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ARROZ PUITÁ INTA CL CULTIVADO NA PLANÍCIE COSTEIRA DO RIO GRANDE DO SUL: COMUNIDADES BACTERIANAS, CARACTERÍSTICAS FÍSICO-QUÍMICAS DOS SOLOS E VALOR NUTRICIONAL

São Leopoldo
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Tese apresentada como requisito parcial para a obtenção do título de Doutor em Biologia, pelo Programa de Pós-graduação em Biologia da Universidade do Vale do Rio dos Sinos - UNISINOS.

Orientador: Dr. Uwe Horst Schulz
Coorientadora: Drª Denize Righetto Ziegler

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BANCA EXAMINADORA

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Com Ele aprendi o que é realmente amor... dedico não somente essa tese, mas toda a minha vida, alma e espírito ao meu Deus.

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"Sua vinda aguardo eu cantando; meu lar no céu; seus passos hei de ouvir soando, além do escuro véu. Passarinhos, belas flores, querem m'encantar; são vãos terrestres esplendores, mas contemplo o meu lar."

Hino da Harpa Cristã – Exilado

RESUMO

As bactérias possuem um papel fundamental na manutenção da vida e do equilíbrio dos ecossistemas. As lavouras de arroz têm diversas ações microbianas, inclusive interações entre plantas e micro-organismos. Os agroecossistemas são compostos por diversos micro-habitats e propiciam a adequação de uma grande diversidade microbiana. O manejo da cultura do arroz promove mudanças das características físicas e químicas da água e devido às alterações de pH, turbidez, temperatura, radiação e quantidade de matéria orgânica, podem estar relacionadas com a dinâmica das comunidades microbianas presentes no solo. Esse trabalho objetivou avaliar as comunidades bacteriológicas presentes no solo, de cultivo mínimo, do arroz tipo Puitá Inta CL semeado em duas regiões orizícolas, nos anos agrícolas 2013/14 e 2014/15, com diferentes classes de solos (Planície Costeira Interna/PCI e Planície Costeira Externa/PCE). Nesse estudo foram identificadas as bactérias heterotróficas que podem auxiliar as plantas no seu desenvolvimento e na produção de grãos mais nutritivos e efetuadas análises dos elementos físico-químicos dos solos, assim como a determinação da composição nutricional dos grãos de arroz Puitá Inta CL, integral e polido, produzidos em cada região de estudo. Os dados dessa pesquisa foram apresentados em quatro capítulos que correspondem às publicações na forma de artigos científicos. Os resultados revelaram no total 29 espécies bacterianas, sendo 27 registradas na PCE e 22 a PCI, nas diferentes fases fenológicas de *Oryza sativa*. Quanto à abundância de Unidades Formadoras de Colônias (UFCs), considerando a região PCE obteve-se 18,5% das espécies no período de pré-plantio, 22,3% na fase vegetativa, 22,3% na fase reprodutiva e 36,9% durante a maturação ($F_{1,9}$ = 7,84, p <0,05). A diversidade estimada pelo índice de *Shannon* foi superior na maturação (M = 3,901) em comparação com os valores obtidos no solo de outras fases de cultivo (M = 2,97). A abundância de UFCs na PCI foi de 23,9% no período de pré-plantio, 21,7% na fase vegetativa, 20,7% na fase reprodutiva e 33,7% durante maturação ($F_{1,9}$ = 7,03, p <0,05). A diversidade estimada foi maior na maturação (M = 3,801) em comparação com os valores obtidos no solo das demais fases fenológicas (M = 2,46). O índice de *Evenness* indica que a distribuição de espécies foi semelhante em ambas as regiões (PCE e PCI) durante a maturação (E = 0,988 e 0,967). Os valores elevados obtidos na análise CCA explicam a variação observada nos três eixos: para o primeiro ano agrícola explicam 67% da variação das espécies e para o segundo ano agrícola explicam 86,5%. Esses dados indicam que a fase de maturação das plantas à diversidade de espécies bacterianas é predominante. Os resultados da PCA demonstram que os três primeiros eixos explicam 74,19%: o eixo 1 (47,12%) inclui as variáveis Argila, pH e Hidrogênio + Alumínio; o segundo eixo (15,79%) inclui Fósforo, Capacidade de troca de elétrons pH 7 e Magnésio; e o terceiro eixo (11,28%) Alumínio, Matéria Orgânica e Índice de análise e correção da acidez. O padrão do resultado espacial demonstra uma diferença significativa entre os solos de cada região, o que pode ser comprovado pela MANOVA (Wilk's Lambda: 0,008, p <0,0001 and F = 61,6). O valor nutricional do arroz, o teor fenólico e o teor de antioxidantes foram testados pelo método de Mann Whitney que revela a diferença do arroz integral e polido, de cada região orizícola (p<0,001), não havendo diferença temporal (p>0,05) entre ambos (2013/14 e 2014/15).

Palavras-chave: *Oryza sativa*, Bactérias heterotróficas, Solos, Conteúdo nutricional.

ABSTRACT

Bacteria has a key role in maintaining the life and balance of ecosystems. Rice crops suffer microbial actions, including interactions between plants and micro-organisms. The agro-ecosystems are composed of several micro-habitats and allow the adaptation of a great microbial diversity. Rice crop management promotes changes in the physical and chemical characteristics of the water and due to changes in pH, turbidity, temperature, radiation and organic matter, may be related to the dynamics of the microbial communities present in the soil. The objective of this project was to evaluate the bacteriological communities present in the soil of the Puitá Inta CL rice, sowed in two rice regions, in the 2013/14 and 2014/15, with different soil types (Internal Coastal Plain / PCI and External Coastal Plain / PCE). In this study, we identified heterotrophic bacteria that can assist plants in their development and in the production of more nutritious grains and in the analysis of the physical and chemical elements of the soils, as well as the determination of the nutritional composition of the Puitá Inta CL rice grains, brown and Polished, produced in each region of study. The data of this research were presented in four chapters that correspond to the publications in the form of scientific articles. The results revealed a total of 29 bacterial species, of which 27 were recorded in PCE and 22 were PCI, in the different phenological phases of *Oryza sativa.* The abundance of Colony Forming Units (CFUs), the PCE region obtained 18.5% of the species in before sowing, 22.3% in the vegetative phase, 22.3% in the reproductive phase and 36, 9% during seed maturation ($F_{1.9} = 7.84$, $p < 0.05$). The diversity estimated by the *Shannon* index was higher at maturity (M = 3.901) compared to the values obtained in the soil of other phenological phase (M = 2.97). The abundance of CFU in the PCI was 23.9% in before sowing, 21.7% in the vegetative phase, 20.7% in the reproductive phase and 33.7% during seed maturation ($F_{1.9} = 7.03$, $P < 0.05$). The estimated diversity was higher in maturation (M = 3.801) compared to the values obtained in the soil of the other phenological phases (M = 2.46). *Evenness* index indicates that species distribution was similar in both regions (PCE and PCI) during maturation (E = 0.988 and 0.967). The high values obtained in the CCA analysis explain the variation observed in the three axes: for the first agricultural year they explain 67% of the variation of the species and for the second agricultural year they explain 86.5%. These data indicate that the maturation stage of plants to the diversity of bacterial species is predominant. The results of the PCA show that the first three axes explain 74.19%: axis 1 (47,12%) includes the variables Clay, pH and Hydrogen + Aluminum; The second axis (15.79%) includes Phosphorus, Electron exchange capacity pH 7 and Magnesium; And the third axis (11,28%) Aluminum, Organic Matter and Index of analysis and correction of acidity. The spatial result pattern shows a significant difference between the soils of each region, which can be verified by MANOVA (Wilk's Lambda: 0.008, $p < 0.0001$ and F = 61.6). Rice nutritional value, phenolic content and antioxidant content were tested by the Mann Whitney method, which reveals the difference between, brown and polished rice from each rice region ($p<0,001$), it is not temporal difference ($p>0,05$) between them (2013/14 and 2014/15).

Keywords: *Oryza sativa*, heterotrophic Bacteria, Soils, Nutritional content

SUMÁRIO

1 INTRODUÇÃO

1.1 Produção de arroz irrigado

Cereais são alimentos de origem vegetal, constituídos de grãos e largamente consumidos pelos povos do mundo todo. Os cereais têm composições semelhante de proteínas, lipídios, fibras, cinzas e carboidratos. O nome “cereal” deriva da *Ceres*, a deusa grega da agricultura e da colheita. Os principais cereais cultivados são arroz, trigo, milho, aveia, centeio, cevada e triticale. Eles são amplamente consumidos por fazer parte dos hábitos alimentares de muitas pessoas; fácil conservação, transporte e renda; por ser de baixo custo e ter bom valor nutricional; além de ter uma grande variedade de formas de uso culinário. A domesticação do arroz aconteceu há cerca de 10.000 anos atrás na Ásia e, no Brasil, a planta veio quando houve a colonização portuguesa. (PANIZZON et al, 2015). O arroz é o segundo cereal mais consumido no mundo com cerca de 2,4 bilhões de pessoas o usando como base na sua alimentação (EMBRAPA, 2016). O consumo de arroz faz parte da rotina de mais da metade da população mundial. A maior produção de arroz ocorre na Ásia (KUENZER; KNAUER, 2013), porém, o Brasil se destaca junto aos países que compõem o mercado desta região (COMPANHIA NACIONAL DE ABASTECIMENTO (CONAB, 2016)) por produzir mais de 80% do arroz, sendo o maior produtor de arroz fora do continente asiático. O Estado do Rio Grande do Sul tem como destaque o cultivo de arroz irrigado na sua economia agrícola. (CONAB, 2016).

Na safra 2015/16 o Brasil teve 2.143.500 ha de área plantada com uma produtividade de 5.425 Kg/ha. Tendo o Estado do Rio Grande do Sul que é o maior produtor do país 1.108.300 ha plantados com 7.350 Kg/ha de produtividade. (CONAB, 2016).

O arroz é uma planta do gênero *Oryza* da família das gramíneas, sendo a *O. sativa* (arroz asiático) e *O. glaberima* (arroz africano), as espécies mais cultivadas. O arroz é um dos cereais mais cultivados no mundo para a alimentação humana direta e como insumo para a indústria de alimentos. Existem tendências destas indústrias a procurarem cada vez mais o arroz, principalmente o orgânico e o integral, devido as boas práticas de alimentação humana. (CONAB, 2016).

A produção de arroz no Rio Grande do Sul tem predominância do cultivo irrigado; (AZAMBUJA et al, 2004). O Rio Grande do Sul é dividido em seis regiões orizícolas, denominadas: Fronteira Oeste, Depressão Central, Zona Sul, Planície Costeira Externa, Planície Costeira Interna e Campanha (AZAMBUJA et al, 2004), como pode ser observado na Figura 1. Essa divisão serve para melhor avaliar os resultados da produção estadual.

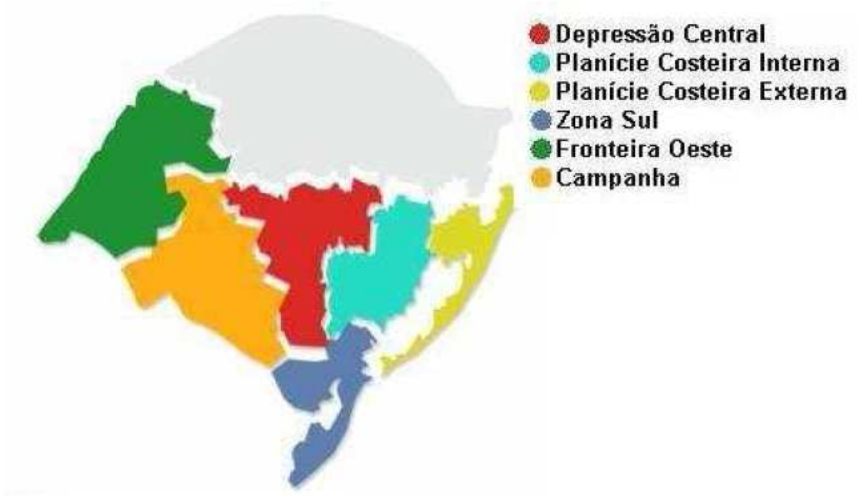

Figura 1. Mapa das regiões orizícolas do Rio Grande do Sul: Fronteira Oeste, Depressão Central, Zona Sul, Planície Costeira Externa, Planície Costeira Interna e Campanha. (Fonte: SOSBAI, 2014).

O Rio Grande do Sul apresenta diversas classificações de solo de várzea, que são adequados ou não ao cultivo de arroz irrigado como: Planossolos, Gleissolos, Chernossolos, Neossolos, etc. O Planossolo representa 11% do total da área de várzea do estado. (PINTO et al, 2004). Os solos de ambas as cidades estudas de Santo Antônio da Patrulha (Gleissolos) e de Charqueadas (Planossolos) apresentam boas condições para a utilização agrícola, considerando-se uma agricultura desenvolvida, ou seja, que atende às correções de acidez, fertilizações e demais práticas de manejo da cultura. A Planície Costeira Externa apresenta em sua maior parte o solo do tipo Gleissolos, que possui uma alta taxa de matéria orgânica. A Planície Costeira Interna apresenta em sua maior parcela o solo tipo Planossolos que em suas camadas mais profundas é compactado com poucos nutrientes que dificulta o crescimento da planta. (IRGA, 2013).

A cultura orizícola se adapta facilmente a diversas condições de solo e clima, o que é muito importante, já que a população mundial está em ampla expansão e a demanda na produção de alimentos deve aumentar cada vez mais para suprir as necessidades nutricionais diárias da população. Mundialmente, este cereal ocupa uma área de 158 milhões de hectares. O consumo médio no Brasil é de 45 kg por pessoa por ano. Estima-se que em 2050 a população mundial esteja entre 7,9 a 10,9 bilhões de pessoas. (PROGRAMA DAS NAÇÕES UNIDAS PARA O MEIO AMBIENTE (PNUMA), 2015). Por isso, a produção de alimentos precisa aumentar proporcionalmente a esse crescimento, logo o desafio hoje é utilizar tecnologias que propiciem uma maior produtividade.

Como toda monocultura, o cultivo de arroz irrigado pode gerar impactos se não for manejado de forma adequada. Como esta cultura utiliza uma quantidade elevada de água e, normalmente, fica nas proximidades de corpos hídricos, preocupações com o mesmo acaba sendo inevitável. Além disso, a demanda de água para outros setores, como o industrial, mas principalmente, para o consumo humano acaba gerando uma série de discussões sobre a manipulação deste recurso tão importante à vida na Terra, podendo ocasionar conflitos pelo uso desses recursos. (MACEDO et al, 2009).

A bacia hidrográfica do Rio dos Sinos que integra Santo Antônio da Patrulha e está localizada no nordeste do Estado do Rio Grande do Sul (RS), entre os 29° e os 30° paralelo sul. Esta bacia abrange uma área de 3820 km^2, cuja nascente do Rio dos Sinos é na cidade de Caraá e estendendo-se por 190 km até o delta do rio Jacuí onde ocorre a sua descarga. (PANIZZON et al, 2012). A cidade de Charqueadas é banhada pelo Rio Jacuí. A Bacia do Jacuí tem uma área de 71 600 km^2, correspondendo a 83,5% da área da região do lago Guaíba. O rio nasce no plátano Jacuí nos municípios de Passo Fundo e Marau, e toda a sua área de drenagem é caracterizada pelo uso intensivo de terras para a agricultura e pecuária. (PRÓ GUAÍBA, 2016).

Tendo em vista a relevância desta cultura e a demanda considerável de água que ela utiliza, é importante que se conheça a dinâmica de micro-organismos que habitam esse agroecossistema, pois eles são responsáveis pela ciclagem de nutrientes e têm interações muitas vezes simbióticas com as plantas. Os micro-organismos representam uma diversidade química e molecular na natureza, constituindo a base de processos ecológicos, como os ciclos biogeoquímicos e a cadeia trófica, além de manterem relações vitais entre si e com os organismos superiores. (ALBIACH et al, 2000; KUNITO et al, 2001; HIRSCH et al, 2010). Além disso, sabe-se que os cultivares escolhidos e também o estágio de desenvolvimento da planta de arroz pode influenciar as populações microbianas associadas a estes agroecossistemas. (PANIZZON et al, 2012).

Aspectos importantes também estão associados aos agroquímicos necessários para que as culturas possam expressar seu máximo potencial produtivo. (SILVA et al, 2009). Por outro lado, a utilização de fertilizantes e pesticidas pode atingir rapidamente mananciais hídricos superficiais por meio de drenagem superficial, percolação lateral, escorrimento superficial e sub-superficial, erosão, deriva, volatilização e, também, para mananciais hídricos subterrâneos por lixiviação e fluxo facilitado, atingindo organismos não-alvo e proporcionando contaminação ambiental. (SILVA et al, 2009).

Os sistemas de cultivo empregados, para a produção de arroz irrigado, são o plantio convencional, o plantio direto, o cultivo mínimo e o pré-germinado. (EMBRAPA, 2016). Cada sistema é caracterizado por diferentes tipos de manejo do solo e da própria cultura. No cultivo convencional, preparo do solo é feito com aração, logo, ocorre um intenso movimento do solo. Para o plantio direto, há pouco movimento do solo, que é realizado muito antes do período de plantio, a fim de utilizar um herbicida de ação total contra plantas que germinam no período entre o preparo do solo e outros tipos de plantio. O plantio pré-germinado é caracterizado pela semeadura de sementes pré-germinadas em solo inundado de antemão. O preparo do solo é feito com máquinas e implementos, trabalhando dentro do quadro plantação totalmente inundada. Já o cultivo mínimo usa menos mobilização do solo. Quando comparado com o sistema convencional, a semeadura é realizada diretamente sobre a cobertura vegetal previamente dessecada com herbicida, sem perturbação do solo. A cultivar utilizada nessa pesquisa foi a PUITÁ Inta CL, no sistema de plantio cultivo mínimo. A cultivar PUITÁ Inta CL juntamente com a variedade IRGA 424 são as sementes de arroz mais usadas no estado do Rio Grande do Sul. É ideal para todas as regiões do Rio Grande do Sul, exceto áreas meridionais do litoral Sul e campanha, devendo evitar áreas com altos teores de ferro.

Cada sistema de cultivo também apresenta uma demanda diferenciada da água de irrigação. Cultivo mínimo e plantio direto têm metodologias semelhantes de irrigação. Porém, o plantio direto pode ter a antecipação do inicio da inundação da lavoura, pois tem efeito positivo no controle de ervas daninhas. (VERNETTI JUNIOR; GOMES, 2004; GOMES et al, 2004). Já o sistema pré-germinado, apresenta um sistema diferenciado de irrigação, com a inundação prévia do solo, antes do seu preparo permanecendo durante 20 dias até a semeadura. De um a três dias após a semeadura a água é retirada, permanecendo assim de três a seis dias. A partir desse estágio, o manejo da água assemelha-se aos demais sistemas mencionados.

