study participant doubted their participation in this field of science. Many were discouraged by the lack of help and support, the amount of work load and extra effort they were putting in their studies without seeing any positive outcome. Advisors who were professors themselves discouraged some of these students by advising them to consider other majors. For instance, they recommended students to switch from pre-med to nursing because they believed medical school could be very intense and hard for the students.
Faculty Undergraduate Experiences
Whether it was an un-manipulated interest test, a high school teacher who made chemistry fun, or whether he lived in a chemistry-oriented environment because his father was a chemist, Drs. Wilson, Smith, and Hedger, respectively, decided to become chemists.

The institutions these three professors attended as undergraduate students were private universities, with the exception of Dr. Hedger who attended a community college and then transferred to the four-year public institution, California Polytechnic University, San Luis Obispo. Dr. Wilson attended the University of Redlands in Southern California and Dr. Smith did his undergraduate work in Carnegie Mellon University because, as he stated, “it had a superb reputation for computer science and mathematics, which was what I intended to major in, until finding my way back to chemistry in my sophomore year.” Lastly, Dr. Rhangel attended Holy Cross College in England because of its quality education and short distance from his home. The institutions these professors attended were not diverse in student population; Dr. Smith is the only one who recalls having a large number of Asian nationalities represented on campus, but not in the division of chemistry.

Academic, Mentoring, and Social Support
Dr. Wilson had a full scholarship and formed part of accelerated classes taught to essentially small classes of 20 students; making his classmates a great support group and allowing for a more personal student-professor relationship; he also worked as a teaching assistant, grading on the side. The relationships of the students with faculty and staff in the chemistry department were very close; Dr. Smith met the department chair and frequently discussed with him how to juggle his schedule to complete a double major in a four-year period. Dr. Hedger was lucky to have one of his chemistry instructors guide and advise him as to how to discipline and apply himself in order to succeed as an undergraduate chemistry major and in the course of his career. Dr. Hedger academically eased his studies by using his networks; although he preferred to work alone, he always had friends, classmates, and of course his own professors to help him whenever he needed help in solving a problem.

Professors’ Undergraduate Research Experience and Challenges
As undergraduate students, the professors in their classes stressed the importance of research and, thus, directed them into conducting research during the summer. They were provided with offices, their own desks, and keys to the science building, therefore, having year-round accessibility. Conducting research significantly impacted their career decisions. Overall,
the professors felt they did not have any challenges as undergraduate students. Dr. Smith and Dr. Hedger, on the other hand, found their biggest challenge to be adjusting to the level of work required in college versus the level of work expected in high school.

Faculty’s Perception of Students
One of the perceptions that Dr. Wilson has of today’s undergraduate students is that they are not as serious about their education as he believes they should be. His conclusion is based on the comparison of his undergraduate institution to the institution at which he is currently teaches, assuming that because education at CSU, Sacramento is not as high as it was at his institution, students do not find it as disastrous as they otherwise would to repeat courses. Dr. Wilson states, “There are some students in my class that are passing time, they shouldn’t be in there, they should be doing something else. I don’t think some of them are not quite ready…they should think about maybe not going to college for a while until they are ready to do it.” Dr. Wilson is aware that he has a reputation of being hard, but fair. In other words, he expects the students to perform at a certain level or they do not get an A or a B. He commented that he tells his students that grades are not a strict percentage, that instead they float based on the student who has a reasonably high grade in class. Professor Rhangel, on the other hand, recognizes the unfairness of comparing his experience with that of today’s students because of the apparent difference in institutions. He notices that students are less prepared than students 20 years ago, mainly with math and study skills.

Dr. Hedger finds that students’ main distractions are cell phones and video games, that they lack respect for the time of academic course work and for their professors. He believes that due to the quantity of work students have, they display their frustrations easily and become less civil. Dr. Hedger expresses that the level of expectations students have of their professors is out of line, “It seems as if they want a personal tutor, but they don’t consider that I have to serve 250 students.” He sees those students as too babied and lacking effort on their part and views himself as a learning facilitator and mediator who makes learning easier, but not someone that can do all of the work for the students.

Accessibility and Faculty’s Teaching Style/Philosophy
For students taking general chemistry, they can obtain help in the “Help Office” where they can always find a graduate student or professor assistant to help them out, between nine in the morning to two in the afternoon. Drs. Wilson, Hedger, and Rhangel believe students do not visit their professors during office hours as often as they should. These professors express that
they make themselves available through emails, when their office hours contradict the students’ school and work schedules.