O desenvolvimento da planta de arroz se classifica em três fases: 1) fase vegetativa, desde a germinação até a iniciação da panícula; 2) fase reprodutiva, que compreende a iniciação da panícula até a floração; 3) maturação, desde o florescimento à maturação dos grãos, conforme a Figura 2. De modo geral, as mudanças de fase provocam uma alteração na composição química e microbiológica do solo, já que a planta libera exsudados que se modificam ao longo do ciclo da cultura. (ASAKAWA; KIMURA, 2008; JACKSON; ILAMURUGU, 2014).

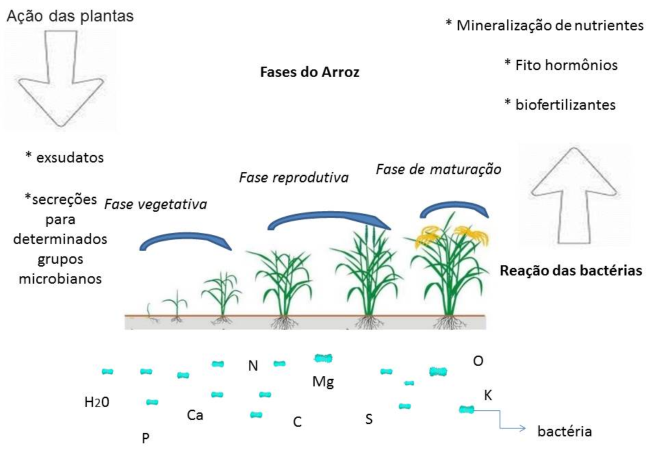

Figura 2. Fases fenológicas do arroz irrigado juntamente com a ação das plantas e a reação dos micro-organismos. (Fonte: Panizzon et al, 2016).

1.2 Micro-organismos do solo e da água

O solo é composto por microhabitats que diferem em suas propriedades físico-químicas. (CARSON et al, 2009). Estas características determinam uma composição diversificada de micro-organismos no solo. Apesar de muitas vezes o solo proporcionar condições mínimas para as necessidades microbianas, esses organismos desenvolvem-se lentamente até o limite permitido pelos parâmetros físico-químicos. (TATE, 1995). Qualquer mudança nas características do solo, sejam elas físicas ou químicas, provocam uma mudança na composição de organismos que habitam esse local, pois sabe-se que os micro-organismos tem grande importância em ciclos bioquímicos e geoquímicos participando ativamente neles. Portanto, o sistema solo pode abrigar muitos grupos funcionalmente importantes no ecossistema, como por exemplo as bactérias diazotróficas, bactérias nitrificantes e fungos decompositores. A composição e riqueza destes micro-organismos neste sistema pode ser afetada por vários fatores, tais como distúrbios físicos, uso de fertilizantes e as espécies de plantas existentes. (PANIZZON et al, 2016). A biodiversidade funcional em agroecossistemas é a chave ecológica para a sustentabilidade da produção e as bactérias têm fundamental importância para esta manutenção do solo. (DICKENS; ANDERSON, 2001).

Micro-organismos podem ser responsáveis por altos prejuízos econômicos na lavoura de arroz. No Rio Grande do Sul, as principais doenças de arroz que causam prejuízos significativos na produção e na qualidade dos grãos são: brusone (*Magnaporthe oryzae*), mancha parda (*Bipolaris oryzae*), mancha-de-grãos (complexo de patógenos) e escaldadura (*Monographella albescens Thümen*). (EMBRAPA, 2016). Os micro-organismos do solo podem ter efeitos negativos, positivos ou neutros para as plantas. (PRABHU et al, 2004). Os micro-organismos positivos atuam na manutenção do solo por meio da produção de substâncias bioativas como enzimas, vitaminas, antibióticos e nutrientes. (KOEPF et al, 1983). Portanto, eles causam efeitos benéficos às plantas, sendo responsáveis pela decomposição dos resíduos orgânicos, pela ciclagem de nutrientes e pelo fluxo de energia do solo (DE-POLLI; GUERRA, 2007), exercendo influência tanto na transformação da matéria orgânica quanto na estocagem de carbono e nutrientes, garantindo boa produtividade agrícola.

Existe uma grande diversidade de bactérias no solo que, quando associadas a plantas hospedeiras, estimulam o seu crescimento, como é o caso das rizobactérias. (SOTTERO et al, 2006). A estimulação do crescimento em plantas ocorre devido ao aumento de disponibilidade de nutrientes minerais no solo (PEÑA; REYES, 2007), produção de hormônios de crescimento como giberelinas e auxinas (KETZNELSON; COLE, 1998; BROWN, 1998), resistência induzida a doenças e da supressão de micro-organismos da rizosfera de plantas. (NANDAKUMAR et al, 2001). Esses efeitos proporcionam um ganho que podem reduzir os custos e minimizar possíveis problemas ambientais, como a contaminação do solo e mananciais hídricos, provocados pelo uso de produtos químicos, como os fungicidas. Dentre os gêneros de rizobactérias mais estudadas pode-se citar *Pseudomonas fluorescens, P. putida, Azospirillum brasiliense, Serratia marcecens, Rizobium* sp*, Bradyrhizobium* sp, *Arthrobacter* sp, *Enterobacter* sp, *Azotobacter* sp dentre outras. (MELO, 1998). *Bacillus* são bactérias Gram-positivas, aeróbias ou anaeróbias facultativas e formadoras de esporos (MORI et al, 2004), que permite o desenvolvimento da bactéria em temperaturas diversificadas. Espécies como *B. thuringiensis, B. subtilis, B. cereus* e *Lysinibacillus sphaericus* podem apresentar atividade inseticida para diversas ordens de insetos (APAYDIN et al, 2005), sendo amplamente utilizados no controle biológico de pragas.

Algumas espécies também estão associadas a raízes das plantas, auxiliando na absorção e fixação de nutrientes presentes no solo. Elas também têm aplicações biotecnológicas e produzem antibióticos. (GARBEVA et al, 2003). Portanto, saber manejar o solo de modo a preservar, ou mesmo melhorar suas características em sistemas sustentáveis, é um dos desafios à agricultura. (REIS JUNIOR; MENDES, 2007). A composição de uma comunidade dificilmente se manterá estática (BEGON et al, 2008), portanto qualquer mudança em um habitat pode provocar a modificação na composição das populações. Sabe-se que práticas agrícolas alteram características físicas e químicas do solo, influenciando diversas populações e comunidades bacterianas. (PEREIRA et al, 2000).

Micro-organismos que habitam o solo, juntamente com processos biológicos, têm sido investigados como indicadores da sustentabilidade da agricultura e da qualidade do solo. (REIS JUNIOR; MENDES, 2007). Eles influenciam a qualidade biológica dos produtos e a produtividade alcançada. (PEREIRA et al, 2000). Há muitas condições que podem alterar a abundância de bactérias no solo. A disponibilidade de oxigênio, as relações de umidade, a faixa de temperatura, a matéria orgânica disponível e o pH são fatores que afetam diretamente estas comunidades. As bactérias também acabam sendo limitadas pela disponibilidade de fontes de carbono no solo. (FEIGI et al, 1998). De um ponto de vista ecológico, organismos decompositores são importantes, pois atuam em moléculas orgânicas degradantes transformando-as em inorgânicas, de modo que podem ser utilizadas pelas plantas. As bactérias também são protagonistas nos ciclos de nitrogênio e enxofre, e algumas vezes podem gerar um estresse hídrico. Este estresse induz o choque osmótico que pode resultar em lise celular e liberação de solutos intracelulares. Portanto, as bactérias podem sobreviver em água uma vez que permaneçam partículas de solo aderidos às raízes e, favorecendo a umidade ideal para as raízes se manterem vivas. (PANIZZON et al, 2016).

Sabe-se que as bactérias contribuem com o equilíbrio metabólico e fisiológico dos agroecossistemas. (NORMANDER; PROSSER, 2000; BLACKWOOD; PAUL, 2003; GELSOMINO; CACCO, 2006; WAKELIN et al, 2008). Bactérias heterotróficas são as principais decompositoras de matéria orgânica no solo e na água. Dentre as espécies que compõe esse grupo, estão as do gênero *Pseudomonas*. As bactérias do gênero *Pseudomonas* sp que se caracterizam por possuírem eficientes mecanismos de ação antagônica, atuando como agentes no controle biológico de várias pragas e doenças, sendo capazes de promover o crescimento de plântulas, além de apresentarem excelente sobrevivência e colonização na rizosfera. (BOTELHO; MENDONCA-HAGLER, 2006). Algumas espécies de *Pseudomonas* são encontradas em diversas regiões, sendo consideradas cosmopolitas. (TIEDJE et al, 2001). Elas estão presentes em água doce, mas principalmente no solo, onde estão associadas às raízes das plantas e, assim como outros grupos, auxiliam na fixação de nitrogênio. (REINHARDT et al, 2008). Outras bactérias conhecidas como promotoras de crescimento em plantas de arroz, já que auxiliam na fixação de nitrogênio, são: *Azoarcus* sp (HUREK et al, 1994), *Herbaspirillum seropedicae* (ELBELTAGY et al, 2001; RIGGS et al, 2001) e a espécie *Burkholderia.* (DIVAN-BALDANI et al, 2000; OLIVEIRA et al, 2002).

A compreensão, portanto, do impacto de diferentes sistemas de cultivo de arroz irrigado na comunidade bacteriana do solo é importante para o conhecimento das tecnologias de menor impacto ambiental do solo e, eventualmente, das que possam contribuir positivamente para a melhoria das interações ecológicas que se processam no solo.

1.3 Arroz na alimentação humana

A alimentação humana é um indicador essencial de qualidade de vida, além de afetar os indivíduos de diversas formas, em virtude da importância de macro e micronutrientes, proteínas, vitaminas, minerais e nutrientes que são necessários para o correto funcionamento do corpo. De acordo com ZHOU et al (2002), a composição nutricional e de antioxidantes dos grãos está sujeita a diversos fatores, como variedade, variações ambientais, manejo, processamento e armazenamento. Além disso, o polimento do grão resulta em redução no teor de nutrientes, exceto de amido, as quais demonstram que o arroz polido apresenta concentrações menores de cinzas, lipídios e fibras insolúveis, quando comparado ao arroz integral.

A dieta é um dos maiores determinantes para a persistência de certas bactérias no trato gastrointestinal, porque a dieta proporciona nutrientes, não somente para o hospedeiro, mas também para as bactérias que estão associadas. Componentes de alimentos não digeríveis servem de fonte de energia e carbono para as bactérias do intestino humano. (BLAUT; CLAVEL, 2007). É conhecido que o corpo humano é habitado por um grande número de micro-organismos, distribuindo-se por vários tecidos e órgãos e que sua abundância é maior do que o número de células humanas, bem como, estarem diretamente ligados ao sistema imunológico e no melhor aproveitamento dos alimentos. (DAVIS; OHLHORST, 2014).

Com a evolução da ciência nutricional foi permitido que se soubesse mais sobre a composição alimentar e a ação dessas substâncias dentro do organismo que a está ingerindo. A nutracêutica estuda os componentes fitoquímicos presentes nos alimentos dispondo a estudar os seus benefícios para a saúde e possíveis tratamentos de doenças. Nutracêuticos, então, são suplementos alimentares que contêm a forma concentrada de um composto bioativo de alimento, apresentado separadamente da matriz alimentar e utilizado com a finalidade de melhorar a saúde, em doses que excedem aquelas que poderiam ser obtidas de alimentos. (RATAJCZAK et al, 2015).

Sabendo-se que o trato gastrointestinal é colonizado por micro-organismos logo após o nascimento do indivíduo e que, também, algumas situações da vida, como o consumo de antibióticos, resultam na redução dos micro-organismos no trato. Faz-se importante a adição de alimentos com características nutracêuticas. Sendo assim, os probióticos são suplementos alimentares adicionados aos alimentos em quantidades adequadas, que acabam influenciando seu valor nutritivo e terapêutico. São representados pelas culturas de micro-organismos vivos (bactérias benéficas e leveduras) que contribuem melhorando o sabor do produto final, além de produzirem substâncias antimicrobianas. (SANTOS; CANÇADO, 2009). Já, os prebióticos são ingredientes alimentares que são os principais substratos de crescimento dos micro-organismos intestinais, não digeridos no intestino delgado que, ao atingir o intestino grosso, são metabolizados seletivamente por um número limitado de bactérias denominadas benéficas, as quais alteram a microbiota do cólon gerando uma microbiota bacteriana saudável. (RAIZEL et al, 2011).

Algumas bactérias têm sido empregadas como probióticas, como as espécies dos gêneros *Bifidobacterium* e *Lactobacillus*, as quais auxiliam da microbiota intestinal humana, quando a flora intestinal é drasticamente reduzida e ficam vulneráveis ao ataque de patógenos. (SANTOS; VARAVALLO, 2011).

Os prebióticos, por sua vez, são definidos como carboidratos fermentáveis capazes de alterar a composição e atividade da microbiota gastrointestinal, conferindo benefícios à saúde do hospedeiro, tendo seus efeitos atribuídos à função de substrato, estimulando o crescimento e estabilização das populações microbianas benéficas, em detrimento às demais. (SANTOS et al, 2016).

Nesse contexto, a presente pesquisa avalia as comunidades bacteriológicas presentes no solo, de cultivo mínimo, de arroz do tipo Puitá Inta-CL semeado em duas regiões orizícolas do RS (Planície Costeira Interna e Planície Costeira Externa). Nesse trabalho foram realizadas análises físico-químicas do solo e identificadas bactérias heterotróficas, as quais auxiliam as plantas no crescimento e na produção de grãos potencialmente com maior valor nutricional.

Os dados da pesquisa estão estruturados na forma de artigos científicos que constituem os quatro capítulos da presente tese:

Capítulo 1: **“Microbial Diversity: Relevance and Relationship Between Environmental Conservation And Human Health”.** Artigo de revisão publicado em Brazilian Archives of Biology and Technology.

Capítulo 2: **“Bacteria-Soil-Plant Interaction: This Relationship to Generate can Inputs and New Products for the Food Industry.”** Artigo de revisão publicado em Rice Research: Open Access.

Capítulo 3: **“Communities of heterotrophic bacteria in gleysoles and planosoles of irrigated rice fields.”** Artigo de pesquisa submetido em Journal of Microbiology Research.

Capítulo 4: **“Can the physicochemical characteristics of the soil of the Coastal Plain of the Brazilian State of RS interfere in the nutritional value of Puita Inta CL rice?”** Artigo de pesquisa submetido na Ciência Rural.

2 CAPÍTULO 1: MICROBIAL DIVERSITY: RELEVANCE AND RELATIONSHIP BETWEEN ENVIRONMENTAL CONSERVATION AND HUMAN HEALTH

Artigo de revisão publicado em Brazilian Archives Of Biology And Technology.

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Microbial Diversity: Relevance and Relationship Between Environmental Conservation And Human Health

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ABSTRACT

This work presents bibliographic data on the role and function of microbial diversity. The increasing use of probiotics and prebiotics foods has led to the studies on their actual functions in the human body. It is known that in the environment, microorganisms are extremely important in recycling of nutrients, balance of trophic chains, vital physiological activities in the plants and animals, as well as the conservation of natural habitats. In human food, these microscopic organisms contribute from flavoring products to the synthesis of antimicrobial substances and vitamins essential to living beings.

Key words: Bacteria, nutraceutical, collective health, review

INTRODUCTION

Environmental and health issues, along with the increase in food production, have been topics of investments in the area of scientific research. Food production with quality and quantity to meet the world's population is a matter related to public health. The defense of biodiversity stimulates the production of foods that provide benefits for the consumers and simultaneously benefit environmental sustainability.

Consumption of processed products with low nutritional value and high levels of trans fatty acids is frequent in the population. In Brazil, ten steps to a healthy diet were included in the National Plan for the promotion of proper nutrition and healthy weight, which aimed to improve people's knowledge about health and encourages the practice of physical exercises. In these ten steps, it is possible to check the improvement in the quality of life of the consumer, so they facilitate and improve the eating habits of the intestinal microbiota, and therefore, a person's health (Vinholes et al. 2009).

The human diet is a key indicator of quality of life and affects individuals in different ways, because of the importance of protein, vitamins, minerals and nutrients that are needed for the perfect functioning of the body. The cereals have similar percentages of protein, lipid, fiber, ash and carbohydrate. Cereals are foods of plant origin, consisting of grains and largely consumed by the people worldwide. The name 'cereal' derives from the *Ceres*, the Greek goddess of agriculture and harvest. The main cultivated cereals are rice, wheat, corn, oats, rye, barley and triticale. They are widely consumed for being part of the food habits of many people; for ease of culture, conservation, transportation and income, for being

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low cost and good nutritional value and for the great variety of forms of use (Coppens 2005). Rice is one of the most cultivated cereals in the world for human consumption and raw material for the food industry. There are increasing trends of these industries to seek rice, mainly organic and whole grain rice, due to health issues and the media, which has an interest in the cultural revival of a power based on wholegrain products (Ley et al. 2005). Rice, despite not being a food rich in vitamins, has a differential to be easily assimilated by the body. Its main component is starch, which provides energy, serving as fuel for the functioning of the body. The protein percentage varies around 3 to 12% depending on the type of rice (Ibge 2013).

Practitioners of macrobiotic and vegetarian diets seek to use rationally, using the rice in order to promote a complementary action of one food over another. In nature, the microbial communities facilitate the flow of nutrients and energy from the earth, but little is known about the interaction, and their diversity is still underestimated. Their patterns of succession and how their spatial distribution also occurs are poorly studied (Barbosa et al. 2010). Therefore, functional biodiversity in agroecosystems is key to the ecological sustainability of production and microorganisms are essential to this process (Johnson et al. 2003).

Bacteria comprise a large microbial community, consisting of species considered pathogenic to humans, animals and plants, or even beneficial species that interact with other organisms. Therefore, the importance of the study of microbial diversity is at stake.

METHODS

The bibliographic search was performed through a systematic search covering the 1900s and up to 2013. The terms “soil bacteria”, “agroecosystem” and “nutraceutical” were utilized to search databases like SciELO and Portal Capes. These databases fulfill the minimum criteria search to conduct a systematic review, in accordance with the literature. The articles written in languages other than English, Spanish or Portuguese were excluded. Then the full text of selected articles and cited references were analyzed, which were selected in additional studies that belonged to the subject. The initial search identified 68 articles, which were used for the preparation of this review article

MICROBIAL DIVERSITY

In preserved ecosystems

Microorganisms represent the richest repertoire in chemistry and molecular diversity in nature, providing the basis for ecological processes such as biogeochemical cycles and food chains, as well as maintaining vital relationships among themselves and with superior organisms (Hunter-Cevera 1998). The diversity of microorganisms is as vast as it is unknown. So far, between 0.1 and 10% of microbial species are known, depending on the habitat studied. In an agroecosystem, the variation in microbial diversity throughout the seasons is still not well understood, since in each season, one microbial community seems to occur more dominantly, accompanied by other less abundant that often are below the level of detection using the current methods of evaluation (Torsvik and Ovreas 2002). The diversity of microorganisms is critical to the functioning of the ecosystem, because there is the need to maintain ecological processes such as decomposition of organic matter, nutrient cycling, soil aggregation and controlling pathogens within the ecosystem (Kennedy 1999). The functional diversity is very important in ecological assessments of microorganisms within the ecosystem, mainly because little is known about the relationship between the structural and functional diversity of these microorganisms. There is, however, a consensus that microbial diversity is directly related to ecosystem stability (Yamanaka et al. 2003).

In agroecosystems

Modern agriculture is characterized by a constant search for increased crop yields through the use of mechanization, irrigation, chemical fertilization and pesticide application, coupled with the improvement of plant genotypes. However, the environmental impact caused by the intensification of farming did not always receive the necessary attention. The lack of detailed knowledge about the ecosystem and/or inadequate planning for land use led to a framework of intense environmental degradation, with loss of non-renewable resources and biodiversity not only in Brazil but in other countries (Eussen 1997). The observation in the increase of degraded agricultural areas has led to a change in the concept of land use, based on a holistic view of the agricultural process, where natural resources (soil, water and biodiversity) are exploited more sustainably.

The diversity of microorganisms as an indicator of the quality of agroecosystems has been widely debated, especially in the last decade, with the advent of molecular biology techniques that have favored the evaluation of microorganisms in environmental samples (Coutinho et al. 1999; Tiedje et al. 2001; Unicomb et al. 2005). The main argument in favor of such environmental feature is the fact that the microbial diversity naturally remains unchanged throughout the year (Dickens and Anderson 2001). Despite the continued use of land for agricultural practices, the soil may be rich in the biological point of view, since many microorganisms found in this environment are considered important in the biological control of diseases and pests of agriculture (Andreoti 2009). Thus, microbial processes play a fundamental importance in the functioning of production systems, performing tasks directly related to their productivity and sustainability (Boncowski and Roy 2005).

Rice is a plant of the genus *Oryza* of the grass family. *O. sativa* (Asian rice) and *O. glaberima* (African rice) are the two most cultivated species. The domestication of rice happened about 10,000 years ago in Asia. In Brazil, the plant was introduced by the Portuguese (Heinrichs 1997; Hunter-Cevera 1998). Rice is still considered the main component of the world's staple diet, although the per capita consumption in Brazil has decreased by 40.5%, according to the study by the Brazilian Institute of Geography and Statistics (FAO) in 2013. However, it is noteworthy that the production of rice in 2013 was 12,816 million tons. Rice cultivation in Brazil is primarily in irrigated mode. The largest producing state of Brazil is Rio Grande do Sul with 1,0666 million hectares, representing 44.5% of the national area and accounting for 66.5% of Brazilian production (Panizzon et al. 2013).

Microbiological monitoring of water and soil depends on the quality of water entering the crops. Part of the diversity of Gram-positive bacteria found in rice ecosystems belong to the groups *Bacillus*, *Micrococcus*, *Staphylococcus*, *Corynebacterium* and *Lactobacillus*, along with a variety of Gram-negative species belonging to the families *Enterobacteriaceae*, and *Peseudomonadaceae* *Aeromonadaceae*, *Vibrionaceae*, *Brucellaceae*, *Burkholderiaceae*, *Xanthomonadaceae* and others (Reche and Fiuza 2005). Among the Gram-negative bacteria, the following species have been identified by the same authors in rice cultivation waters: *Escherichia coli*, *Enterobacter* sp. *Citrobacter freundii*, *Klebsiella pneumoniae*, *K. oxytoca*, *Proteus mirabilis*, *P. vulgaris*, *P. penneri*, *Citrobacter* spp, *Enterobacter cloaceae*, *Providencia rettgeri*, *Stenotrophomonas maltophilia*, *Ochrobactrum anthropi*, *Chromobacterium violaceum*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *P. fluorescens* and *Burkolderia cepacia*.

In Human microbiota

The human body is populated by a large number of microorganisms, being distributed in various tissues and organs. It is believed that the abundance of microorganisms is greater than the number of cells in the body and is directly related to the immune system and better utilization of feed (Brandt et al. 2006). The digestive system consists of the alimentary tract associated with organs that partially perform digestion, mechanically and chemically. The purpose of the digestive system is the breakdown of food into smaller particles in order to be absorbed in human body.

The gastrointestinal tract of mammals maintains a highly diverse microbial population that plays an important role in nutrition metabolism, protection against pathogens, and the development of the immune system. It is estimated that at least 1000 different bacterial species coexist the human intestinal tract. Although often considered as pathogens, the majority of microorganisms in intestinal tract have beneficial effects. They play multiple roles in the human host, as they are directly involved in the synthesis of vitamins and cofactors, help to degrade complex lipids and polysaccharides and also have detoxifying action (Kennedy 1999; Douglas- Escobar et al. 2006). It is known that bacteria intensely populate the most part of the enteric tract. However, the stomach and the small intestine have fewer microorganisms due to the presence of hydrochloric acid (Bedani et al. 2009). The large intestine has numerous bacteria, especially *Lactobacillus* sp., *Escherichia coli*, *Klebsiella* sp. and *Proteus* sp. that assist in the synthesis of degradation of the food (Camargo et al. 2011; Odonkor and Ampofo 2013).

The intestinal microbiota is a dynamic mixture of microorganisms, whose composition varies along the gastrointestinal tract (GIT) and between the mucosa and the intestinal lumen. The microbiota develops all the time, due to the interaction of genetic factors, contact with the environment, diet and disease, thus explaining the fact that each individual presents a unique microbiota (Bedani et al. 2009).The genotype of the individual may also be a factor that influences the bacterial community in the gut, following a hypothesis that in the gut there are sites of specific adhesion to some bacteria in the host, selecting the most important. These adhesion sites are probably genetically predetermined (Brandt et al. 2006). Figure 1 represents didactically the factors that can influence the intestinal microbiota.

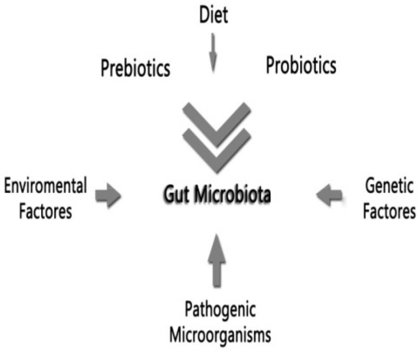

Figure 1 - Factors influencing the bacterial community in the human gut.

The highest concentration of microorganisms and metabolic activity is found in the large intestine. Starting from the ileum the concentration of bacteria increases gradually, reaching 10^{11} to 10^{12} CFU/g in the colon. The adult and stable microbiota are composed of autochthonous species (permanent members) and allochthonous (transient members that are acquired from an external source). The microbiota are diverse, comprising 400-1000 species, of which over 60% are not cultivable outside the intestine (Moreira 2012). The intestinal microbiota plays different roles that are important for the host such as they exert a trophic effect on the intestinal epithelium, favoring the development of microvilli, which in turn promotes the absorption of the nutrients (Moreira 2012). The diet is a major determinant for the persistence of certain bacteria in the gastrointestinal tract, because the diet provides nutrients, not only for the host, but also for bacteria that are in it. Components from indigestible food serve as carbon and energy source for the human gut bacteria (Blaut and Clavel 2007).

The intestinal biota is increasingly considered a symbiotic partner for the maintenance of health. Over the years, the intestinal microbiota has become recognized in the development of the immune system. The cells of the intestinal mucosa and microbiota (enterocyctes, dendritic cells, lymphocytes, macrophages) regulate the production of several cytokines and chemokines). The homeostasis of the intestinal tract is dependent on characteristics of the host (age, gender, genetics, etc) and environmental conditions (stress, drugs, gastrointestinal surgery, infectious and toxic agents). Changes in the gut microorganisms can be reversed by diet and weight loss. Qualitative and quantitative changes in the consumption of specific food components (fatty acids, carbohydrates, micronutrients) have effects not only on the composition of intestinal microbiota, but can modulate the expression of genes in the host tissues such as the liver, adipose tissue, intestine, and muscle. This in turn can lead to the development or decrease of fat mass and metabolic disorders associated with the intestinal barrier and systemic immunity. These intestinal microbiota are composed of various bacterial groups. Therefore, it is essential to keep them balanced to preserve human health.

Some bacteria have been used as a probiotic, such as species of the genera *Lactobacillus* and *Bifidobacterium*, which help in the restoration of the human intestinal microbiota, when the intestinal biota is drastically reduced and is vulnerable to attack by the pathogens (Santos and Cançado 2009).

Human nutraceutical

The evolution of nutritional science made it possible to know a little more about the food composition and action of these substances in the body. Studies have been carried out on the phytochemical constituents of the nutraceuticals in the food in order to study their health benefits. Nutraceuticals are dietary supplements that contain a concentrated bioactive compound in the food. They are used for improving the health that could be obtained from the food (Zeisel 1999).

The Brazilian Health Surveillance Agency of the Ministry of Health in the ordinance n° 398, April 30, 1999 defined the functional food as the ingredient that claimed functional or health properties. It may, in addition to basic nutritional functions, in the case of a nutrient, and produce metabolic or physiological effects and or health benefits, and should be safe for consumption without medical supervision (Vigilância Sanitária 1999). The importance of the adequacy of food with respect to microorganisms in order to reduce the cost, increase quality regarding the taste and nutrition must be kept in mind. They produce a more flavorful food, with less cost and less waste of chemicals, which is of great significance for the food industry (Ley et al. 2005; Panizzon et al. 2012).

Prebiotic and probiotic microorganism

The gastrointestinal tract is colonized by the microorganisms soon after the birth of the individual and also after certain life situations, such as the consumption of antibiotics, resulting in the reduction of microorganisms in the gut, which makes it important to understand when this type of food should be used.

The enterobacteria and some species of *Clostridium* spp. are harmful to human health. The gut biota balance is maintained by the interrelations between the bacteria themselves and the host. Accordingly, the probiotics increase the number of microorganisms in the host; in addition, they induce pro-inflammatory, anti-inflammatory and anti-carcinogenic actions. The majority of probiotic microorganisms are lactic acid bacteria, which are Gram-positive and microaerophilic (Rodrigues and Barroso 2011). Probiotics are dietary supplements added to the food in adequate quantities influencing their nutritional and therapeutic value. They are represented by cultures of live microorganisms (beneficial bacteria and yeasts), which contribute to improve the flavor of the final product and produce antimicrobial substances (Santos 2009). Prebiotics are food ingredients that are primarily substrates for the growth of the intestinal microorganisms, not digested in the small intestine that, upon reaching the large intestine, are selectively metabolized by a limited number of bacteria, called beneficial, which alter the microbiota of the colon generating a healthy bacterial microbiota (Raizel et al. 2011).

A healthy diet by adding probiotics and prebiotics in food benefits the people. In today's society, with the increasing population life expectancy and drug costs, nutraceutical should unify scientific knowledge about the public health and nutrition suitable to these new challenges by promoting health and not just curing diseases. The healthy microbiota promotes well-being and with the addition of probiotics, it is possible that they could produce antagonistic effects against unwanted microorganisms. Therefore, knowledge of the gut biota leads to the development of numerous public health strategies (Saad 2006). The importance of probiotic approach to the management of obesity in humans is supported by some intervention studies in humans so far, but the experimental data obtained with the compounds help to elucidate potential new molecular targets associated with the diet and the existing bacteria (Delzenne et al. 2011).

Recent studies involving rice bran and its oil have shown that they possess prebiotic potential, aiding in the growth of important microorganisms in the intestine, such as those of the genus *Lactobacillus* that are highly effective in controlling the intestinal infections by *Salmonella* and are catalysts of non-digestible molecules (Kumar et al. 2012; Tamura et al. 2012).Changes in the bacterial composition of obese versus lean people relate to bacterial phyla such as, for example, a reduction in *Bifidobacterium* spp. or an increase of *Staphylococcus aureus* (Neyrink and Delzenne 2010).

Pathogenic microorganisms

The members of the normal microbiota can cause diseases under certain circumstances. Since they have a non-invasive way of life defined by limitations of the medium, unless they are held they can become pathogenic. Population levels of microorganisms are determined by the exogenous and endogenous multifactorial processes (Griffiths 2001).The bacteria of the intestinal tract have heterogeneous distribution. The colonization of the intestinal tract depends on the ability of bacterial adhesion. There are bacteria on the adhesion sites on the intestinal mucosa, which need not to be periodically reintroduced. However, there is the native biota that is external to the gut ecosystem, thus is transient. The microbiota has the following functions: antibacterial, immunomodulatory and metabolic. Antibacterial prevents the establishment of pathogenic bacteria. Immunomodulatory activity helps the immune system and metabolic function contributes to facilitate the nutrition (Brandt et al. 2006). It is important to highlight the impact that pathogenic bacteria can cause in the public health issue, resulting serious intestinal diseases such as diarrhea - considered as the most common disease caused by viruses and bacteria and one of the diseases that affects large no of children in the world. Hence, it is important to know the bacteria that may possibly compromise the gut and the human organism as a whole (Clotildes 2007). The use of antibiotics in the rats can increase intestinal microbiota associating to some changes that affect the acquisition of energy from compounds in the diet and how it is spent and stored (Ley et al. 2005).*Salmonella* is represented by more than 40 serogroups and 2000 serotypes and may be classified as typhoid and non-typhoid. This genre is usually associated with food. *Salmonella enteritidis* (Fig. 2B) is one of the serotypes most widely distributed in the world and one of the major contaminants in food, usually beef, pork, poultry and eggs. This bacterium usually causes fever, abdominal cramps and diarrhea, which can present blood clots. A study by the Center for Epidemiological Surveillance - SES / SP showed that from 1999 to 2007, *S. enteritidis* was responsible for 42.3% of outbreaks of diarrhea, showing the attention in public health that this bacterium should be given (Kirk et al. 2004; Unicomb et al. 2005; Paiao et al. 2013).

Escherichia coli (EPEC) (Fig. 2C) causes gastroenteritis in almost all age groups. It is similar to *Shigella* sp (Fig. 2D) because it penetrates directly into the intestinal epithelium where it can multiply, causing dysentery. It can be transmitted by the consumption of water and many foods such as milk and milk products. The importance of EPEC as a cause of diarrhea has declined since the 1960s, but is the primary infectious agent in children in developing countries, including South America, Africa and Asia. EPEC outbreaks are sporadic, emerging in places where sanitary conditions are poor (Silva et al. 2001; Maltick et al. 2010). Other bacteria that are not involved directly with the human gut, but are cause of worry in the matter of public health, are related to urogenital infections, such as *Proteus vulgaris* (Fig 2A), which inhabits the human gut, but causes urinary tract infections and other complications (Rodrigues and Barroso 2011).

Having a prebiotic diet can help treating the intestine infections, such as those caused by the bacteria of the genus Salmonella because these prebiotic serve as substrate for the growth of bacteria, which has the potential to eradicate other pathogenic bacteria such as Lactobacillus (Kumar et al. 2012).

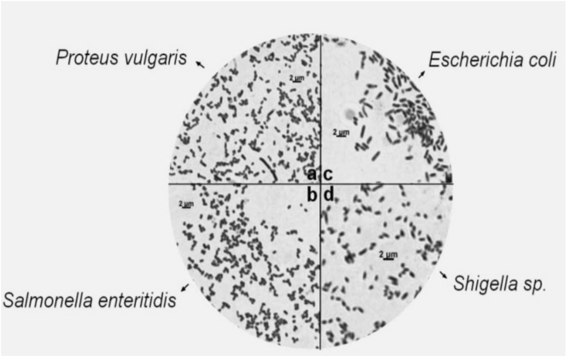

Figure 2 - Photomicrographs of bacteria (100x) for representing their morphology.

Microbial diseases of the large intestine are second only to the respiratory system diseases. Pathogens are able to cross the digestive system and extend to organs, causing numerous diseases, e.g., gastroenteritis caused by *Salmonella* and rotavirus. *Bacillus cereus* is common in the soils and vegetables. Rice has vast abundance of this bacterium. It is generally harmless, but when found in the foods, can cause illnesses such as gastroenteritis. The rice, for being a plant that is cultivated in water, has the risk of receiving numerous microorganisms. Water is characterized by low nutrients. Therefore, bacteria tend to grow on standing surfaces in particular materials, as is the case with rice (Pomeroy 1974a; Pomeroy et al. 2007b; Andreoti et al. 2009). Therefore, paddy fields are important for local biodiversity conservation because they support a rich biodiversity and high productivity feature. Rice is one of the most important cereal crops in the world. Therefore, the conservation of biodiversity in agriculture is a challenge of great importance. Several studies have demonstrated the contribution of ecosystems such as rice, providing habitats for creation of numerous microorganisms (Hofman et al. 2003; Maltick et al. 2010).

The microorganisms in the biosphere perform important functions, for example, the influence on biogeochemical processes. In aquatic environments, there is an important chain of interactions that affect the elements involved in the environment (Comte et al. 2006). It is noteworthy that quality evaluations in health should always aim at the welfare of the patient, which is always the focus of the studies. Therefore, monitoring allows detecting the faults and correcting them in order to not compromise the product and the consumer (Abrantes et al. 2007).

It is possible to relate the bacteria of rice with their intake and activity within the human body. Microorganisms can generate many by-products through fermentation, which may even have commercial value and are easily produced. The cost/price margin is small because they can be easily replaced by cheap products and chemicals; however, they are naturally formed in the body.

The development of non-dairy probiotic products is a major challenge in the food industry because many are lost during the processing and storage of the product. Cereals such as rice have been widely studied and can be used as fermentable substrates that favor the growth of probiotic microorganisms (Pomeroy et al. 2007b; Oliveira and Jurkiewicz 2009). Microorganisms have a high degree of specificity, acting separately in chemical reactions. Therefore, with the development of biotechnology, it could be possible to manufacture the food and perform transformations that can affect people's lives.

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3 CAPÍTULO 2: BACTERIA-SOIL-PLANT INTERACTION: THIS RELATIONSHIP TO GENERATE CAN INPUTS AND NEW PRODUCTS FOR THE FOOD INDUSTRY

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Review Article

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Bacteria-Soil-Plant Interaction: This Relationship to Generate Inputs and New Products for the Food Industry

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Abstract

A thorough study of microbial communities that inhabit aquatic agro-ecosystems is crucial to a better understanding of what happens in the soil, since these microorganisms play important roles for the maintenance of the habitat. Irrigated rice culture is very common in Brazil. However, the incorrect use of these environments can affect the soil and cause damage. The bacteria-soil-plant interaction has been used in order to support biotechnology, as the rhizosphere possesses a different microbial ecology from the rest of the soil. Microorganisms from this region are directly related to plant growth. Bacterial diversity in soil is extremely diverse, with its population changed rapidly as new nutrients are made available or the existing ones are exhausted. The bacteria that live there receive both the plant nutrients and antimicrobial agents, which are selective and inhibit certain undesirable microorganisms. Aiming at this data, this article has an objective to review existing literature on the interaction between microorganisms and their relationship with the plants, which can be transmitted to food through minerals and/or enzymes, thus enabling the generation of inputs or new products.