In terms of their teaching philosophy, Dr. Wilson articulated, “I try to present every point in a clear and understandable way that allows students to ask questions. I am willing to help them as long as I see they are putting in sufficient effort on their own part, which implies them reading the notes and doing their homework.” This is what worked for him as an undergraduate student; therefore, he believes that using this method of teaching is the best way for most students to get a good grasp of the material. Dr. Smith’s philosophy has been influenced by his strongest student experiences, best and worst. He has adopted many of the practices from his professors whose courses he enjoyed and learned the most from. Although he is still in the process of developing his teaching philosophy because he has been teaching for only two years, his principal goal in teaching chemistry is to instill in his students a through knowledge that reaches beyond memorization to application, associated with problem solving skills as well as appreciation of the subject material. Dr. Smith gained additional ideas through a “Preparing Future Faculty” course he took prior to beginning his pedagogy at CSU, Sacramento, in which he became more aware of the diversity of students, their backgrounds and their different learning styles. Dr. Hedger prefers to have students approach learning as a whole and so that they begin to make connections among individual topics being taught. Through this standpoint he tries to develop critical thinking skills where students move beyond knowledge comprehension and instead learn about critical analysis, evaluations, and higher level thinking. Dr. Hedger mentioned that he approaches teaching in away that is natural to him.

Undergraduate Research Mentorship and the Culture of Science
Dr. Wilson sees research as a form of student retention, stating, “Most of the students that get into research really get into it, there are a few students that are having conflict between living and surviving and that might prevent them from sticking with it, but if they actually do research then that definitely helps them keeps them here.” Dr. Smith echoes Dr. Wilson in that through research students not only get a chance to know their faculty members more closely but also apply what they have learned in their courses. He encourages students to conduct research by talking to them when they demonstrate a potential interest; he displays research posters to heighten their interest and directs them to other faculty members if their research interest lies outside of his area of expertise. Dr. Smith, on the other hand, is interested in researching the best practices for teaching chemistry, especially as students demonstrate their difference in learning styles.
All of the professors had a very distinct viewpoint of what the culture of science meant for them. Dr. Wilson, sarcastically, responded, “I don’t think that there is a culture of science really, unless you are talking about nerds. What comes to my mind is that there is some cult of a little group of people in science, which is not the case. Well, it depends on the level as a professor, if you are a professor at Harvard or Berkeley, then you eat, drink, and breathe science. I don’t choose to do that, I prefer to do other things.” Dr. Wilson, though, agrees that science is a “survival of the fittest environment,” if a student goes to a high school that emphasizes science and mathematics, the student has a big advantage. In Dr. Hedger’s perspective, the culture of science belong to those who are very curious; curiosity is the common denominator to find out if a student can do science.

Faculty’s Challenges and Advice
Dr. Wilson recalls that 30 years ago class sizes were maxed out at 30 students, but today that figure has risen to 80 students per classroom. Due to this increase in students, professors have adjusted their exams. Dr. Wilson used to administer his exams in such a way that students could receive partial credit, using short-answer questions. With larger class sizes, he has implemented multiple choice exams to make grading easier and meeting deadlines to turn in grades, especially for finals exams, possible.

Professor Rhangel’s main advice to students is to work hard and not be afraid to ask for help; to identify their weaknesses and begin to strengthen them as soon as possible. Dr. Smith recommends always attending class and keeping up with assignments, to work hard and devote proper time to the course. He expects students to see him when they have questions, instead of just letting things go. In science overall, he believes students should develop the proper foundation (i.e., good coursework, good mathematical and communication skills to get involved with research be creative, and find the area that they are personally excited about).

Dr. Hedger’s advice to students is to “choose two.” He presents to them “The Triangle” as part of his class instruction, every semester. The triangle is divided into three parts: school, work, and social life/family. Students are then advised to pick two sides of the triangle. If the student chooses to go to school and work, then that student should not have much of a social life or family time. If the student decides to have a social life and work, then that student is not going to have enough time for school, lastly, if the student decides to go to school and have a social life, then that student had better not work, in order to be a successful science student. Choosing two and being able to negotiate time for sleep are the key components, in Dr. Hedger’s eyes.
Clearly there are differences in the professors’ and the students’ undergraduate experiences. The background and values of the professors, the type of institution they attended, and the quality of education they received is very distinct from that of the students’. The students did not have the social capital, the networks, faculty and peer support and resources that the professors had, thus, their experiences as science students were more demanding.

Mexican descent college students cannot neglect one of the sides of the triangle, Dr. Hedger had previously mentioned; education, family, and work are all interrelated and essential to each other. Therefore, students find themselves balancing out an agenda that demands triple the time and effort the professors had to consider as undergraduate students. Some of the professors seem to undervalue the academic achievement and progress of this particular ethnic-minority group of students and fail to execute their role as institutional agents of the university. Faculty has the capacity and ability to transmit or negotiate the transmission of institutional resources and opportunities in the university, such as research and academic information, programs, and opportunities (Stanton-Salazar & Dornbush, 1995).