Keywords: Bacteria; Soil plant; Food microbial comunity

Introduction

Rice is one of the most consumed foods in the world, being one of the most important grains in global economic terms. It is also an important product in the economy of many Latin American countries because it is a staple in the diet of the population [1].

Brazil is among the 10 countries with the highest production of rice, reaching the significant amount of 13 million tons per year, representing about 82% of production in the Mercosul bloc. Rio Grande do Sul is the largest producing state in Brazil, accounting for 61% of total production. Along with the state of Santa Catarina, the southern region accounts for 70% of total production in the country, ensuring the supply of this grain to the entire population of Brazil. The average consumption in the country is 45 kg per person per year [2].

The irrigated rice culture is the largest agricultural user of water worldwide. It is believed that it may cause environmental impacts and this not completely known. Some of the possible impacts of rice culture may be linked to phytosanitary treatments, water, and culture management in a general way [3]. New rice varieties were introduced in the second half of the 20th century that provided increased yields, and these yield gains were almost doubled when synthetic fertilizers were used [4]. Therefore, anthropogenic activities, such as the expansion of cities, pesticides and pollution may directly affect the microbiota of water and soil. However, little is known about how these factors may influence the action of these microorganisms in the ecosystems [5].

The key aspect of this review is to obtain answers to the following questions; (I) how the literature shows the interaction between soil, plant and bacteria; and (II) how the bibliography relates microorganisms with the generation of inputs for the food industry.

Rice

Rice is a plant of the genus *Oryza* belonging to the Gramineae family, who most cultivated species are *O. sativa* (Asian rice) and *O. glaberima* (African rice). The rice domestication happened about 10,000 years ago in Asia and, in Brazil, the plant came through Portuguese colonization [2].

Rice is an annual monocot and can be planted in wetland or highland ecosystems, but has variations in its yield, since the plant needs to adapt to the environment [6]. The plant grows in a period of 100 to 140 days, depending on the chosen crop [7]. The rice development cycle is separated into three phases: the vegetative stage; the reproductive phase; and the grain filling stage [8].

Because it is a culture of easy adaptation, which develops in many soils and climate conditions, the rice is being cultivated more, as the world population is in broad expansion and demand in food production should continually increase to meet the daily nutritional needs of the people, since rice is a mandatory component in the Brazilian meal [9]. The soil microbiota helps in the development of the rice plant. Such as the establishment of plant-microorganism association in the root system that is critical to the chemotactic response of the endophyte to root exudates.

The rice is a cereal that is recommended to people with coeliac disease for its lack of gluten, which is the element of some flour that gives softness to the food. Brown rice is a food that, for each 100 g, consists of 79.3 g of starch, 6.61 g mg of protein and some mg of vitamins such as vitamin B1, B2, B6, niacin, phosphorus, magnesium, iron, potassium and zinc [10,11].

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The rice grain is divided into three parts: Bark, containing the embryo, the endosperm and a fiber complex; - Bran, a thin layer of differentiated tissues such as fibers, proteins, fats, and vitamin B; - Embryo, containing starch, amylose and amylopectin [2].

Wet Lands

Irrigated agriculture shows significant growth in Brazil [12]. The rice fields may be considered wetlands because, according to Maltchik et al. [13], wetlands are environments created by men that help promote the proliferation of microorganism populations on substrates such as sand, gravel or other material in association with plants.

Each cultivation system also presents a different demand of irrigation water. Minimum tillage and no-till systems have similar methods of irrigation [14]. The pre-germinated system presents a different irrigation system of soil, with a flooding prior to their preparation, remaining for 20 days before sowing.

Soil

The soil is a complex mixture of chemicals, including inorganic such as minerals, and the changes taking place in its composition are called biogeochemical transformations. The rate of mineralization grade depends on the availability of oxygen. Compared with the anaerobic metabolism, aerobic is more versatile. Many organic materials are mineralized only if there is oxygen. When the soil is dry and loose, oxygen penetrates more easily, up to about 30 cm depth. Yet, small soil particles are anaerobic, since microorganisms that use oxygen consume it quickly. The mineralization happens slowly in flooded soils because of this oxygen deficit. Soil fertility depends on adequate supply of oxygen, nitrogen, phosphorus and potassium whereas the inorganic forms of these minerals are produced by microorganisms as they mineralize organic material. Natural fertilizers are added to enrich the soil with these elements [15,16]. Necessary the maintain of vegetation, because it avoids the loss of soil nutrients and contamination, and the rational use of pesticides, as it preserves the food chain and also prevents contamination of water resources in the region. It also requires soil analysis before planting to make proper fertilization, since the answer will be different because the cultivation system chosen requires specific conditions [17]. Other important aspects are associated with the use of agrochemicals, once they can quickly reach surface water sources through surface drainage, lateral seepage, surface and subsurface draining, drift, volatilization and also reaching underground water sources by leaching and facilitated flow, reaching non-target organisms and causing environmental contamination [17].

The most widespread cropping systems for irrigated rice are: no-till, conventional, pre-germinated and minimum tillage. Each system is characterized by different types of soil management and of culture itself. In conventional cultivation, soil preparation is done with plowing, in other words, occurs very intense movement of topsoil. For no-till, there is little soil movement, which is held far in advance of planting period, in order to use an herbicide of total action against competing plants that germinate in the period between soil preparation and planting. The pre-germinated planting is characterized by the sowing of pre-germinated seeds in soil flooded beforehand. The soil preparation is done with machinery and implements, by working within the fully flooded plantation frame. Minimum tillage cultivation uses less soil mobilization. When compared to the conventional system, the seeding is performed directly on the vegetation cover previously desiccated with herbicide without soil disturbance [2].

In paddy fields, the first fertilization occurs with nitrogen coverage in a dry ground. After the development of 3 or 4 leaves in the plants, irrigation starts. Another detail that should be noted is that early irrigation, up to two days after herbicide application, results in greater weed control [2]. Knowing how to handle the soil to preserve or even improve their characteristics in sustainable systems is one of the challenges to the current agriculture [18]. Agricultural practices affect physical and chemical characteristics of the soil, influencing diverse populations and bacterial communities [19]. The frequent use of the soil, over time, tends to lead to a reduction of its heterogeneity [20], resulting in the decrease of nutrients available to the microorganisms and consequently the plants. In addition to the common use of soil, the use of chemicals may also have antimicrobial effects [21].

The continuous use of the soil, through the planting of rice, can cause loss of important resources for maintaining this culture, like any other culture that uses it, and the loss of nutrients ends up being inevitable. The soil microbiota promotes important processes for their maintenance and less impacted soil have positive influence on crop development and demand for fewer inputs, which represent costs in the production of grain [22].

Microorganisms

In the soil, it is possible to find various types of microorganisms, such as fungi, protozoa, bacteria and yeasts, which live in symbiosis with others. Some of these relationships are mutualistic, also with plants [23]. The bacteria are differentiated by size and cell structure, and may be spherical, rod-shaped, helical and others. Some obtain energy by processing organic compounds (food), others get nutrients from the environment where they live, using intermediates of glycolysis and other degradation routes [24]. Microorganisms have great importance in biochemical and geochemical cycles and have a great chemical and molecular arsenal. In the context of rice cultivation, bacterial abundance can be considered wide by its variety of microhabitats, caused by constant irrigation, resulting in complex bacterial communities [25].

Because it is a heterogeneous environment, the soil system may harbor the development of many important groups (diazotrophs, nitrifying bacteria, decomposer fungi and antagonists) that can be affected by several factors such as physical disturbances, use of fertilizers and plant species [26]. Most bacteria in the rhizosphere are highly dependent on associations with plants. Therefore, the bacterial diversity plays a key role in the agricultural environment. The result set shows that the irrigated rice culture influences the bacterial density present in water samples used for soil irrigation [27].

From an ecological point of view, decomposer organisms are the most important, which act degrading organic molecules and turning them into inorganic, so that can be used by plants. They are also protagonists in nitrogen and sulfur cycles [28]. Microorganisms are also of great importance in biotechnology, such as the use of bacteria in the bioremediation of pollutants and toxic wastes generated by the industry. Some bacteria are able to use pollutants as energy sources or to convert toxins into less harmful substances. The effect of these bacteria in the environment is highly positive, since the toxins may be removed from different environments, thus being possible their use in oil spills and locations with the presence of toxic waste. Microbial communities are particularly affected by the management and impact in the ground. Agricultural practices such as soil alterations during preparation and irrigation can modify bacterial communities. Water stress is a physiological state of the bacterial community. This stress induces osmotic shock that may result in cell lysis and release of intracellular solutes. Therefore, bacteria can survive under water since they remain in soil particles adhered to the roots, and thus can provide the ideal moisture to the roots to keep them alive [1]. There are groups of microorganisms that produce endospores, which stay dormant and viable in the environment for years, remaining so during adverse situations waiting for a favorable environment for their development, when they return to active form. Some organisms use the energy of chemical interactions, others are even able to photosynthesize and produce their own energy [29]. Most studies of microbial communities in irrigated rice are focused on mass of populations from soil experimentally developed, as many microorganisms are closely linked to the ground by removing nutrients and interfering in its composition [30].

Diazotrophic Bacteria

The endophytic bacteria play a fundamental role in plants and do not cause disease symptoms in which they are associated. These species are able to invade the internal tissues providing a systemic dissemination. The population of viable endophytic diazotrophs in cultivated rice varies with the type of soil, the growth phase of rice culture, and plant tissue. In general, bacterial populations are larger in the roots, compared with stems and leaves [30]. The rice roots harbor endophytes equivalent to 10^8 cultivable nitrogen-fixing bacteria by root gram of dry weight, and an even larger number of non-cultivable bacteria [31]. Nitrogen is one of the most important nutrients for achieving high productivity of annual crops due to high demand of the plant for this nutrient. Therefore, the low availability of this nutrient limits the productivity of the crop. Most of the nitrogen fixation from air takes place through diazotrophs such as *Azospirilium*, *Herbaspirilium* and *Burlkoderia* [32]. The diazotrophs, which are inserted in nitrogen utilization in the soil, are important organisms that can be used as an alternative to nitrogen fertilization [33].

The fact of being able to process organic and inorganic substrates successfully, bacterias become critical for the dynamics of aquatic ecosystems. The interaction between plant and microorganism is little explored in agriculture, despite having global and local importance in the dynamic equilibrium of ecosystems.

The plant and microorganism association in the root system is essential for the chemotactic response of the endophyte to root exudates. In the colonization process, several stages occur, beginning with the displacement of the microorganism into the root system, clinging and distributing through roots [34].

The endophytic bacteria, in order to penetrate the roots, first need the formation of intra and intercellular microcolonies. The different associations of endophytic bacteria can cause changes in plant colonization processes. Accordingly, the microorganisms migrate to the rhizosphere in response to root exudates, which are rich in amino acids, organic acids, sugars, vitamins, purines/pyrimidines, among others. In addition to providing nutritious substances, the plants can also eliminate secretions that facilitate colonization of specific groups of bacteria [35,36]. Microorganisms allow the recycling of nutrients such as lost carbon that can be reintroduced into the food web. Aquatic environments differ in physical and chemical aspects as there are microbial differences. Fungi and bacteria are mainly responsible for the decomposition process in aquatic ecosystems by converting organic matter into inorganic substances. Rainfall, when high, appears to contribute to the high bacterial rates [37].

Heterotrophic Bacteria

Bacteria that inhabit the rhizosphere promote the growth of host plants through the production of phytohormones such as auxins, the phosphate solubilization, the production of iron chelators (siderophores), the release of antimicrobial metabolites and for competition for nutrients [38]. The bacteria found in soil are highly diversified. In hot soils, for example, there is the presence of thermophilic microorganisms and microbial population changes very quickly as the available nutrients are modified [39].

The soil has great spatial variability composed of many micro habitats that may differ in their physicochemical properties [40], (Figure 1). These characteristics provide a diverse composition of microorganisms, which accomplish the primary decomposition, cycling and regulation of nutrients and minerals retention. Bacteria

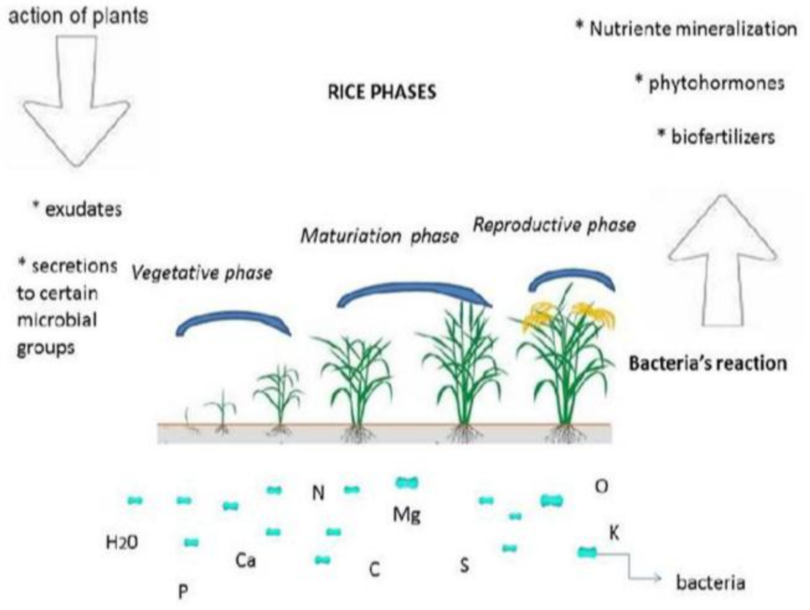

Figure 1: Interactions between bacteria and the rice plants in their different development stages.

also secrete various enzymes such as catalase, urease, cellulose, among others [41], also important for the plants. The cultivars and also the stage of the irrigated rice crop development can influence the microbial populations associated with these agro-ecosystems [17], as well as the different soil management systems, the application of pesticides and the fertilizers used for better development of this culture (Figure 1).

The composition of a community hardly will remain static, so any change in the habitat can cause changes in the composition of populations. Species such as *Bacillus thuringiensis*, *B. subtilis*, *B. cereus* and *Lysinibacillus sphaericus* may have insecticidal activity to different orders of insects [42] and are widely used in biological pest control. Some species are also associated with plant roots, aiding in the absorption and retention of nutrients in the soil.

They also have biotechnological applications and produce antibiotics [43], among other important functions. Therefore, understanding the impact of different rice cultivation systems in the bacterial community in the soil is important for the knowledge of lower environmental impact soil technologies and, eventually, the ones that can contribute positively to the improvement of ecological interactions that take place under sunshine [39]. Cosmopolitan microorganisms isolated from soil, water and vegetables such as bacteria of the Enterobacteriaceae family can also be found in the digestive tract of animals and humans, although it is also possible to find them in transient or normal microbiota. Among them include: *Escherichia* spp., *Klebsiella* spp., *Salmonella* spp., *Enterobacter* spp., *Serratia* spp., *Hafnia* spp., *Citrobacter* spp., *Yersinia* spp., *Proteus* spp., *Rhanella* ssp., *Providencia* spp., *Morganella* spp., *Shigella* spp., *Edwarsiella* spp., *Ewingella* ssp., *Budvicia* ssp., *Tatumella* spp., *Erwinia* spp., *Koserella* ssp., *Kluyvera* ssp., *Hoganella* ssp., *Moellenella* ssp., *Leminorella* ssp., *Buttiauxella* spp. and *Pantoea* spp. They can be pathogenic or opportunistic, occasionally forming components with physicochemical and biological properties beneficial to humans and the environment in which they operate [44]. Pereira et al. [45] points out that there is a wide variety of bacteria in the soil that, when associated with plant hosts, stimulate their growth, such as the rhizobacteria [46]. Stimulation in plants growth is mainly due to increased availability of mineral nutrients [47] and production of growth hormones such as gibberellins and auxin (induced resistance to disease and suppression of harmful microorganisms from the rhizosphere of plants). These direct effects provide a high gain to producers, allowing them to lower their use of inputs in farming or monoculture, reducing costs and also potential environmental problems such as contamination of soil and water sources, caused by the use of chemicals for pest control [48].

Pseudomonas fluorescens, *P. putida*, *Azospirillum brasiliense*, *Serratia marcecens*, *Bacillus subtilis*, *B .megaterium*, *Rizobium* sp., *Bradyrhizobium* sp., *Arthrobacter* sp., *Enterobacter* sp., *Azotobacter* sp. among others are heterotrophic bacteria genres most cited in the literature [49]. Microorganisms that inhabit the soil, along with biological processes, have been investigated as indicators of the sustainability of agriculture and / or soil quality [18]. They also influence the biological quality of products and the productivity achieved [19].

Food

Many diseases that affect plants and impair food production in crops can be caused by bacteria. Some examples well known for rice farmers are the brown spot and the stem rot caused by the plant pathogens *Helminthosporium oryzae* and *Sclerotium oryzae*, respectively [2]. However, besides being studied by the negative aspects, recent scientific research has given great importance to microorganisms for other functions. As an example in the food industry, the fermentation, this allows the manufacture of cheeses, dairy products, breads, meat products, alcoholic beverages and plants. Microorganisms present in the soil can influence the nutrients the food provides being ingested by humans, thus being able to bring beneficial changes to health. Most food is chemically stable, rotting only when contaminated by microorganisms. Factors such as pH, temperature, water availability and addition of chemical substances help in the preservation of food [24].

In the case of rice, its processing ensures quality control. It undergoes different processes leading to each type of rice: whole, parboiled, polished and white rice. White rice is polished and the starch is what predominantly stays in the grain. Parboiled rice is sterile in which the nutrients of the husk migrate into the grain. Whole rice has a medium and long grain, being darker than the processed, because it retains the film and the germ, which is more nutritious and rich in vitamins [11].

The rice is responsible for 27% of the daily calorie and 20% of the protein needs of the population, containing thiamine, riboflavin and niacin. It is easily digestible, being a good fiber regulator. The rice-based diet selects fermentative bacteria that are resistant to pathogens. By not containing gluten, it does not cause eating disorders. It has low sodium content, 5 mg in 100 g, and high potassium content, 92 mg in 100 g. It is also useful for osteoporosis because it has selenium (an antioxidant that acts against free radicals and unstable molecules of high reactivity) and silicon that helps forming collagen, elastin and protein of the cognitive tissue [50]. Foods considered new are resulted from new techniques of biotechnology and plant breeding. There are few genetically modified plants being commercialized. The genetic traits that are changed confer resistance to insects, herbicides etc. In order to arouse consumer interest in the products, there must be a higher quality of these that improves health or that has a greater shelf life. Biotechnology can improve organoleptic properties such as flavor and pigmentations [51].

Molecular Biology in Food

At present, the microorganisms have been used in the production of enzymes, antibiotics, solvents, amino acids and dietary supplements. Microbial diversity is a source of genetic resources, with each microorganism playing a unique and particular function. Therefore, first the decoded gene from the specific enzyme is identified and then isolated and transferred by recombinant DNA techniques into a known microorganism. The main bacteria used are: *Escherichia coli* and *Bacillus* sp, The main fungi used is: *Aspergillus* sp. and the main yeast is: *Saccharomyces cerevisiae*. Enzymes are biological catalysts present in all beings, therefore are used in various fields, including the bioremediation. The biocatalysts are protein molecules with an associated catalytic power. These biocatalysts degrade the molecules present in the environment such as cellulose, starch, lignin etc. [52,53].

Application of molecular biology techniques has greatly contributed to the exploration of new enzymes and new enzymatic properties and certainly continued to promote and develop production. Enzymes generate added value to the product in line with the demand of technological, market and environmental preservation characters. The use of catalysis is critical in Brazil, as it helps in environmental renewal, with the increase of enzymes used in pharmacy and food [38].

Conclusion

Agriculture is one of the most important activities, but it is necessary to observe some points, as food production is directly linked to environmental quality. Rice is one of the most consumed foods in the world, one of the most important grains in global economic terms. Rice is a culture that besides being simple is a great resource for human consumption. Rice, although not a food rich in vitamins, has a differential, it is easily assimilated by the starch, which provides power, serving as fuel for the operation body. Currently, crops cultivation methods seek to optimize the potential of agricultural production through the application of fertilizers and pesticides and which consequently cause human health problems and an imbalance in agricultural ecosystems, especially in the communities that inhabit the soil. The rice crops suffer and benefit from various microbial actions, including interactions between plants and microorganisms [54,55]. Rice agroecosystems consist of several micro-habitats and provide the suitability of a wide variety of microorganisms. The management of rice promotes changes of physical and chemical characteristics of the water and due to changes in pH, turbidity, temperature, radiation and amount of organic matter that may be related to the dynamics of microbial communities in the soil. Soil is a habitat full of living microorganisms that directly influence the development of the plant. The bacteria that act in it are inserted in the process of chemical transformations that facilitate nutrient cycling and can be added to the food, generating inputs that provide functionality and well-being to the human being. The challenge of scientists in the area is still the search for a better quality of life, environmental protection and sustainable development, because rice is one of the grains produced in the world and extremely important for the world economy.

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4 CAPÍTULO 3: COMMUNITIES OF HETEROTROPHIC BACTERIA IN GLEYSOLES AND PLANOSOLES OF IRRIGATED RICE FIELDS.”

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Communities of heterotrophic bacteria in gleysoles and planosoles of irrigated rice fields.

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Abstract This work evaluated the effects of physical and chemical variables in the diversity of bacterial communities present in rice field soils in two areas of different regions of southern Brazil. The samples were collected along 2013/14 and 2014/15, in rice producing areas of the regions: External Coastal Plain (PCE) and Internal Coastal Plain (PCI), Rio Grande do Sul, Brazil. The results were analyzed using Component Principal Analysis (PCA), Canonical Correspondence Analysis (CCA), Diversity Index, and Analysis of Variance. Were recorded 29 Colony-Forming-Units (CFUs) in both regions and the diversity was similar differentiated only for different phenological phases of plants. Bacterial abundance of colonies corresponding CFUs in OCP was ($F_{1.9}$ = 7.84 $p < 0.05$): 18.5 % in before sowing; 22.3 % in vegetative phase; 22.3 % in reproductive phase and 36.9 % during seed maturation. Bacterial abundance of colonies in ICP was ($F_{1.9}$ = 7.03 $p < 0.05$): 23.9 % in before sowing; 21.7% in vegetative phase; 20.7% in reproductive phase and 33.7 % during seed maturation. The first two axes of each crop fields generated by CCA in the 2013/14 crop explain 67 % of the variation observed and in 2014/15 explain 86.5%. The most important variables for ordination and correlation with the axis were content of organic matter and clay. With an analysis of the entire dataset, the PCA explained 64.84% and a CCA explained 70.7% enter the main two axes, highlighting clay and organic matter. In both types of soils, gleysoles and planosoles, the CFUs of the following communities of heterotrophic bacteria were higher: *Bacillus thuringiensis, Bacillus cereus, Lisinibacillus sphericus, Pseudomonas fluorescens, P. putida*, *Corinebacterium* spp.

Keywords Microorganisms, Rice producing regions, Physicochemical parameters”

1. Introduction

Rice (*Oryza sativa*) is one of the most consumed foods in the world, being one of the most important grains in global economic terms. The rice agro-ecosystems in Brazil produce about 11,000 kg/ha annually, and over 60% of this production is in the state of Rio Grande do Sul [1]. The biodiversity in these agroecosystems refers to all species of plants, animals and microorganisms that interact with the environment [2]. Studies on the factors that influence the distribution, diversity and structure are relatively recent. In-depth study of microbial communities living in aquatic agro-ecosystems is fundamental to a better understanding of what happens in the soil, since these microorganisms play important roles for habitat maintenance [2]. The diversity present in the rhizosphere is different from the rest of the soil because the microorganisms of that region are directly related to plant growth. The bacteria that live there receive plant nutrients as well as antimicrobial agents which are selective and that inhibit certain undesirable microorganisms. A better understanding of environmental factors that influence changes in the diversity of the bacterial communities is very important because they realize functions that are essential for the maintenance of ecosystems [3-4]. Several studies have analyzed the effects of chemical substances used for fertilization and pest control on bacterial diversity [5] associated with cultivation [6-7]. These studies mainly analyze the impact of different factors on biodiversity of the bacteria in soil. Other works deal mainly with the relationship of bacteria in soil of rice culture, which are involved in the processing of waste from rice plants [8-9]. This study aimed to evaluate the diversity of bacterial communities present in the two types of soil: Gleysoles and Planosoles: i) Physical-chemical and microbiological analysis of gleysoles (External Coastal Plain) type soil; ii) Physical-chemical and microbiological analysis of planosoles (Internal Coastal Plain) type soil; iii) Comparison between the two soils types studied through statistics elements.

2. Materials and Methods

2.1 Study Area

The town of Santo Antonio da Patrulha (External Coastal Plain -PCE) is bathed by the Sinos River, in the northeastern region of the state of Rio Grande do Sul (RS). It covers an area of 3,820 km^2, and flows into the delta of the Jacuí River. In the Sinos Basin, there are thirty-two districts with 975,000 inhabitants, which directly obtain water for consumption and disposal of sewage. Rice fields take water from the same river for irrigation [10]. The city of Charqueadas (Internal Coastal Plain -PCI) is bathed by the Jacuí River. The Jacuí Basin has an area of 71 600 km^2, corresponding to 83.5 % of the area of the Guaíba region. The river originates in Jacuí Plateau in the municipalities of Passo Fundo and Marau, and all its drainage area is characterized by intensive use of land for agriculture and livestock [11]. The analyzed sites (Figure 1) were the districts of Santo Antônio da Patrulha (PCE)- RS: 29 ° 50 '18' 'S and 50 ° 30' 58''W and Charqueadas (PCI) - RS: 29 ° 97 '04.9' 'S and 051 ° 31 '33.2' 'W.

Figure 1. Location of the areas of Santo Antônio da Patrulha / RS and Charqueadas /RS, Brazil, during the agricultural years of 2013/14 and 2014/15, evaluated in this study.

2.2. Soil Sample

Data collection took place during both years: 2013/14 and 2014/15. Two rice-growing areas with different soils were chosen. The External Coastal Plain (Santo Antônio da Patrulha) has for the most part soil of the Gleysoles type, which has a high rate of organic matter. The Internal Coastal Plain (Charqueadas) has for the most part soil of the Planosoles type, in which its deeper layers are cemented with few nutrients, hindering plant growth. In each farm, soil samples were collected in triplicate. Each sample was made up of sub-samples collected randomly, covering a representative area of the property. The crops were divided into homogeneous areas identified by relief, vegetation, soil type, use and management. They were collected between 10 and 20 sub-samples to form a single sample, crossing a zigzag path at random, covering the entire area. The rocks, roots and debris from the soil surface cultures were eliminated at the collection point. Sampling was done with auger at a depth of 20 cm at each collection point, and the soil was stored in sterile vials. After harvesting, the soil was homogenized and a sample of 500 grams was gathered [12]. After collection, the samples were placed in sterile vials and sent to the Microbiology and Toxicology Laboratory of UNISINOS University.

2.3 Stutied Rice Plant

The sampled cultivar was Puitá INTA-CL in crops of minimum tillage, which are areas that are not heavily manipulated throughout the planting process. Cultivar Puitá INTA-CL is a variety resistant to herbicides, with an average cycle of 125 days, having low height, with an average height of 86 cm which makes it resistant to lodging. Samples were collected into four periods of the crop cycle: one collection after soil before sowing, two collections in the vegetative phase, two collections in the reproductive phase and two during seed maturation, with a total of seven collections in each crop year for each crop [12].

2.4 Diversity

Bacteria growth initiated from 100 mL water with soil (10g), in which an aliquot of 500 µL was withdrawn in 4.5 mL saline (NaCl 10%) dilution reaching 1.10^{-3}. Then, 100 µL was applied in a Petri dish containing Nutrient Agar [digestible gelatin enzyme (5 g / L), beef extract (3 g / l) and agar (15g / L)] [13], which was incubated in bacteriological incubator at 30° C for 24 hours. The individual colonies grown were counted using a colony counter and the bacterial cells were examined cytologically by differential interference phase microscopy (1,000×). The bacterial spores were separated by pasteurization, and both groups (sporulating and non-sporulating) were identified by cell morphology, physiological and biochemical characteristics, according to international methods of bacterial classification described in Bergey (2010) [14] and the adapted identification key. Gram-negative bacteria were identified by *API Biomerieux® 20 E* method with subsequent use of *APIWEB* software for the determination of species.

2.5 Analyzed parameters

Physicochemical elements of the soil were analyzed in the EEA-IRGA and the Soil Analysis Laboratory: Clay (%), pH, Index of analysis and correction of acidity - SMP, Phosphorus (mg/L), Potassium (mg/L), Organic Matter - MO (mg/L), Aluminum (mg/L), Calcium (mg L), Magnesium (mg/L), Hydrogen + Aluminum (mg/L), Effective electron exchange capacity – CTC E (mg/L), Electron exchange capacity pH 7 – CTC 7 (mg/L), Saturation (Sat) Bases (%), Sat Al (%) Sat K (%), Relationship (R) Ca/Mg (mg/L), RCa/K (mg/L) e RMg/K (mg/L). The procedures used to estimate the physical and chemical variables were defined by Tedesco et al. (1999) [15].

2.6 Statistical Analysis

Firstly, to determine the variation of the soil quality, the results were organized into two banks of different data and analyzed separately. The first databank was utilized to evaluate the Gleysoles and the second for planosoles. Analysis of variance (ANOVA - $p < 0.05$) was used to evaluate the differences in the abundance of bacterial colonies in the soil of each field studied, at different periods of plant cultivation. Diversity in the rice fields was analyzed using the *Shannon* index [16] and *Eveness*, calculated separately for each town, using the totals for each environmental abundance. The expression values were transformed by $\log 10\ (X + 1)$ to compensate for deviations caused by lower or higher abundance [17]. The influence of physicochemical parameters on the abundance of the main species was quantified by canonical correspondence analysis (CCA) method using the PC-ORD 6.0 software [18]. With the CCA it was possible to produce an analysis of direct ordination gradient, explaining the distribution of species in relation to environmental variables. The meaning of the main axis of the canonical ordination was evaluated by Monte Carlo [17] permutation test. For this analysis it was used species with more than five colonies per point. After the separate analyzes, we did analyzes with the whole database. For the PCA analysis we used a correlation matrix, because the variables are estimated in different units of measurement. Only autovalues greater that 1 were utilized as criteria for extraction of the principal components. The varimax rotation, was used to simplify the expression of a particular sub-space in terms of just a few major items each, the actual coordinate system is unchanged, as to facilitate the interpretation of the data generated by the principal component analysis. We used PCA in the PAST 3 program.

3. Results

In the analysis of the results it was recorded a total of 29 species; of these, 27 were found in External Coastal Plain-PCE and 22 in Internal Coastal Plain-PCI in different periods of crop cultivation. Abundance of bacterial colonies in External Coastal Plain ($F_{1,9} = 7.84$, $p < 0.05$) was 18.5 % in the pre-planting period, 22.3 % in the vegetative phase, 22.3 % in the reproductive phase and 36.9 % during maturity. The *Shannon* index estimated diversity was more pronounced at maturity (M = 3,901) compared to the values obtained in the soil of other phases (M = 2.97). Abundance of bacterial colonies in Internal Coastal Plain ($F_{1,9}= 7.03$, $p < 0.05$) was 23.9% in the pre-planting period, 21.7% in the vegetative phase, 20.7% in the reproductive phase and 33.7% during maturity. The *Shannon* index estimated diversity was more pronounced at maturity (M= 3,801) compared to the values obtained in the soil of other phases (M= 2.46). The *Evenness* index indicates that the distribution of species is similar in both locations during maturity (E= 0.988 and 0.967). Figure 2 identifies the microorganisms found in the soil samples in the areas of rice cultivation in the studied cities.

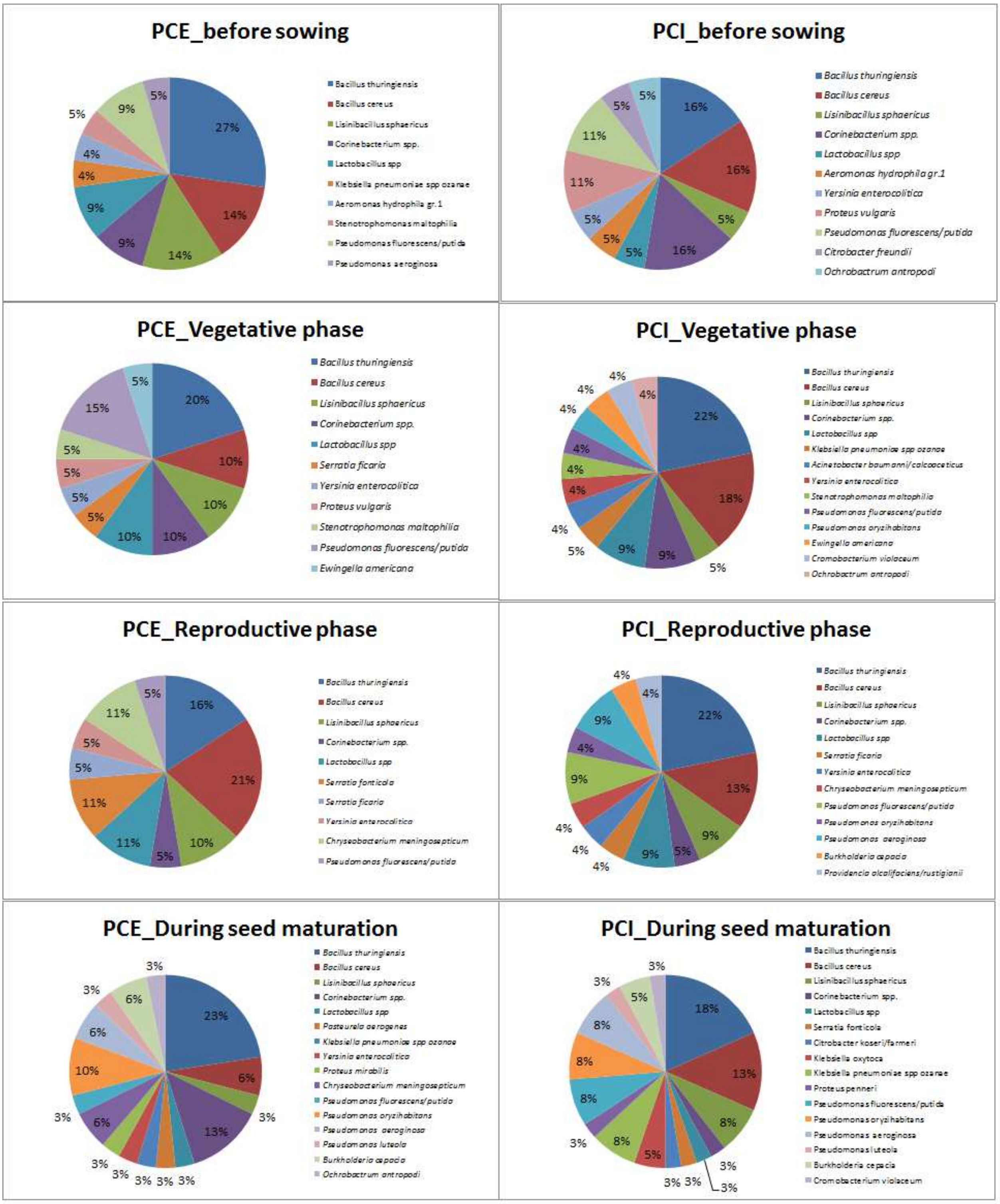

Figure 2. Frequency of bacteria (CFU / 100 ml) present in soil samples in areas of rice cultivation in External Coastal Plain and Internal Coastal Plain in the state of Rio Grande do Sul Brazil, in the agricultural years of 2013/14 and 2014 / 15.

The species *Aeromonas hydrophila* gr.1 and *Ewingella americana* were observed during the pre-planting and the vegetative phase, respectively. The largest records were colonies of *Bacillus thuringiensis, Bacillus cereus, Lisinibacillus sphericus, Corinebacterium* spp. and *Pseudomonas fluorescens/putida* found in both crops evaluated. The high values, obtained in the CCA analysis, indicate the contribution of each axis, explaining the observed variation in the data. The three axes for the first agricultural year explain 67% of the variation of species and for the second agricultural year, explain 86.5%. The species-environment correlation for axis 1 indicates a good relationship between species and environmental variables used for sorting. The CCA analysis was performed by joining the two years (2013/14 and 2014/15) (Figure 3) explain 77,07% of variation between species and Physical-chemical variables. The Monte Carlo test (p<0,001) suggests that the occurrence by chance of this ordination is low. The most important variables for ordination, correlated with the first axis, were organic matter, calcium and Potassium.