There is a huge contradiction between the professors’ perceptions of their students and what the students perceive from their professors. Professors believe they are providing the students, as a whole, with the necessary instruction and resources for them to succeed in their courses. Mexican descent students, though, experience the opposite; they feel ignored, undervalued, and some confront a language barrier. Oftentimes professors give examples to students to make a point in regards to a concept being taught, using certain American expressions not familiar to Mexican descent students. As a result, the students do not grasp the concept(s) to the fullest. Consequently, the students who ask their professors for help report being discouraged in pursuing a science career because they are expected to know what was “taught” in class. In the end, no clarification is made to the student’s question and the students get discouraged in further seeking faculty for help. When professors perceive the students ambivalence in help seeking as a lack of effort on the students behalf, the students also began to internalize those viewpoints, therefore increasing their social stigma. It is then fair to say, that non-colored faculty do not and cannot understand the experiences of minority students. For this reason the presence of faculty of color is fundamental.

The way in which professors present themselves to students can also determine the level of confidence students have towards them. Sadly, the
intimidating personality students perceive from their professors can inhibit them from interacting with faculty. By applying the teaching style that best worked for the professors is a faulty pedagogical practice, however some professors acknowledge that such teaching styles may not be effective for all students. Notwithstanding, they have not devised a way to assure the needs of all the students they serve.

Moreover, students and professors perceive false cues presumed by both parties in terms of prioritizing education, through unspoken words and misinterpreted behavior. The professors were described as culturally insensitive and gave less than supportive advice to the students, but they can become more effective student advocates if they provided access to information in an approachable manner, as it is the role of a true mentor. To make grading easier, professors accommodated their exams, but one thing to consider is that test taking skills is a crucial means for success, especially if “success” is defined by high scores in exams. Students can be knowledgeable on the material being tested, but not knowing how to tackle the exams can be devastating.

Some of the implications higher education institutions can implement to better serve its student body include having student affairs administrators consider the multiple social experiences and implement mentoring and research programs, promote the retention of faculty of color, and diversity education. Tutoring, science exposure, role models and mentors that can provide a nurturing environment, especially for first-generation college students, has a great impact on minority students. It is crucial for universities as a whole to be able to acknowledge that negative and culturally insensitive attitudes and behaviors affect all incumbents in the university, and affects the students’ academic performance, satisfaction, and retention. Therefore, faculty members in particular should assess their interactions in the classrooms and monitor their delivery and cultural sensitivity while not minimizing the role of presenting curriculum and learning experience, and be held accountable for their actions in the classroom. Faculty promotion guidelines should include evaluations based, not only on demonstrated knowledge of their area of expertise, but on cultural sensitivity and multicultural competency (Jones, Castellanos, & Cole, 2002). University administrators, staff, faculty, and students need to collaborate and effectively communicate with each other for the institution as a whole to understand the needs and expectations of their students of color, and to meet their needs.
LIMITATIONS
There are a number of limitations to be noted. First, data collection was difficult to obtain because the researcher, herself an undergraduate biology student, had a great deal of difficulty outreaching to her former and prospective professors in her field of study, hence, the lack of professor participation did not allow for proper data analysis. Second, the purposeful sampling technique confines the generalization of this study’s results to Mexican descent science majors because of the limited representation of ethnic minority voices in this study, and its findings can apply only to this particular institution, CSU, Sacramento, where the study was conducted. Third, there was no statistical data available on undergraduate students conducting research at this university, which was intended to be used to analyze its impact on students’ academic achievements.

CONCLUSION
The intent of this research study was to identify and bring about an understanding of the experiences of Mexican descent undergraduate students in the sciences with the objective of identifying possible factors that affect their representation in this field. The researcher concludes accountability structures and leadership must move in the direction of centrally incorporating discussions of diversity. In other words, asking and supporting faculty to create more inclusive curriculum (Figueroa & Garcia, 2005). Furthermore, students should not conform to the current academic cultural system and infrastructure within the sciences. Instead the system should be reconstructed and adjusted by adopting strategies, conditions, and structures that meet the need of different cultural contexts among students, particularly in the area of science. Otherwise, Mexican descent students’ negative experiences in the field of science will continue to be dismissed or unrecognized by faculty, staff, administrators and other students who uphold the problem students of color face, thus, they will remain underrepresented in the sciences. It is, then, worth stating that, in higher education, degree granting is a measure of accountability.
REFERENCES


Sacramento State University FactBook. http://www.csus.edu/portfolio/Factbook