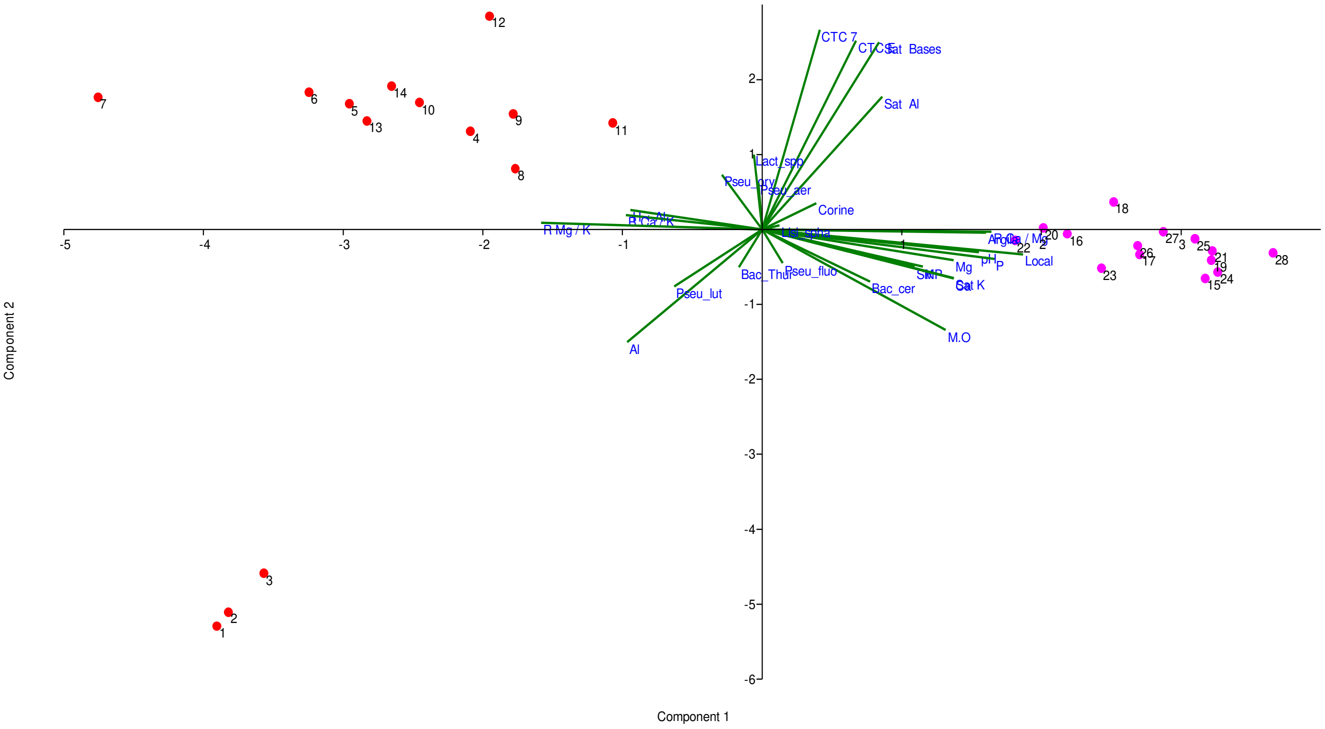

Figure 3. Biplot graph showing the separation between Internal Coastal Plain and External Coastal Plain and principal components.

The 18 physical-chemical variables (Table 1) and the bacterias were analyzed according to the Principal component analysis (PCA) technique. The results of the PCA (figure 4) show that the three axes explain 64,84%: axis 1 (30,79%) includes Clay, Local, pH, Phosforus, Organic Matter, *Bacillus cereus*; The second axis (14,49%) includes Aluminium and Calcium; and the third axis (9,78%) *Corinebacterium*. The separation of the points which were analyzed based only Physical- chemical elements, shown in Figure 4, were observed a separation between soils. It is indicate that the soils has differents physical-chemical caracteristics.

Table 1. Average of environmental variables and physicochemical parameters of soil sampling of rice crops in External Coastal Plain and Internal Coastal Plain, RS / Brazil, during the agricultural years of 2013/14 and 2014/15

	Clay	pH	SMP	P	K	MO	Al	Ca	Mg	H+Al	CTC E	CTC 7	SAT Bases	Sat Al	Salt K	R Ca/ Mg	R Ca/ K	R Mg /k
INTERNAL COASTAL PLAIN																		
2013/14	13	4,9	7,1	16,6	175	1,3	0,1	4,2	1,8	1,2	6,5	7,7	83,9	1,5	5,8	2,2	9,3	4,1
	13	4,9	7,2	14,4	184	1,4	0,1	2,5	1,3	1,1	4,4	5,4	79,7	2,3	8,7	1,9	5,4	2,8
	13	5,5	6,9	14,7	182	2,3	0	2,4	1,8	1,6	4,7	6,3	75,2	0	7,4	1,4	5,3	3,8
	13	5,1	6,6	21,6	230	0,8	0,1	2,4	1,3	2,2	4,4	6,5	66,4	2,3	9	1,8	4,1	2,3
	14	5,1	7,2	10,7	132	1,1	0,1	5	1,8	1,1	7,2	8,2	82,7	1,4	4,1	2,7	14,7	5,4
	14	5,3	6,8	15	182	0,4	0,2	2,8	1,6	1,7	5	6,6	73,6	4	7,1	1,7	6	3,5
	15	5,3	7,1	11,7	99	1,3	0,2	1,4	1	1,2	2,8	3,9	68,1	7,1	6,5	1,3	5,4	4,1
2014/15	14	5,1	7	17,8	195	0,9	0	2,7	1,5	1,4	4,8	6,1	77,5	0	8,1	1,8	5,5	3,1
	14	4,8	7	14,6	178	0,9	0	4	1,7	1,4	5	7	86,7	0	7,6	2,1	5,5	3,1
	12	5	7,2	17,5	167	0,8	0,3	3	1,7	1,3	5,1	6	85,4	1,3	7	1,9	5,7	3,4
	14	5,1	7,3	16	189	1,2	0,2	3	1,6	1,2	4,6	6	87,9	1,5	9	1,8	5,4	2,8
	13	5,2	6,8	15,8	178	1,1	0,1	2	1,9	1,3	4,6	5,4	69,9	1,7	5,8	2,3	5,8	3,2
	13	4,8	7	14,7	187	1	0	1,7	1,4	1,1	4,7	7	60,2	1,6	7,4	2,1	8	2,9
	13	4.7	7,1	13,9	190	0,9	0	1,4	1,5	1	4,3	5	89,1	1,8	4,1	2,1	8	2,8
EXTERNAL COASTAL PLAIN																		
2013/14	15	6,1	7,5	27,9	200	1,8	0	4	2	1,1	4	4	74,2	0	9,8	3	4	2,1
	16	5,9	7,1	21,6	210	1,8	0	4,1	1,9	1	4,5	4	74,3	0	9,7	2,9	7	2,1
	17	5,5	7,3	22,1	201	1,9	0	4,5	2	1,2	3,9	4	73,2	1,3	9,8	3	6	2,2
	18	6,2	7,3	23,6	198	2	0	4,7	1,9	1	4,3	5	75,5	1,5	9	2,8	7	2,6
	16	6,1	7,7	25,1	189	1,9	0,1	4	1,8	1	4,5	4,6	70,1	1,7	9,6	2,9	6	2,1
	18	6,4	7,2	19	201	1,8	0,2	4	1,9	1,2	4,6	5,2	69,9	1,6	9,7	3	5	2,9
	17	6,3	7,5	19,2	220	1,7	0	4,5	1,9	1	4,1	4	70,1	1,8	9,9	3,1	5	2,1
2014/15	19	5	7	24,9	203	2	0,1	4,8	2	1,1	4	4,2	72,1	0	8,9	2,1	4	2,1
	17	6,7	7,4	24,7	220	2	0,1	3,9	1,9	1,2	3,8	4,1	72,5	0	9	3	4	2,1
	16	6,6	7,3	21,7	200	1,9	0	6	1,8	1,2	3,2	4,3	68,2	1,9	9,1	3,1	4	2
	15	6,2	7,1	21,9	200	2	0	5,4	1,9	1,1	3,9	4,3	68,5	0	9,6	3,2	4	2,9
	18	5,7	7,1	19,9	201	1,8	0	4,1	2	1	4	5	68,4	0	9,7	3,1	4	2,1
	17	5,8	7,3	31,2	202	1,9	0	4	1,9	1	3,9	4,2	70,1	1,6	9,9	3	4	2
	15	6,1	7,5	17,9	200	1,8	0	4	2	1,1	4	4	74,2	0	9,8	3	4	2,1

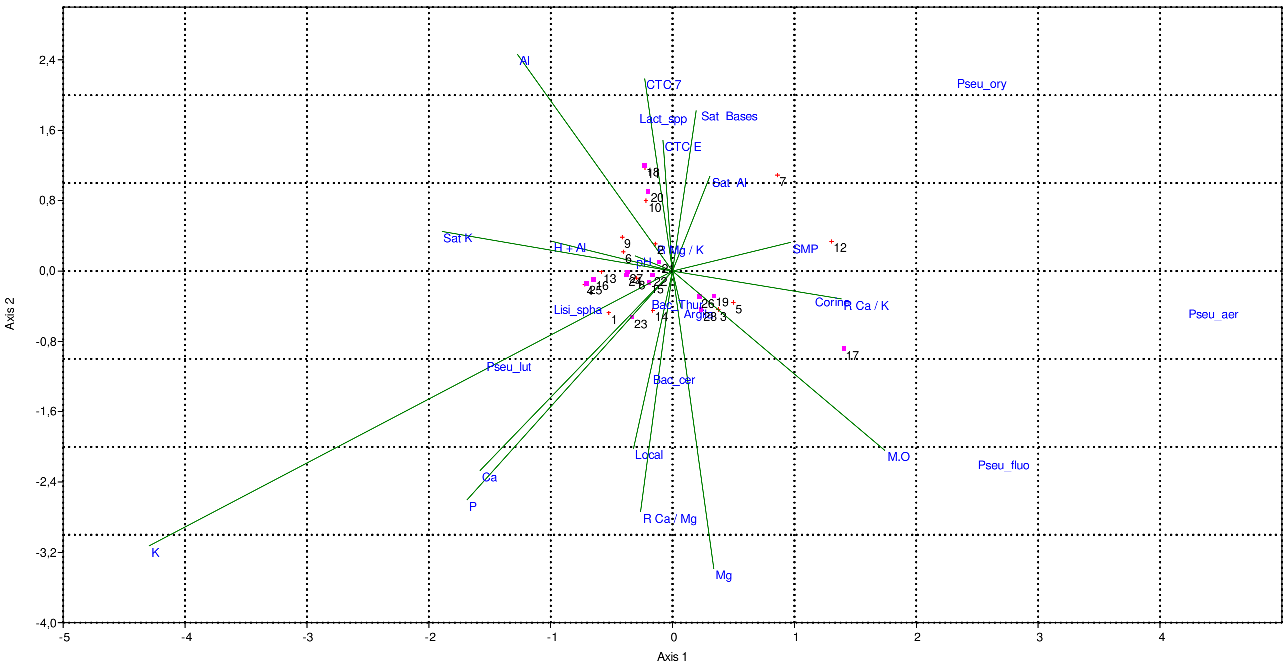

Figure 4. Canonical correspondence analysis (CCA) of bacterial communities obtained from soil of rice crops in External Coastal Plain and Internal Coastal Plain, RS / Brazil.

4. Discussion

The bacterial communities found in agricultural soils of External Coastal Plain and Internal Coastal Plain / RS are composed of several species, but with low abundance - such as *Bacillus cereus, Bacillus thuringiensis, Pseudomonas fluorescens* and *Lactobacillus* spp. Other authors also found similar results evaluating the bacterial diversity in soil [9 – 19,20]. In an agroecosystem, the diversity variation throughout the seasons of the year is still not well understood, as in every season it seems to occur dominant microbial community accompanied by other less abundant that often are below the detection level of the current evaluation methods [21] . The species *B. thuringiensis* was the most frequent in soils of rice cultivation. These bacteria can act as entomopathogens in the biological control of pests, especially in rice culture. This species was also prevalent in the work of Panizzon et al. (2012) [10] in water samples from rice cultivation areas. Bacteria from the genus *Pseudomonas* are a major group from soil, especially because of their ability to act as endophytes contributing significantly to the development of plants [22-23]. Microorganisms have great importance in biochemical and geochemical cycles and have a great chemical and molecular arsenal. In the context of rice cultivation, bacterial abundance can be considered by its wide variety of microhabitats, caused by constant irrigation, resulting in complex bacterial communities [24]. In a similar study, Panizzon et al. (2012) [10] evaluated the effects of physical and chemical variables on the diversity of bacterial communities present in the water used to irrigate rice fields, the water from the central part of the crop and the water drained back into the river. They found that the abundance of bacterial colonies was higher in the irrigation water than in the rice field or the drainage. In addition, the bacterial community in the water samples from rice growing areas in the Sinos River Basin, RS, Brazil, contains several species, but few are abundant, such as the genus *Bacillus*, *Lactobacillus* and *Pseudomonas*, confirming the results found in this study in rice culture soil (Table 1). Cortés-Sánchez et al. (2015) [25] report that cosmopolitan microorganisms isolated from soil, water and vegetables such as bacteria of the Enterobacteriaceae family can also be found in the digestive tract of animals and humans, although it is also possible to find them in transient or normal microbiota. Among them include: *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., *Yersinia* spp., *Proteus* spp., *Providencia* spp., *Shigella* spp., *Ewingella* ssp., *Erwinia* spp. and *Pantoea* spp. They can be pathogenic or opportunistic, occasionally forming components with physicochemical and biological properties beneficial to humans and the environment in which they operate. According CCA analysis, the clay is associated with bacterial diversity. This element is essential for the production of annual crops and its low availability impairs productivity. The impact of nitrogen and clay in bacterial communities in soil is difficult to analyze and restrains the visualization of patterns, although it is associated in some studies with a reduction in biomass [26-28]. Microbial communities are particularly affected by the management and impact in the ground. Agricultural practices such as soil alterations during preparation and irrigation can modify bacterial communities. Being the organic matter one of the main elements for good survival of microorganisms [29]. The bacteria found in soil are highly diversified. In hot soils, for example, there is the presence of thermophilic microorganisms and microbial population changes very quickly as the available nutrients are modified [30]. The amount of organic matter significantly influenced the microbial growth (Table 3). Usually the rivers that irrigate the rice fields receive large amounts of sewage, which can contribute with antimicrobial substances that help forming the bacterial community in the studied region. Heavy metals also present in the sources supplying the crops may inhibit growth of some bacteria, such as the case of mercury. However, many *Pseudomonas* survive in environments and thus convert the mercury into methylated mercury, which is volatile and escapes to the atmosphere [31-32]. Thus, a soil with high organic matter content tends to maintain the microbial population more stable throughout the year, probably due to the richness of ecological niches, through the heterogeneity of carbon sources [33]. The CCA analysis shows an explanation of 67% for the year 2013/14 and 86.5% for the year 2014/2015. Such variations are directly linked to the water system and climate of the region, the structure and soil management, and the content and quality of accumulated plant residues [34]. In this case, the small difference found in relation to the two agricultural years evaluated may be related to high rainfall during the agricultural year of 2013/14. However, the differences are shown in only a few species which may be present or absent at any place to a greater or lesser degree. If we remove those species, the other diversity values of the two areas are similar. The CCA analysis (Fig. 3) shows an explanation of 77,07% for the two years together. The PCA analysis explanation of 64,84% (Fig.4). There are two different soils, so the physical-chemical variables are different. The External Coastal Plain has a more nutritive soil than Internal Coastal Plain. This situation directly affects the development of the plant. But, with the data obtained in this study we concluded that the bacterial diversity is similar in the two areas studied, regardless of soil type. The difference is for bacteria in each soil. According to Reche et al. (2016), phosphorus, potassium and calcium are the nutrients that have most appeared to be influencing the development of the rice plant. The authors presented a reduction of potassium in the maturation period, similar to what happened in our research. It is known that pH, phosphorus and potassium help the plant to develop.

5. CONCLUSIONS

The bacterial diversity was influenced by the parameters Clay and organic matter. The findings are a contribution to the study of factors that influence the diversity of heterotrophic bacterial communities in rice fields. Microorganisms are essential in the natural processes responsible for the quality of soil and, therefore, knowledge on microbial diversity of the system and the factors influencing them are of considerable importance.

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5 CAPÍTULO 4: CAN THE PHYSICOCHEMICAL CHARACTERISTICS OF THE SOIL OF THE COASTAL PLAIN OF THE BRAZILIAN STATE OF RS INTERFERE IN THE NUTRITIONAL VALUE OF PUITA INTA CL RICE?

Artigo de pesquisa submetido na Ciência Rural.

Can the physicochemical characteristics of the soil of the Coastal Plain of the Brazilian State of RS interfere in the nutritional value of Puita Inta CL rice?

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ABSTRACT

Rice (*Oryza sativa*) is one of the most consumed foods in the world, being one of the most important grains in global economic terms. The purpose of this research was to evaluate if the physicochemical characteristics of the soil in rice fields of the Internal and the External Coastal Plain of the Brazilian State of RS have the potential to modify the nutritional value of the rice cultivar Puita Inta CL. This study evaluated the nutritional composition, the total phenolic compounds content and the antioxidant activity of brown and polished grains of Puita Inta CL rice from two different areas. Samples were collected during two crop years, 2013/14 and 2014/15, in rice producing areas of the Coastal Plain, RS-Brazil. The nutritional content, phenolic content and antioxidant content were tested by the Mann Whitney method, in which samples are compared, obtaining the result that brown rice and polished rice have different characteristics, depending on the place where it is cultivated ($p < 0.001$), and that there is no temporal difference ($p > 0.05$). The 18 physicochemical variables were analyzed by principal component analysis (PCA), followed by MANOVA. Results from PCA showed that the first three axes explain 74.19%. The first axis (47, 12%) includes the variables: regions, phosphorus, pH, potassium saturation, calcium / magnesium ratio, clay and organic matter. The second axis (15.79%) includes: calcium / potassium ratio and effective electron exchange capacity. The third axis includes: (11, 28%) potassium. The resulting spatial pattern shows a significant difference between the soils of each region as demonstrated by MANOVA (Wilk's Lambda: 0.008, $p < 0.0001$ and $F = 61.6$).

Key words: *Oryza sativa*, Coastal Plain, physicochemical compounds, soils, nutrients.

INTRODUCTION

Rice is one of the most cultivated cereals in the world for direct human nutrition and as an input to the food industry. Trends in the food industry are making rice, especially organic and brown, to be increasingly sought after due to its health benefits (CONAB, 2016).

The cultivation systems employed for the production of irrigated rice are: conventional, direct, minimum and pre-germinated (EMBRAPA, 2016). Each system is characterized by different types of soil management and the crop itself. The State of Rio Grande do Sul presents several lowland soil classifications, which may be suitable or not for irrigated rice cultivation such as Planosols, Gleysols, Chernosols and Neosols, among others (PINTO et al., 2004). The soil is composed of microhabitats that differ in their physicochemical properties (CARSON et al., 2009). These characteristics determine a diversified composition of microorganisms where any change, physical or chemical, causes modifications in the community of organisms (PANIZZON et al., 2016). The availability of oxygen, moisture ratio, temperature range, available organic matter and pH are factors that directly affect the soil and consequently the plants and their products that are used in both animal and human feeding (FEIGI et al. 1998).

In foods, antioxidants, for example, are molecules capable of reducing or preventing oxidation. Due to the risks of consuming synthetic antioxidants, research studies on natural products that contain antioxidant activity have increased with the aim of replacing the synthetic antioxidants (MENDIOLA et al., 2010). Among the sources of phenolic compounds, rice plays an important role in diet because it contains distinct phenolic compounds, such as tocopherols, tocotrienols and g-oryzanol, mainly associated with the pericarp. However, grain polishing reduces the concentration of phenolic compounds in the endosperm, which remain in the bran from where be limited to carbohydrates or proteins making the hydrolysis process important to obtain the maximum yield of phenolic acids if you want to recover them (OLIVEIRA et al. 2012).

The present work aimed to examine if the physicochemical characteristics of the soil can influence the nutritional composition of polished and brown rice of the cultivar Puitá INTA-CL, cultivated in the crop years 2013/14 and 2014/15, in the rice-growing regions of the Internal Coastal Plain and the External Coastal Plain, RS-Brazil.

MATERIAL AND METHODS

The analyzed sites (Fig. 1) were the districts of Santo Antônio da Patrulha (PCE)- RS: 29 ° 50 '18' 'S and 50 ° 30' 58"W and Charqueadas (PCI) - RS: 29 ° 97 '04.9' 'S and 051 ° 31 '33.2' 'W.

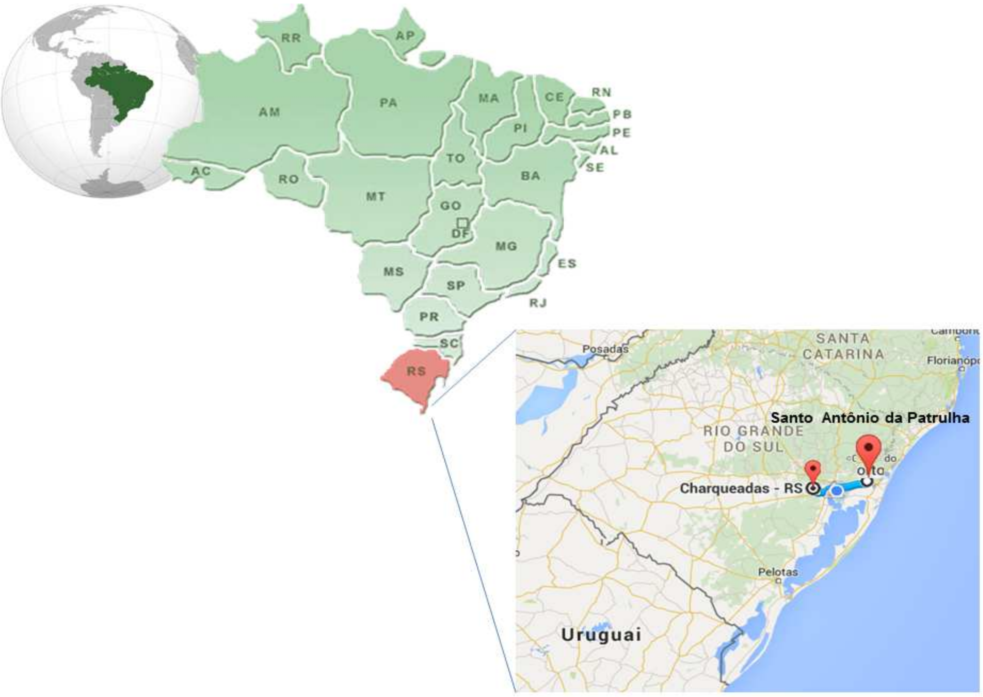


Figure 1: Sampling sites in the External Coastal Plain(large dot) and Internal Coastal Plain (Small dot) of Rio Grande do Sul State, areas in Brazil, during the crop years of 2013/14 and 2014/15, evaluated in this study.

Soil samples: Soil samples were collected during two years: 2013/14 and 2014/15, it is totaling 28 samples. Two rice-growing areas with different soils were chosen. The External Coastal Plain (Santo Antônio da Patrulha) has for the most part soil of the Gleysoles type, which has a high rate of organic matter. The Internal Coastal Plain (Charqueadas) has for the most part soil of the Planosoles type, in which its deeper layers are cemented with few nutrients, hindering plant growth. In each area, soil samples were collected in triplicate. Each sample was made up how many subsamples collected randomly, covering a representative area of the property. They were collected between 10 and 20 sub-samples to form a single sample, crossing a zigzag path at random, covering the entire area. The rocks, roots and debris from the soil surface cultures were eliminated at the collection point. Samples were collected by an auger at a depth of 20 cm at each collection point, and the soil was stored in sterile vials. Soil samples were homogenized and a subsample of 500 grams was retained (Irga 2013). After collection, the samples were placed in sterile vials and sent to the Microbiology and Toxicology Laboratory of UNISINOS University.

Studied rice plant: The sampled cultivar was Puitá INTA-CL in crops of minimum tillage, which are areas that are not heavily manipulated throughout the planting process. Cultivar Puitá INTA-CL is a variety resistant to herbicides, with an average cycle of 125 days, having low height, with an average height of 86 cm which makes it resistant to lodging. Samples were collected in four periods of the crop cycle: one collection after soil before sowing, two collections in the vegetative phase, two collections in the reproductive phase and two during seed maturation, with a total of seven collections in each crop year for each crop (Irga 2013).

Culture management: Both cultures presented the same management and treatments, with three applications of basic fertilization, 300 kg / ha – 7-14-27 days. Around 25 days after this application, 150 kg of white urea 46-00 was added to the soil. The herbicides were applied 1 day before the urea, following the manufacturer's dosage. The fungicides were applied in the middle of the rice plant cycle.

Analyzed parameters: Physicochemical elements of the soil were analyzed in the EEA-IRGA and the Soil Analysis Laboratory: Clay (%), pH, Index of analysis and correction of acidity - SMP, Phosphorus (mg/L), Potassium (mg/L), Organic Matter - MO (mg/L), Aluminum (mg/L), Calcium (mg L), Magnesium (mg/L), Hydrogen + Aluminum (mg/L), Effective electron exchange capacity – CTC E (mg/L), Electron exchange capacity pH 7 – CTC 7 (mg/L), Saturation (Sat) Bases (%), Sat Al (%) Sat K (%), Relationship (R) Ca/Mg (mg/L), RCa/K (mg/L) e RMg/K (mg/L). The procedures used to estimate the physical and chemical variables were defined by Tedesco et al. (1999).

Rice samples: The grains were collected by tractor in each of the studied properties and were taken to the Rice Experimental Station (IRGA), which carried out actions such as grain transport, reception, pre-cleaning, drying and storage. Essentially, consisting of the industrial process of turning the rice grain from the crop into a processed food product: polished rice and brown rice (IRGA, 2013).

Centesimal composition: The analyses of nutritional composition were performed according to the norms of the Adolfo Lutz Institute (2008). The dry extract was obtained by determining the humidity by subjecting the samples to oven heating at 105 ° C to constant weight. The ashes were obtained by incineration in a muffle furnace at 550 ° C. Lipids and proteins contents were obtained by the methods of Gerber and Kjedhal, respectively. Fiber content was determined by the AOAC (2012) method and the determination of carbohydrates was obtained by the difference of the others. All analyses were performed in triplicate.

Rice extract: 2 g of milled rice was added to 40mL of the acetone / water / acid solution (70:29:1, v/v/v) and left to stand for 1 hour in an ultrasonic water bath. The mixture was centrifuged at 5000 rpm at 20 ° C for 20 minutes. The supernatant was collected for the determination of antioxidants and phenolic content. This extract was used for the determination of DPPH and phenolic compounds (QIUA et al, 2010).

DPPH method: Antioxidant activities were determined using DPPH as a free radical. For each antioxidant, different concentrations were tested (expressed as the number of moles of antioxidant / mole of DPPH). The antioxidant solution of methanol (0.1 mL) was added to 3.9 mL of 6 x 10-Smol / L of DPPH solution. The decrease in absorbance was determined at 515 nm at 0min, with every 15 min the reaction being expected to reach a plateau. The initial DPPH index was calculated by concentration (CDPPH) in the reaction medium from a calibration curve with the equation whose absorbance of 515nm = 12509 × (CDPPH) - 2.58 x 10^{-3} as determined by linear regression. For each antioxidant concentration tested, the kinetic reaction was plotted. From these graphs, when the percentage of DPPH is maintained at steady state the values of DPPH residues are determined as the function of the molar ratio of antioxidant to DPPH anti-radical activity, with the result expressed in mM / g m.s (BRAND-WILLIAMS et al.1995).

Folin-Ciocalteu method: This method was used to determine the total phenolic content. Each sample of rice extract was diluted in 50 mL with distilled water and filtered. This solution (0.5 mL) was then mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent for 5 minutes and 2 mL of 75 g / L sodium carbonate (Na_2CO_3). After incubation for 2 h, the absorption of the reaction was measured at 760 nm against a blank of methanol. Gallic acid (0-200mg / L) was used as the standard to produce a calibration curve, with the result expressed in: Mg EAG / g m.s (MEDA et al. 2005).

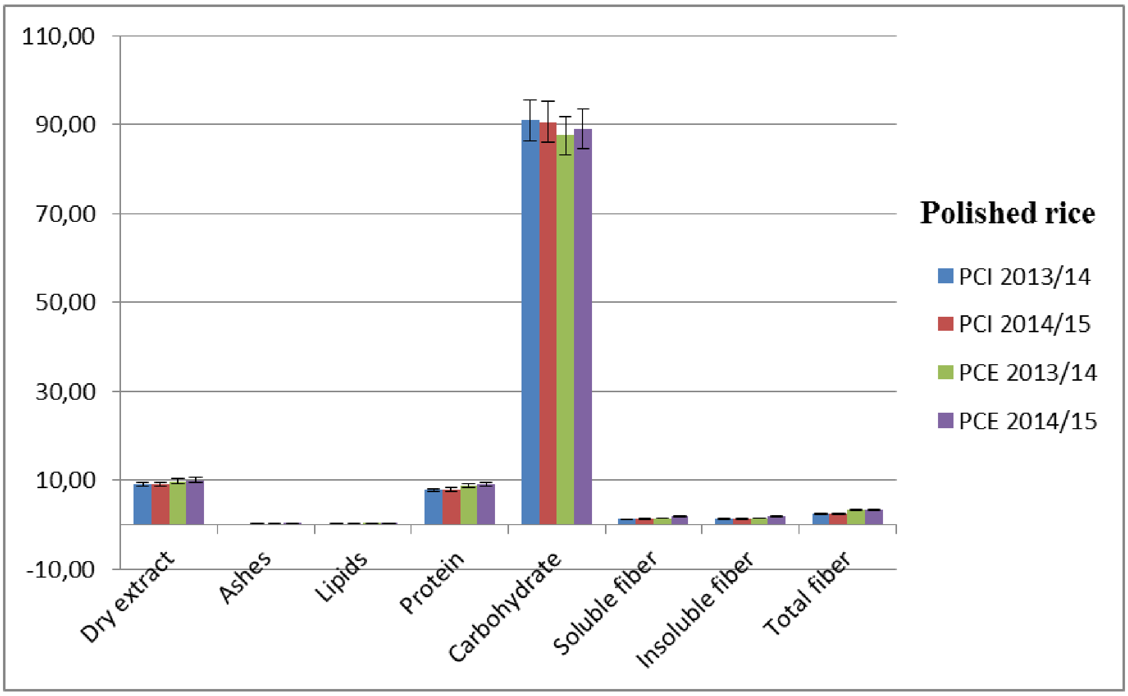
Proteins BCA: A second method (BCA) of protein determination was also tested using the colorimeter-spectrophotometer, with the result being expressed in µg / mL. A curve is made with a blank, generating a standard curve, where x is the concentration and y is the absorbance. The higher the concentration of proteins, the greater the coloration. The rice samples were filtered, then centrifuged. A dialysis of 12 hours was made and the measurement was done on the device. (ZAIA et al. 1998).

Statistical analysis: To determine the grouping of the physicochemical elements, a principal component analysis (PCA) was performed. A MANOVA was then performed to verify if there was a difference in soil characteristics among the studied regions that could nutritionally modify the rice consumed. Since the data were not normalized, a Mann Whitney analysis was performed with the grains produced in the areas to evaluate if there was difference of nutritional components between the rice of each region and between the studied cycles (ZAR, 1999).

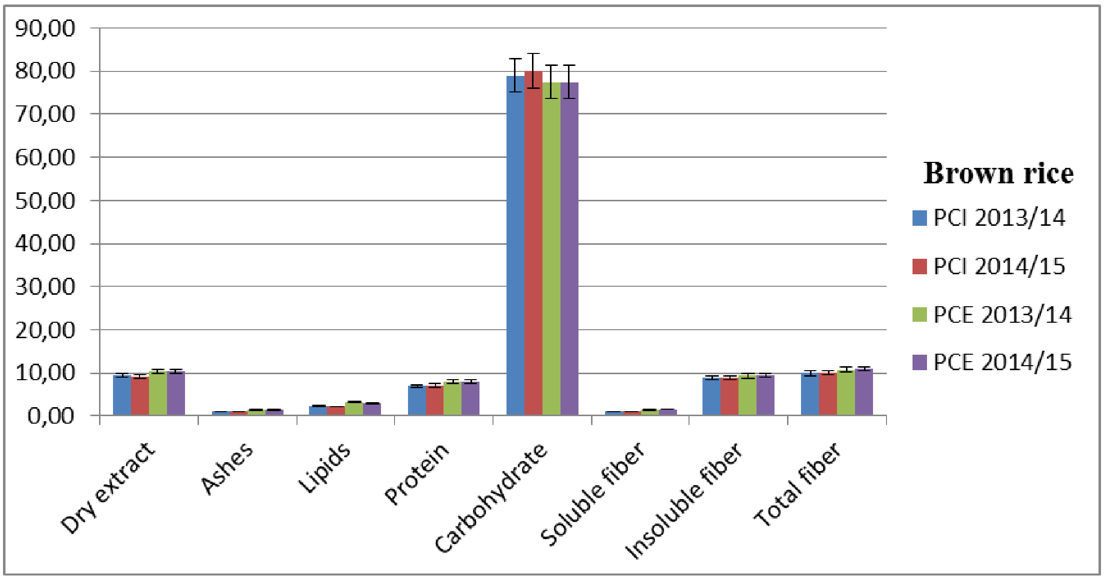
RESULTS AND DISCUSSION

The parameters evaluated in the nutritional composition of polished and brown rice (Figure 2 A and B) were maintained in the two regions, Internal Coastal Plain (PCI) and External Coastal Plain (PCE), RS / Brazil. According to ZHOU et al. (2002), the composition of the grains and their portions is subject to several factors, such as variety, environmental variations, handling, processing and storage. In addition, grain polishing results in a reduction in nutrient content, except for starch, which can be observed in Figure 2 A and B, which shows that polished rice had lower concentrations of ash, lipids and insoluble fibers when compared with brown rice. According to MANO (1999), dietary fiber exerts different functions in the human body. Studies associate the reduction in blood pressure, the concentration of total cholesterol, LDL cholesterol and triglycerides, and the control of blood glucose, aiding in the prevention and in the control of some chronic diseases, such as diabetes and cardiovascular diseases.

Carbohydrates are the main constituents of rice as can be seen in the analyzes (Figure 2A and B). The protein content in rice is considered low, on average 7%, and lipids have their highest concentration in the germ. Thus, the lipid concentration is higher in the brown rice, being reduced with the polishing, corroborating with values found experimentally (WALTER et al., 2008). The concentration of minerals differs in the grain fractions. While in husked rice silicon is a dominant component, in brown and polished rice phosphorus, potassium and magnesium stand out. Mineral content is greatly influenced by growing conditions, including fertilization and soil conditions, and by processing (ITANI et al., 2002). The content of phenolic compounds, antioxidant index (DPPH) and proteins BCA of both polished rice and brown rice were higher in PCE, where the soil is higher in nutrients, indicating that the most fertile soils produce foods with higher nutritional quality.



A



B

Figure 2: Nutritional composition in percentage (%) of rice produced in two rice regions of RS / Brazil. (A) Polished rice; (B) Brown rice. PCI = Internal Coastal Plain; PCE = External Coastal Plain.

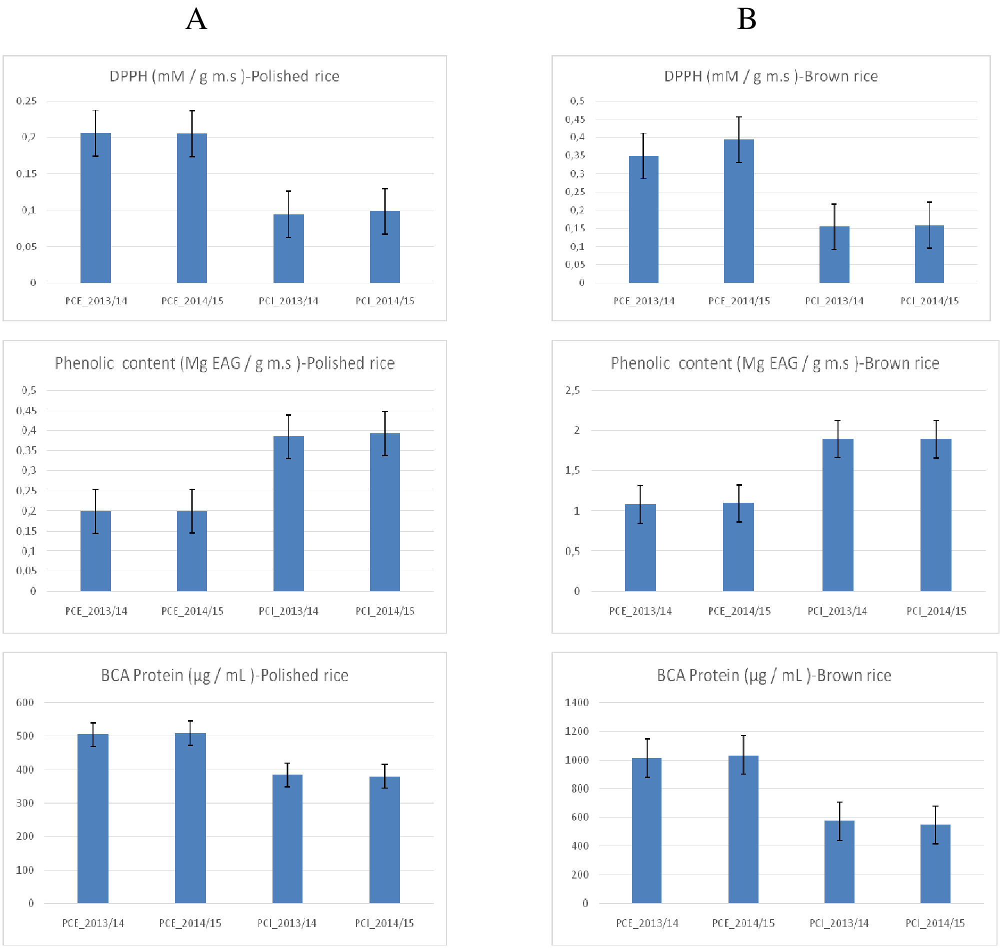


Figure 3: Content of phenolic compounds, antioxidants (DPPH) and proteins BCA of rice produced in two rice regions of RS / Brazil. (A) Polished rice; (B) Brown rice. PCI = Internal Coastal Plain; PCE = External Coastal Plain.

The Mann Whitney tests showed that the nutritional content, phenolic content and antioxidant content of the grains grown in the crops did not vary significantly between the crop years ($p > 0.05$). However, a variation of the same nutritional components was detected between the grains generated in the Internal and the External Coastal Plain ($p < 0.0001$). All nutritional components analyzed were higher in the External Coastal Plain, except carbohydrates, because starch, despite being the predominant polysaccharide in rice, is a result of the sum of the other nutritional components, indicating that if the other components are higher, its content will be reduced. Starch is the main form of energy storage of plants, being used in the industry due to its low cost.

Among the various classes of natural occurrence of antioxidant substances, phenolic compounds have attracted special attention because they inhibit lipid peroxidation and lipo-oxygenation *in vivo* (KRISTINOVA et al., 2009). Therefore, the chemical structures that allow the neutralization or sequestration of free radicals, as well as the chelation of transition metals, prevent the propagation of oxidative processes. Vegetable tissues are good sources of these compounds, and rice, despite not having many of these food features, is a food consumed by a large part of the population (OLIVEIRA et al. 2007).

The 18 physicochemical variables (Table 1) were analyzed by the principal component analysis (PCA) technique, after which a MANOVA was performed to test if the regions had similar values of the variables. Results from the PCA (figure 4) showed that the first three axes explain 74.19%: the first axis (47, 12%) includes local variables, phosphorus, pH, potassium saturation, calcium / magnesium ratio, clay and organic matter; the second axis (15.79%) includes calcium / potassium ratio and effective electron exchange capability; and the third axis (11, 28%) includes potassium.

TABLE 1: Physicochemical and environmental parameters of the soils of the Internal Coastal Plain and the External Coastal Plain (RS / Brazil), crop years of 2013/14 and 2014/15.

	Clay	pH	SMP	P	K	MO	Al	Ca	Mg	H+Al	CTC E	CTC 7	SAT Bases	Sat Al	Salt K	R Ca/ Mg	R Ca/ K	R Mg /k
									Internal Coastal Plain									
2013/14	13	4,9	7,1	16,6	175	1,3	0,1	4,2	1,8	1,2	6,5	7,7	83,9	1,5	5,8	2,2	9,3	4,1
	13	4,9	7,2	14,4	184	1,4	0,1	2,5	1,3	1,1	4,4	5,4	79,7	2,3	8,7	1,9	5,4	2,8
	13	5,5	6,9	14,7	182	2,3	0	2,4	1,8	1,6	4,7	6,3	75,2	0	7,4	1,4	5,3	3,8
	13	5,1	6,6	21,6	230	0,8	0,1	2,4	1,3	2,2	4,4	6,5	66,4	2,3	9	1,8	4,1	2,3
	14	5,1	7,2	10,7	132	1,1	0,1	5	1,8	1,1	7,2	8,2	82,7	1,4	4,1	2,7	14,7	5,4
	14	5,3	6,8	15	182	0,4	0,2	2,8	1,6	1,7	5	6,6	73,6	4	7,1	1,7	6	3,5
	15	5,3	7,1	11,7	99	1,3	0,2	1,4	1	1,2	2,8	3,9	68,1	7,1	6,5	1,3	5,4	4,1
2014/15	14	5,1	7	17,8	195	0,9	0	2,7	1,5	1,4	4,8	6,1	77,5	0	8,1	1,8	5,5	3,1
	14	4,8	7	14,6	178	0,9	0	4	1,7	1,4	5	7	86,7	0	7,6	2,1	5,5	3,1
	12	5	7,2	17,5	167	0,8	0,3	3	1,7	1,3	5,1	6	85,4	1,3	7	1,9	5,7	3,4
	14	5,1	7,3	16	189	1,2	0,2	3	1,6	1,2	4,6	6	87,9	1,5	9	1,8	5,4	2,8
	13	5,2	6,8	15,8	178	1,1	0,1	2	1,9	1,3	4,6	5,4	69,9	1,7	5,8	2,3	5,8	3,2
	13	4,8	7	14,7	187	1	0	1,7	1,4	1,1	4,7	7	60,2	1,6	7,4	2,1	8	2,9
	13	4.7	7,1	13,9	190	0,9	0	1,4	1,5	1	4,3	5	89,1	1,8	4,1	2,1	8	2,8
									External Coastal Plain									
2013/14	15	6,1	7,5	27,9	200	1,8	0	4	2	1,1	4	4	74,2	0	9,8	3	4	2,1
	16	5,9	7,1	21,6	210	1,8	0	4,1	1,9	1	4,5	4	74,3	0	9,7	2,9	7	2,1
	17	5,5	7,3	22,1	201	1,9	0	4,5	2	1,2	3,9	4	73,2	1,3	9,8	3	6	2,2
	18	6,2	7,3	23,6	198	2	0	4,7	1,9	1	4,3	5	75,5	1,5	9	2,8	7	2,6
	16	6,1	7,7	25,1	189	1,9	0,1	4	1,8	1	4,5	4,6	70,1	1,7	9,6	2,9	6	2,1
	18	6,4	7,2	19	201	1,8	0,2	4	1,9	1,2	4,6	5,2	69,9	1,6	9,7	3	5	2,9
	17	6,3	7,5	19,2	220	1,7	0	4,5	1,9	1	4,1	4	70,1	1,8	9,9	3,1	5	2,1
2014/15	19	5	7	24,9	203	2	0,1	4,8	2	1,1	4	4,2	72,1	0	8,9	2,1	4	2,1
	17	6,7	7,4	24,7	220	2	0,1	3,9	1,9	1,2	3,8	4,1	72,5	0	9	3	4	2,1
	16	6,6	7,3	21,7	200	1,9	0	6	1,8	1,2	3,2	4,3	68,2	1,9	9,1	3,1	4	2
	15	6,2	7,1	21,9	200	2	0	5,4	1,9	1,1	3,9	4,3	68,5	0	9,6	3,2	4	2,9
	18	5,7	7,1	19,9	201	1,8	0	4,1	2	1	4	5	68,4	0	9,7	3,1	4	2,1
	17	5,8	7,3	31,2	202	1,9	0	4	1,9	1	3,9	4,2	70,1	1,6	9,9	3	4	2
	15	6,1	7,5	17,9	200	1,8	0	4	2	1,1	4	4	74,2	0	9,8	3	4	2,1

The studied soils presented physicochemical characteristics significantly different, as detected by MANOVA (Wilk's Lambda of 0.008, p <0.0001 and F = 61.6). Figure 4 is a detail of the data found in the PCA.

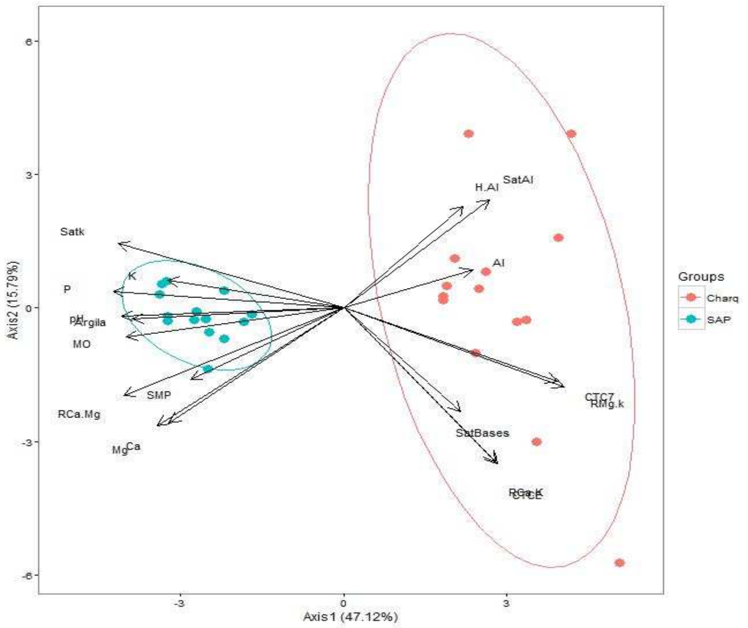


Figure 4: Principal component analysis of physicochemical elements of the soil of the Internal Coastal Plain (Charq) and the External Coastal Plain (SAP) – RS, Brazil, in the crop years of 2013/14 and 2014/15.

In this way it is possible to observe that the parameters are highly correlated with the axis levels totaling 74.19%. With the PCA analysis it is evident that the most important elements are the regions, phosphorus, pH, clay and organic matter for the difference between the points, especially since the correlation between the variables is greater than 0.8. Among the variables of the second component the correlations higher than 0.7 were considered, which consisted of calcium / potassium ratio and effective electron exchange capability. For the third axis, the variable above 0.6 was potassium, according to Table 2.

In a study on the quality of water in rice fields, similar results were found, including the variables that mostly affect rice: pH, phosphorus, potassium and calcium (PANIZZON et al., 2013). A similar study to the previous one found the values of electrical conductivity, pH and phosphorus as signifiers for the growth of the plant, mainly in the phase of maturation, which is when it begins to form the grain of rice (RECHE et al. 2016).

TABLE 2: Matrix of correlation of physicochemical variables of this study, and the three main components of the varimax rotation method.

Variables PCA	Axis 1	Axis 2	Axis 3
Local	0,9713	0,09171	-0,1107
Clay	0,8145	0,04894	-0,2
pH	0,821	0,02829	-0,1501
SMP	0,5461	0,3305	-0,4891
P	0,8459	-0,09832	0,1616
K	0,6351	-0,1443	0,6573
M.O	0,8075	0,124	-0,1512
Al	-0,4517	-0,1625	-0,3084
Ca	0,6606	0,5112	0,03162
Mg	0,6842	0,527	0,232
H + Al	-0,4459	-0,4615	0,5451
CTC E	-0,5533	0,7105	0,2812
CTC 7	-0,7817	0,3461	0,3756
Sat Bases	-0,4406	0,4645	0,04213
Sat Al	-0,5141	-0,4751	-0,5816
Sat K	0,8208	-0,3098	0,123
R Ca / Mg	0,8176	0,3933	-0,04307
R Ca / K	-0,5303	0,7134	-0,1546
R Mg / K	-0,7983	0,3762	-0,2179

The spatial pattern resulting from the distribution of the samples at two distinct locations demonstrates the soil difference and thus defines a different pattern for each region, resulting in grains with statistically different nutritional qualities (Table 2, Figure 4). Other studies show that soil spatial variability occurs naturally (ZHAO et al., 2011). These variations can be affected by human activity, flora and fauna. The soil affects the agricultural activities and is capable of generating foods with distinct nutritional characteristics in the same plant species (RODRÍGUEZ-GARAY et al. 2016).

Currently, there are researches that add silicon to the soil through the straw and the husk of rice. Although silicon is not considered an essential element for plants, its effects on monocotyledons, including rice, have been evaluated as positive. It is known that silicon comprises up to 10% of the dry matter of straw and rice husk, being much higher than other mineral nutrients. If this husk is applied before preparation of the soil, it is possible to increase the initial soil pH throughout the cycle (PINHEIRO, et al., 2016). This is a good option to try to regulate the level of pH in the Internal Coastal Plain that presented values lower than the External Coastal Plain. Another important factor is early irrigation, which can bring advantages to the nutrition of the rice plants, because the availability of nutrients in the soil solution increases with the flood. Therefore, the earlier irrigation is started, more readily rice plants will be able to receive this benefit (NASCIMENTO et al. 2009), making rice more regular in the requirements for its production, thus generating more uniform grains with better nutritional value.

Studies have shown that the presence of phosphorus and potassium, even in soils with low organic matter, results in good grain yield (BEUTLER et al., 2016), as observed in the Internal Coastal Plain. In addition, soil macroporosity is an important physical attribute in soil pore distribution analysis (SIMIONI et al., 2016). With greater porosity the nutrients are more accessible to plants. The amount of calcium needed for monocotyledonous growth is lower than for dicotyledonous. However, calcium was an element that appeared with relative importance in PCA (Figure 4), because it reduces soil acidity and facilitates root growth (GIONGO et al. 2016).

CONCLUSIONS

Under the conditions in which these studies were carried out, based on collections of soil samples and rice grains from the crop years of 2013/14 and 2014/15, it can be inferred that: (i) the two rice regions, the Internal and the External Coastal Plain, presented different physicochemical characteristics of soil, being the values of pH, organic matter and clay higher in PCE; (ii) soil physicochemical elements produce grains with significantly different nutritional qualities; (iii) the polished and brown rice of the External Coastal Plain, for having a more fertile soil, produced better quality Puita Inta CL rice grains.

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6 CONSIDERAÇÕES FINAIS

A agricultura moderna é caracterizada pela busca constante do aumento da produtividade das culturas agrícolas por meio da utilização da mecanização, irrigação, adubação química e aplicação de pesticidas, aliadas ao melhoramento dos genótipos vegetais. Entretanto, o impacto ambiental causado pela intensificação da exploração agrícola nem sempre recebeu a atenção necessária. A ausência de conhecimento aprofundado sobre o ecossistema e/ou planejamento inadequado na utilização das terras levou a um quadro de intensa degradação ambiental, com perda de recursos não renováveis e da biodiversidade não só no Brasil como em outros países. A constatação do aumento de áreas agrícolas degradadas tem levado a uma mudança de concepção do uso da terra, baseada em uma visão do processo agrícola, no qual os recursos naturais (solo, água e biodiversidade) são explorados de forma mais sustentável.

A diversidade de micro-organismos como indicador da qualidade dos agroecossistemas tem sido bastante debatida desde o final do século passado. (COUTINHO et al, 1999; TIEDJE et al, 2001). Esta diversidade de micro-organismos é fundamental para o funcionamento do ecossistema, porque há necessidade da manutenção de processos ecológicos como a decomposição da matéria orgânica, ciclagem de nutrientes, agregação do solo e controle de patógenos dentro do ecossistema. (KENNEDY, 1999).

Apesar da utilização contínua do solo para as práticas agrícolas, o mesmo pode ser rico do ponto de vista biológico uma vez que muitos micro-organismos encontrados nesse ambiente são considerados importantes no equilíbrio das comunidades e suas sucessões.

A união da boa produção do alimento sem gerar grandes impactos na alimentação saudável pela adição de probióticos e prebióticos nos alimentos traz benefícios ao homem. Na atual sociedade, a expectativa de vida populacional e o crescente aumento dos custos com medicamentos, a nutracêutica deve unificar os conhecimentos científicos sobre a saúde humana e a fonte de nutrição adequada a estes novos desafios promovendo a saúde e não apenas a cura de doenças. A microbiota saudável promove o bem estar, quando avaliadas com um alimento comum de toda a população, torna ainda mais acessível a grande parte da população. Portanto, o conhecimento do alimento juntamente com as condições de cultivo, levam ao desenvolvimento de inúmeras estratégias de saúde coletiva que podem ser usadas para minimizar o impacto da natureza e melhorar a qualidade nutricional do que está sendo produzido.

O presente trabalho abordou os principais aspectos relacionados à cultura de arroz irrigado dos anos agrícolas 2013/14 e 2014/15, descrevendo a abundância e diversidade de bactérias heterotróficas presentes no solo de duas regiões orizícolas, bem como avaliação nutricional dos grãos produzidos em cada área de estudo.

O capítulo 1 apresentou dados bibliográficos sobre o papel e a função da diversidade microbiana. O uso crescente de alimentos probióticos e prebióticos levou aos estudos sobre suas funções reais no corpo humano. Portanto, foi realizada uma pesquisa sobre os micro-organismos ambientais e intestinais, que podem contribuir em produtos como aromatizantes, na síntese de substâncias antimicrobianas e vitaminas essenciais aos seres humanos.

O capítulo 2 trouxe uma revisão da literatura sobre as comunidades microbianas que habitam os agroecossistemas aquáticos que são fundamentais para a compreensão do que acontece no solo, uma vez que estes micro-organismos desempenham papéis importantes para a manutenção do habitat. A interação bactéria-solo-planta tem sido usada para apoiar a biotecnologia, pois a rizosfera possui uma ecologia microbiana diferente do resto do solo. Os micro-organismos desta região estão diretamente relacionados ao crescimento das plantas.

O capítulo 3 avaliou os efeitos de variáveis físico-químicas na diversidade de comunidades bacterianas presentes em solos de arroz, em duas regiões do sul do Brasil. Foram registradas o total, 29 Unidades de Formação de Colônias (UFCs), sendo a diversidade bacteriana similar. Em ambos os tipos de solos, gleissolos e planossolos, as seguintes comunidades de bactérias heterotróficas foram maiores: *Bacillus thuringiensis, Bacillus cereus, Lisinibacillus sphericus, Pseudomonas fluorescens, P. putida, Corinebacterium* spp.

O capítulo 4 avaliou as características físico-químicas do solo, em áreas orizícolas da Planície Costeira Interna e Externa do RS, e o valor nutricional do arroz Puita Inta CL. O conteúdo nutricional, teor fenólico e teor de antioxidantes foram testados pelo método de Mann Whitney, em que se comparam amostras obtendo o resultado de que o arroz integral e o polido têm características diferentes, dependendo da região onde é cultivado ($p<0,001$), porém sem diferença temporal ($p>0,05$). As 18 elementos físico-químicos dos solos das duas regiões de estudo apresentaram diferença significativa ($p<0,001$), porém não houve diferença temporal ($p>0,05$). Portanto, o tipo de solo pode afetar a qualidade nutricional do grão de arroz, mesmo que a cultura tenha recebido o mesmo manejo.

Os resultados apresentados nessa pesquisa revelam sua contribuição científica por meio dos quatro artigos publicados e/ou submetidos, assim como servem de base para futuros estudos relacionados às interações dos agroecossistemas quanto aos elementos: solo, água, micro-organismos e plantas.

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